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Diversity and Structure of Hoverfly (Diptera: Syrphidae) Communities in Agricultural Areas in Vojvodina Province (Serbia) A Case Study on *Brassica napus* L.

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ABSTRACT

To gain some insight into the structure of the hoverfly community in fields of oilseed rape (*Brassica napus* L.), we conducted field experiments over three years (2011-2013) at two localities in the province of Vojvodina, northern Serbia. We recorded a total of 20 hoverfly species. Three species-*Episyrphus balteatus* (De Geer, 1776)*, Eristalis tenax* (Linnaeus, 1758) and *Eupeodes corollae* (Fabricius, 1794)-were the most abundant in both localities and in each year. In order to determine the effect of climatic and non-climatic parameters on abundance and diversity of hoverflies, two separate PCA analyses were carried out. Multiple linear regressions were used to examine the relationships between abundance and extracted PC axes, while ordinal multinomial regressions were conducted to determine the relationships between species diversity and extracted PC axes. We did not detect statistically significant correlations between climatic and non-climatic parameters and overall hoverfly abundance. PC axes exhibited slight correlation with species diversity. The first PC axis clearly showed that overall species diversity increases with increasing temperature, relative humidity and diversity of crops surrounding the surveyed plots, whereas PC2 related species composition with monthly average rainfall and the season and year of observation. Synecological analysis of our data indicated that only a few species are major contributors to hoverfly communities on the oilseed rape crops we investigated. However, the importance of less abundant hoverfly species should not be underestimated, as sometimes these species play an important role in pollination in a specific part of the day or season, and this should be considered when creating agricultural policies and regulations, especially when it is known that abundances of hoverflies and pollinators in general are positively correlated with floral abundance and abundance flowering plant species.

Key words: Hoverflies, oilseed rape, PCA, pollinators, species diversity, synecological analysis.

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INTRODUCTION

Pollinators contribute to the sustainability and stability of ecosystems. Additionaly, ecosystem services, such as pollination, are immensely important for human prosperity. alarming declines in some pollinator groups, such as bees (Hymenoptera: Apidae), have been reported worldwide (Westrich, 1989; Buchmann & Nabhan, 1997; Allen-Wardell et al, 1998; Bacandritsos et al, 2010; Genersch et al, 2010; Potts et al, 2010a, b). Understanding the consequences of these declines has become a priority, not only because of the biodiversity loss, but also for their considerable impact on agriculture and economy (Costanza et al, 1997). About 70% of tropical crops have at least one variety that is dependent on animal pollination (Roubik, 1995). This figure is even greater for European crops, 84% of which rely on animal pollination at least to some extent (Williams, 1994).

In the U.K. and Netherlands, declining wild plant diversity is mirrored by a decline in wild bee populations, whereas this pattern was not found for hoverflies (Biesmeijer et al, 2006). Although traditionally placed in the pollinator guild and sometimes considered the second most important pollinators after wild bees (Larson, Kevan, & Inouye, 2001), hoverflies have not received much attention as such. For example, *E. tenax* (Linnaeus, 1758) has been suggested as an adequate pollinator for sweet pepper in greenhouses (Jarlan, de Oliveira, & Gingras, 1997) and for apple trees (Kendall, Wilson, Guttridge, & Anderson, 1971), but the efficiency of other hoverfly species regarding pollination of other plants and especially crops has not been assessed properly (Larson et al*,* 2001).

Hoverflies are a diverse group, with over 6000 species described to date (Thompson, 2013). Hoverflies include generalist and cosmopolitan species due to having different larval feeding types and the heterogeneous ecological requirements of adults (Vujić et al*,* 2002), occurring in various habitat types from seashores to mountains. This insect group is not only important because of its role in pollination, the larvae of many species are aphidophagous and can be used in biological control, but only few species are commercially bred for that purposes (Pineda & Marcos-García, 2008).

Brassica napus (common names: rapeseed, rape, oilseed rape) is a member of the family *Brassicaceae*. It has entomophilous flowers capable of both self- and cross-pollination. The open corollas of the flowers allow almost every group of pollinating insects to feed on it, and their yellow colour and the shallow, visible placement of nectar attracts bees, flies and butterflies (Kunin, 1993). Production of oilseed rape has rapidly increased in recent decades (Rathke, Behrens, & Diepenbrock, 2006). It has become the dominant flowering crop in the European Union, mostly as a result of increased demand for energy crops (van der Velde, Bouraoui, & Aloe, 2009). Due to the high oil and protein content of its seeds, oilseed rape is mainly grown for the production of vegetable oil for human consumption, as an animal food and for the production of biodiesel (Milošević et al, 2016). The area under this important crop in Serbia increased from 17860 ha in 2015 to 37500 ha in 2016 (Association for the Promotion of Production and Exports of Grains and Oilseeds, 2017).

Serbia's northern province, Vojvodina, is composed mainly of agricultural land (83%), almost half of which is high quality chernozem soil (Hadžić, Nešić, & Sekulić, 2005). This province therefore represents one of the most fertile areas in Serbia. The majority of the region's agriculture is focused on the production of cereal grains, constituting 66% of all crops, but oilseed crops have increased in the past decade (Gligorov et al, 2010). As a result of shifting production, Vojvodina now produces 96% of the oilseed crop in Serbia (Gligorov et al, 2010). Considering its great economic value, we assessed the composition of hoverfly community found in *B. napus* fields. We investigated two different localities with oilseed crops in Vojvodina (Crvenka and Rimski Šančevi), considering them as a case study for this region, in order to: 1) detect species of hoverflies found on oilseed rape crops; 2) determine the structure of established hoverfly communities; 3) examine the possible influence of climatic and non-climatic parameters on those communities and 4) gain as much information about these species and their relationship to oilseed crops in order to complement the existing knowledge, which would contribute to better management practice.

MATERIAL AND METHODS

Two study areas were chosen to assess hoverfly diversity, one in the central part of Vojvodina at Rimski Šančevi, (Fig. 1A: ▲) and another in its northwestern part, in the agricultural district of Crvenka (Fig. 1A: ●). Both sampling locations are experimental fields of the Institute of Field and Vegetable Crops, Novi Sad. Sampling was carried out between 2011 and 2013. Specimens were collected in spring (from late February/ early March to July), and in the autumn (from September to November).

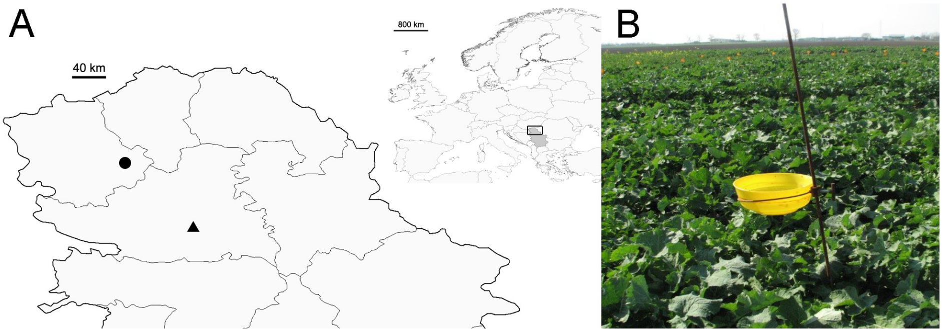

Fig. 1. A. Map of Vojvodina province (northern Serbia) with the sampling locations: ● Crvenka, ▲ Rimski Šančevi. B. Example of a yellow pan trap used for capturing pollinators in fields of *Brassica napus.*

Sampling was carried out using yellow pan traps details in (Leong & Thorp, 1999). The advantage of these traps is that they are easy to use, and the data collected is independent of the weather conditions at the moment of sampling. A set of four traps was placed at each locality. The traps were set on stands, about 10 cm above crop level to prevent overshadowing by the plants (Fig. 1B). Specimens were collected every seven days, stored in 75% ethyl-alcohol and labelled (locality, date, trap number, etc.). Examination of material and species determination was conducted in the

Laboratory for Biodiversity Research and Conservation of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, by Zorica Nedeljković. All specimens were identified to species level, except for three individuals of the genus *Cheilosia* Meigen, 1822, that could not be identified due to damage of taxonomically important characters. We used Van Veen (2004) and Bartsch, Binkiewicz, Rådén, & Nasibov (2009) to aid identifications based on morphological characters. Specimens were examined using a Nikon SMZ 745T binocular microscope.

Assessment of ecological indices

We conducted synecological analysis on our data to detect changes in community structure between seasons and to determine the position of each species in the local assemblages. The indices used here can be divided into analytical ecological indices (abundance, dominance, constancy) and the synthetic index of ecological significance (Grall & Coic, 2005).

Abundance (A) represents the number of specimens collected from a particular species. Species constancy (C_A) represents the percentage of samples in which a particular species occurs and is calculated according to the formula:

$$C_A=(N_P A/N_P)*100$$

Where N_PA is number of samples in which species A occurs and N_P is the total number of samples (Grall & Coic 2005). There are four classes of constancy: C_1 (1-25%)-accidental species; C_2 (25-50%)-accessory species; C_3 (50-75%)-constant species; C_4 (75-100%)-euconstant species.

Dominance reveals the degree to which each species contributes to biomass production in biocenosis and is indicative of relative abundance (Grall & Coic, 2005). Species dominance can be calculated according to the formula:

$$D_A=(N_A/N_1)*100$$

Where N_A is total number of individuals of a particular species, and N_1 is the total number of individuals of all species. Species can be divided into five categories of dominance: D1 (<1%)-subrecedent, D2 (1-2%)-recedent, D3 (2-5%)-subdominant, D4 (5-10%)-dominant, D5 (>10%)-eudominant.

The index of ecological significance (W) represents the relationship between the structural and productive indicators, showing more clearly the position/importance of each species in the assemblage. We calculated the ecological significance index according to the formula:

$$W_A=(C_A*D_A)*100/10000$$

Where C_A is the constancy of species A, and D_A is the dominance of species A. There are five categories of ecological significance: W1 (<0.1%)-accidental species, W2 (0.1-1%)-accessory species, W3 (1-5%)-accompanying species, W4 (5-7%)-constant species, W5 (>10%)-edifying species (Grall & Coic, 2005). Calculation of ecological indices was done using Microsoft Office Excel.

We also calculated the Shannon-Wiener (H’) index according to the formula:

$$H'=-\sum p_i \ln p_i$$

Where pi is the proportion of individuals belonging to a certain species in the dataset.

The H’ index accounts for both species abundance and evenness. Values of this index range from 0 to 4, with the index increasing as both richness and evenness of the community increases and higher values indicating that richness is evenly distributed among species.

Variation in the abundance and composition of hoverfly species

Climatic variables were represented as monthly averages for temperature, relative humidity, insolation, cloudiness/cloud cover, rainfall, and wind velocity. This information was obtained from the Meteorological Yearbook for each year of our research, available from the Hydrometeorological Service of the Republic of Serbia (RHMZ, 2011-2013). Non-climatic parameters included season (spring and autumn), year of observation, sampling locality and crop diversity around the sampling locality.

The influence of both climatic and non-climatic variables on species diversity and abundance was assessed by principal component analysis (PCA) to reduce observed variables into a smaller number of principal components (PC axes) accounting for most of the variance. PCA was carried out by applying a normal varimax rotation of factor loadings. PCs with eigenvalues >1 were retained as predictor variables. Variables with factor loadings >0.8 were interpreted as meaningfully correlated with the PC axes. PCA analysis was carried out in Statistica (StatSoft, Inc. v. 13.2).

In order to examine the effect of interacting climatic and non-climatic parameters on species abundances, we carried out multiple regression analysis on extracted PCs. To inspect the effect of analysed parameters on species diversity, we conducted ordinal multinomial logistic regression on PCs. Analyses were conducted using the R statistical platform (version 3.3.1., R Core Team, 2016).

RESULTS

Species composition

In total, 294 specimens of 20 species were detected in our two oilseed rape field locations. At Crvenka, 13 out of 20 (65%) species were recorded, whereas 14 out of 20 species (70%) were present in Rimski Šančevi.

Calculation of the ecological indices

The most abundant species at both localities was *Eupeodes corollae* (Fabricius, 1794), followed by *Eristalis tenax* (Linnaeus, 1758), and *Episyrphus balteatus* (De Geer, 1776) (Table 1).

Overall species abundance ranged from 1 to 80 individuals per species. In the first year of study (2011), the most abundant species in Crvenka was *E. balteatus* (24 individuals, 35% of all registered species), whereas *E. corollae* was the most numerous in Rimski Šančevi with 16 individuals (94%). In 2012, *E. tenax* was the most abundant species in Crvenka (5 individuals, 24%), and two species were most abundant in Rimski Šančevi (*E. tenax* n=37 (30%) and *E. corollae* n=55 (34%)). In 2013, the most abundant species in Crvenka were *Eristalis arbustorum* (Linnaeus, 1758) and *E. tenax* (each with 3 individuals, 37.5%), and in Rimski Šančevi it was *E. tenax* (21 individuals, 49%).

Table 1. List of species and their abundances recorded in two Brassica napus fields in Vojvodina province, Serbia.

	Sampling localities	
Species	Crvenka	Rimski Šančevi
Cheilosia grossa (Fallen, 1817)	3	0
Cheilosia latifrons (Zetterstedt, 1843)	1	0
Cheilosia sp.	3	0
Dasysyrphus friuliensis (van der Goot, 1960)	1	0
Episyrphus balteatus (De Geer, 1776)	26	17
Eristalinus aeneus (Scopoli, 1763)	2	3
Eristalis arbustorum (Linnaeus, 1758)	7	9
Eristalis pertinax (Scopoli, 1763)	1	0
Eristalis similis (Fallen, 1817)	0	3
Eristalis tenax (Linnaeus, 1758)	28	59
Eupeodes corollae (Fabricius, 1794)	22	80
Eupeodes luniger (Meigen, 1822)	0	1
Helophilus trivittatus (Fabricius,1805)	1	3
Melanostoma mellinum (Linnaeus, 1758)	0	5
Melanostoma scalare (Fabricius, 1794)	0	1
Myathropa florea (Linnaeus, 1758)	0	1
Parhelophilus versicolor (Fabricius, 1787)	1	0
Sphaerophoria scripta (Linnaeus, 1758)	4	7
Syritta pipiens (Linnaeus, 1758)	0	2
Syrphus vitripennis (Meigen, 1822)	0	3
Total	100	194

Slight changes in species composition were recorded throughout the seasons at both localities. The most noticeable difference occurred in the first year of our field study. The most common species (*E. balteatus, E. arbustorum, E. tenax, E. corollae*) were all detected at Crvenka, whereas only two species were recorded (*E. tenax, E. corollae*) at Rimski Šančevi. At Crvenka, species were evenly distributed among the different categories of all three indices (dominance, constancy, and ecological significance index), whereas at Rimski Šančevi only edifying species were detected. In the second year of the study, the gap in species abundance and composition between the two localities was smaller, with Crvenka exhibiting its greatest abundance (10 out of 20 species). Even so, most of the species were in the lower categories of constancy (accidental and accessory species), dominance (subrecedent, recedent, subdominant), and ecological significance (accidental, accessory, accompanying species). *Eristalis arbustorum* and *E. tenax* made a significant contribution to species constancy, while *Cheilosia grossa* (Fallen, 1817) and *Cheilosia* sp. contributed to species dominance. The pattern of species composition at Rimski Šančevi was similar to the previous year. *Sphaerophoria scripta* (Linnaeus, 1758) and *Syritta pipiens* (Linnaeus, 1758) contributed to dominance and ecological significance, along with the three most common species (*E. balteatus, E. tenax* and *E. corollae*). This scenario was reversed for the final year of the study. Crvenka exhibited lower species abundance compared to Rimski Šančevi, it had fewer species in higher categories (e.g. no edifying species), and only one species (*Parhelophilus versicolor* (Fabricius, 1787)) was detected apart from the three most typical species (*E. balteatus, E. tenax, E. corollae*). In the final year, Rimski Šančevi exhibited both its greatest number of species and number of individuals per species. Almost every category of all indices was detected over the course of the three years. The results of our synecological analyses are detailed in Tables 2, 3 and 4.

Shannon-Wiener's index of diversity values ranged from 0.22 to 2.14. The lowest value was recorded in 2011 for Rimski Šančevi, and the highest in 2012 in the same locality. However, overall, Crvenka presented higher values of the Shannon-Wiener index than Rimski Šančevi (Table 5).

Effects of climatic and non-climatic parameters on species assemblage structure

In order to identify factors that influence species composition and abundance, we conducted PCAs. The first PCA generated two PC axes, and the second one generated three PC axes, all with eigenvalues >1 (Table 6). None of the extracted PCs were related to abundance at the significance level of $p<0.05$ ($p=0.4305$, $r=0.0552$) based on multiple linear regressions.

Both PC axes of the first PCA showed a slight correlation with species diversity (PC1: Wald statistic=5.8616, df=1, p=0.01547; PC2: Wald statistic=9.8790, df=1, p=0.00167). The first PC axis was negatively correlated with monthly average temperature and insolation, and positively correlated with monthly relative humidity, wind velocity, cloud cover, locality and surrounding crop diversity. The second PC axis was correlated with monthly average rainfall, season, and year of observation (Table 6, Fig. 2).

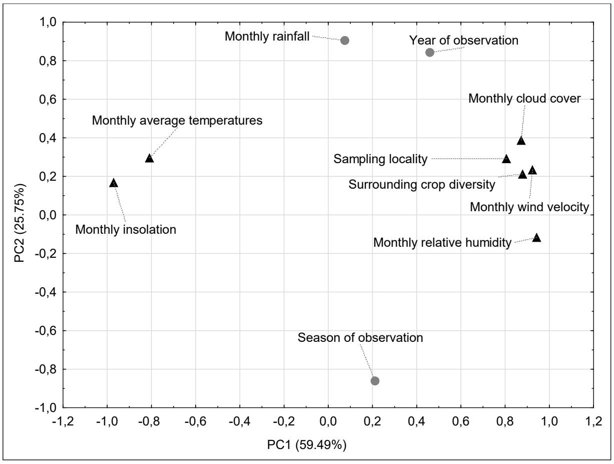

Fig. 2. Projection of analysed variables onto the PC1 and PC2 ordinations. ▲ represents variables significantly correlated with PC1; ● represents variables significantly correlated with PC2.

Table 2. Values of ecological indices for the hoverfly community in Crvenka and Rimski Šančevi in 2011.

	Constancy		**Dominance**		**Significance**	
Species	**%**	**Category**	**%**	**Category**	**%**	**Category**
Crvenka, 2011						
Cheilosia latifrons (Zetterstedt, 1843)	50	C3	0.95	D1	0.48	W2
Episyrphus balteatus (De Geer, 1776)	100	C4	24.65	D5	24.65	W5
Eristalis arbustorum (Linnaeus, 1758)	100	C4	3.89	D3	3.89	W3
Eristalis pertinax (Scopoli, 1763)	50	C3	0.95	D1	0.48	W2
Eristalis tenax (Linnaeus, 1758)	100	C4	22.03	D5	18.26	W5
Eupeodes corolla (Fabricius, 1794)	100	C4	38.85	D5	38.6	W5
Helophilus trivittatus (Fabricius,1805)	50	C3	0.95	D1	0.48	W2
Sphaerophoria scripta (Linnaeus, 1758)	100	C4	7.74	D4	7.74	W4
Rimski Šančevi**, 2011**						
Eristalis tenax (Linnaeus, 1758)	50	C3	10	D4	5	W5
Eupeodes corolla (Fabricius, 1794)	100	C4	95	D5	95	W5

Table 3. Values of ecological indices for the hoverfly community in Crvenka and Rimski Šančevi in 2012.

	Constancy		**Dominance**		**Significance**	
Species	**%**	**Category**	**%**	**Category**	**%**	**Category**
Crvenka, 2012						
Cheilosia grossa (Fallen, 1817)	16.6	C1	4.63	D3	0.77	W2
Cheilosia sp	16.6	C1	4.63	D3	0.77	W2
Dasysyrphus friuliensis (van der Goot, 1960)	16.6	C1	1.52	D2	1.53	W3
Episyrphus balteatus (De Geer, 1776)	33.34	C2	7.07	D4	2.36	W3
Eristalinus aeneus (Scopoli, 1763)	16.6	C1	3.03	D3	0.5	W2
Eristalis arbustorum (Linnaeus, 1758)	33.34	C2	4.85	D3	1.62	W3
Eristalis tenax (Linnaeus, 1758)	33.34	C2	18.19	D5	6.3	W4
Eupeodes corollae (Fabricius, 1794)	16.67	C1	5.56	D4	0.93	W2
Eupeodes luniger (Meigen, 1822)	16.67	C1	16.67	D5	2.78	W3
Myathropa florea (Linnaeus, 1758)	16.67	C1	16.67	D5	2.78	W3
Rimski Šančevi, 2012						
Episyrphus balteatus (De Geer, 1776)	50	C3	10.34	D5	5.15	W4
Eristalinus aeneus (Scopoli, 1763)	16.67	C1	0.48	D1	0.08	W1
Eristalis arbustorum (Linnaeus, 1758)	33.34	C2	0.86	D1	0.27	W2
Eristalis similis (Fallen, 1817)	33.34	C2	1.15	D2	0.21	W2
Eristalis tenax (Linnaeus, 1758)	33.34	C2	9.37	D4	9.37	W4
Eupeodes corollae (Fabricius, 1794)	33.34	C2	32.13	D5	10.72	W5
Helophilus trivittatus (Fabricius,1805)	16.67	C1	0.95	D1	0.16	W2
Melanostoma mellinum (Linnaeus, 1758)	16.67	C1	0.6	D1	0.09	W1
Melanostoma scalare (Fabricius, 1794)	16.67	C1	0.2	D1	0.03	W1
Sphaerophoria scripta (Linnaeus, 1758)	83.34	C4	0.98	D1	0.51	W2
Syritta pipiens (Linnaeus, 1758)	33.34	C2	8.33	D4	8.86	W3
Syrphus vitripennis (Meigen, 1822)	33.34	C2	0.86	D1	0.29	W2

Table 4. Values of ecological indices for the hoverfly community in Crvenka and Rimski Šančevi in 2013.

	Constancy		**Dominance**		**Significance**	
Species	**%**	**Category**	**%**	**Category**	**%**	**Category**
Crvenka, 2013						
Eristalis arbustorum (Linnaeus, 1758)	20	C1	20	D5	4	W3
Eristalis tenax (Linnaeus, 1758)	20	C1	15	D5	3	W3
Eupeodes corollae (Fabricius, 1794)	20	C1	20	D5	4	W3
Parhelophilus versicolor (Fabricius, 1787)	20	C1	5	D3	1	W2
Rimski Šančevi**, 2013**						
Episyrphus balteatus (De Geer, 1776)	40	C2	8.71	D4	3.47	W3
Eristalinus aeneus (Scopoli, 1763)	20	C1	1.21	D3	0.24	W2
Eristalis arbustorum (Linnaeus, 1758)	20	C1	3.64	D3	0.73	W2
Eristalis tenax (Linnaeus, 1758)	60	C3	35.3	D5	21.18	W5
Eupeodes corollae (Fabricius, 1794)	40	C2	12.43	D5	2.5	W3
Helophilus trivittatus (Fabricius,1805)	20	C1	0.61	D1	0.12	W2
Melanostoma mellinum (Linnaeus, 1758)	20	C1	1.21	D3	0.24	W2
Sphaerophoria scripta (Linnaeus, 1758)	40	C2	3.11	D3	1.24	W3

Table 5. Values of Shannon-Wiener's index of diversity in two *Brassica napus* fields from Vojvodina, Serbia.

	Shannon-Wiener's index	
Year of observation	Crvenka	Rimski Šančevi
2011	1.51	0.22
2012	2.14	1.98
2013	1.25	1.68

Table 6. Principal component analysis of 10 climatic and non-climatic variables associated with hoverfly species diversity and abundance in oilseed rape fields from Vojvodina, Serbia. Significant factor loadings are in bold.

	PC1	PC2	PC3	PC1	PC2
Variables	Species abundance			Species diversity	
Monthly average temperatures	-0.18	**0.87**	0.17	**-0.80**	0.29
Monthly relative humidity	0.05	-0.19	**-0.97**	**0.94**	-0.11
Monthly insolation	0.14	-0.27	**0.94**	**-0.96**	0.16
Monthly cloud cover	**0.99**	0.14	0.00	**0.87**	0.38
Monthly rainfall	**0.99**	0.04	-0.02	0.07	**0.90**
Monthly wind velocity	0.41	0.89	0.20	**0.92**	0.23
Sampling locality	0.09	0.90	-0.31	**0.80**	0.29
Year of observation	**0.91**	0.33	0.12	0.45	**0.84**
Season of observation	-0.73	0.16	-0.65	0.21	**-0.86**
Surrounding crop diversity	0.34	**0.84**	-0.28	**0.87**	0.21
Eigenvalue	4.44	3.14	1.91	5.94	2.57
Total variance %	44.40	31.37	19.14	59.49	25.75
Cumulative variance %	44.40	75.77	94.91	59.49	85.25

The influence of variables related with PC1 and PC2 on species diversity is illustrated in Fig. 3. Overall, species diversity increases with increasing temperature, relative humidity and surrounding crop diversity. The left part of the PC1 ordination represents lower surrounding crop diversity (i.e. Crvenka: maize and sugar beet), whereas the right part represents species occurring at the sampling locality with higher crop diversity (Rimski Šančevi: maize, sunflower, sugar beet, wheat, soybean, vegetables, field pea and alfalfa).

DISCUSSION

The most abundant species at both localities over all three years of the study were *E. corollae, E. tenax* and *E. balteatus*. This is not surprising, considering that all of these species are anthropophilic and almost ubiquitous (Speight, 2017). Additionally, these species occur over a wide temperature range (with a tolerance to lower temperatures), and are not conditioned by the diversity of surrounding crops (Fig. 2). Similar findings were noted in a recent study of oilseed rape pollinators in Ireland (Stanley, Gunning, & Stout, 2013). In that study, alongside bumblebees (*Bombus,* Latreille, 1802) and honeybees (*Apis mellifera* Linnaeus, 1761), *Eristalis* Latreille, 1804 hoverflies were one of the most important pollinators of winter crops of oilseed rape based on the amount of pollen they carried, visitation rates and their abundance. Moreover, *E. tenax* is recognised as an efficient pollinator of various cultivated plants (vegetables, fruits, crops), including oilseed rape (Solomon & Kendall, 1970; Kendall et al, 1971; Nye & Anderson, 1974; Jarlan et al*,* 1997; Schittenhelm, Giadis, & Rao, 1997; Jauker et al*,* 2012). This species visits a wide range of flowers, and it can be active even in extremely cold conditions (Speight, 2017). Additionally, it is an anthropophilic species, often found in different types of farmland due to saprophagous larvae (Rotheray & Gilbert, 2011).

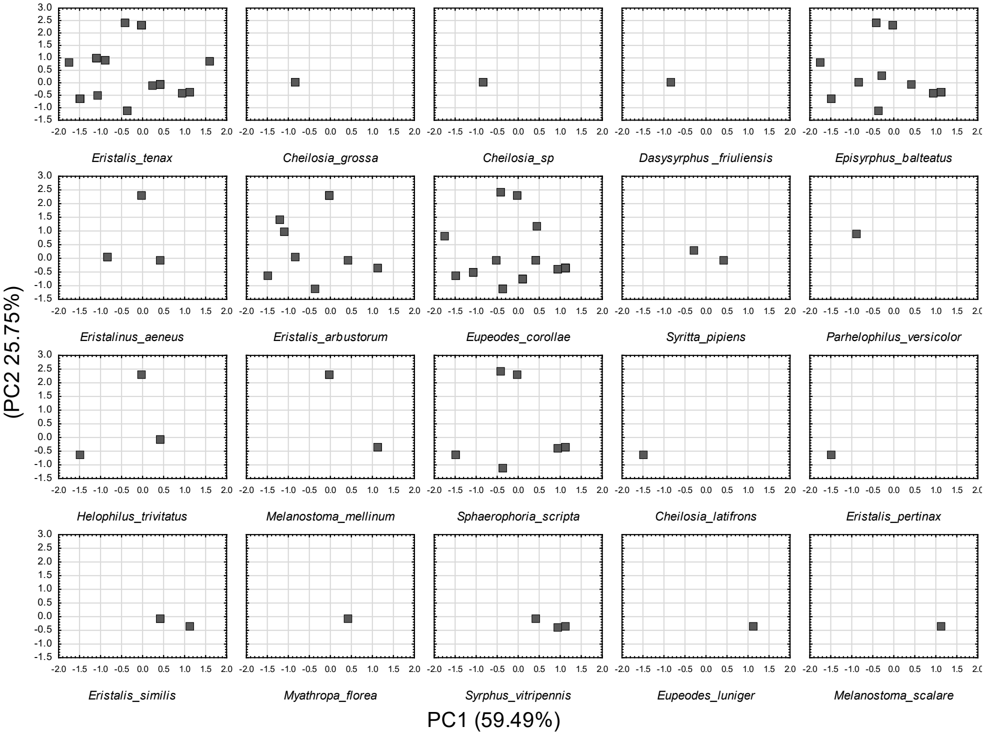

Fig. 3. Scatter plot of factor loadings for the two PC axes, showing the positions of the investigated hoverfly species in the environmental space.

Suitable microhabitats for these larvae are present throughout Vojvodina, so it is not surprising that *E. tenax* is present in the studied area.

It is somewhat expected that two of the most abundant species in our study localities were *E. corollae* and *E. balteatus* since they are predatory hoverflies commonly associated with agricultural habitats (Bergen, Soudhof, & Poehling, 1998; MacLeod, 1999; Sutherland, Sullivan, & Poppy, 2001; Jauker, Diekötter, Schwarzbach, & Wolters, 2009). Larvae of these species can be very useful as biocontrol agents as they feed on aphids, but as adults they are also pollinators for some crops (Hickman & Wratten, 1996). In addition, Jauker et al (2009) recorded that flowers of oilseed rape visited by *E. balteatus* produced significantly more seeds per pod. These authors proposed that apart from pollen transfer, *E. balteatus* enhanced plant self-pollination due to its characteristic behaviour on the flowers.

Our synecological analysis indicated that only a few species (*E. balteatus, E. tenax* and *E. corollae*) make a major contribution to constituting the hoverfly communities on oilseed rape crops. These species, along with *E. arbustorum* and *S. scripta*, may be characterized as the dominant hoverfly visitors of *B. napus*.

It is possible that relatively small number of species recorded is due to use of pan traps as the sampling method. This method has its limitations, since hoverflies are good fliers and do not fall into traps that easily. On the other hand, using pan traps allows simultaneous sampling of multiple locations, coverage of large number of sites, and laboratory identification of specimens (Westphal et al, 2008)

The importance of less abundant hoverfly species should not be underestimated. Gibson, Nelson, Hopkins, Hamlett, & Memmott (2006) found that even though *Platycheirus albimanus* was the least abundant species on their surveyed plots, it carried the highest percentage of pollen. In the same study, they concluded that *S. scripta* was not the most abundant species, but had the highest pollen fidelity. Some species like *Eristalinus aeneus* (Scopoli, 1763) have been found to be better pollinators of *B. napus* than *E. balteatus* and *E. corollae* due to their larger size and their foraging preference for nectar and pollen (Ali, Saeed, Sajjad, & Whittington, 2011). Another such example is *Melanostoma mellinum* (Linnaeus, 1758) that has been found foraging on flowers of *Rosa carolina* L. before sunrise, so it could contribute to pollination during parts of the day when most other pollinators are inactive (Morse, 1981). In our case, only few specimens of these two species were found, but this could be due to methodological limitations of pan trap, or relatively small sample size. Despite certain environmental constraints (solar radiation, temperature, cloud cover, etc.), hoverflies are known to forage under conditions when bees and butterflies are not active (Levesque & Burger, 1982). Thus, even non-abundant hoverfly species should be taken into account when creating agricultural policies and regulations, especially since it is known that abundance of hoverflies (and pollinators in general) is positively correlated with floral abundance and abundance of flowering plant species (Kleijn & van Langevelde, 2006; Meyer, Jauker, & Steffan-Dewenter, 2009; Sajjad et al, 2010). However, in modern agroecosystems, hoverflies face considerable challenges because agricultural intensification negatively affects the heterogeneity and quantity of hoverfly resources at various spatial scales (Benton, Vickery, & Wilson, 2003). In this era of increasing agricultural production, ecologically important habitats are being progressively eroded. Even if the increased area under crops could benefit certain species adapted to this type of habitat, in general it influences negatively the diversity of hoverflies and other pollinators. Therefore, it is of great importance to enhance overall plant species richness through crop rotation and, more importantly, by enlarging field margins and preserving patches of natural habitat within fields. Such areas have been shown to act as important population sources from which pollinators can disperse and thereby contribute to higher densities and species richness of pollinators in adjacent agricultural areas (Duelli & Obrist, 2003; Öckinger & Smith, 2007).

We did not find a statistically significant correlation between climatic parameters and overall abundance of hoverfly species, perhaps due to a lack of variation in the climatic parameters we measured, or relatively small sample size. However, it is possible that these parameters still affected some species or certain stages of their development (e.g. low temperatures at the beginning of the flight period, excessive rainfall at the peak flight period, not enough sunlight, etc.). Also, many factors other than climate can affect species diversity and seasonal patterns, such as food abundance and predation (Wolda, 1988; Abrahamczyk, Kluge, Gareca, Reichle, & Kessler, 2011). Despite the lack of a correlation for overall abundance, our PCA analysis showed that increased surrounding crop diversity contributed to higher numbers of individuals of *E. tenax*, *E. balteatus*, *E. corollae* and *S. scripta* (Fig. 2). Furthermore, the PCA indicated that variation in abundance is also related with temperature range for these four species (Fig. 2).

Focusing on species richness, it is clear that relative humidity and insolation (variables very strongly correlated with PC1) have a major influence on species composition. These variables, together with monthly average temperature, had the greatest impact on species diversity, describing 59.5% of total environmental variation (PC1). Species that can tolerate lower temperatures (up to approximately 10° C) are *Dasysyrphus friuliensis* Goot, 1960, *C. grossa*, *E. tenax, E. arbustorum, Eristalis similis* (Fallen, 1817), *E. balteatus, E. corollae, Eupeodes luniger* (Meigen, 1822), *S. scripta, Syrphus vitripennis* (Meigen, 1822) and *Melanostoma scalare* (Fabricius, 1794). Species only occurring at temperatures above 20° C are *Cheilosia latifrons* (Zetterstedt, 1843) and *Eristalis pertinax* (Scopoli, 1763).

Our results also highlight the importance of surrounding crop heterogeneity for species diversity. Some species such as *S. pipiens*, *S. vitripennis*, *M. mellinum*, *M. scalare*, *Myathropa florea* (Linnaeus, 1758), *E. similis* and *E. luniger* were recorded only at Rimski Šančevi (Table 1, Fig. 2). There, unlike in Crvenka, surrounding crops were changed annually, amounting to eight different crops over the three-year study period (maize, sunflower, sugar beet, wheat, soybean, vegetables, field pea and alfalfa).

The relatively recent discovery that Diptera can be a very important component of temperate pollinator communities (Inouye, Larson, Ssymank, & Kevan, 2015), especially at high altitudes, indicates the potential for further investigation. Even though honeybees are considered one of the most efficient pollinators, their presence can sometimes depend on the proximity of hives since their populations are often managed. Thus, their richness and diversity can be low or they can be completely absent from some fields. In such cases, the Syrphidae, solitary bees and other pollinators can be very important. It is crucial that the importance of other pollinator groups is recognized so that they can be taken into consideration when certain agricultural regulations and policies are made. We assert that the significance of Diptera as pollinators should engender the same concern about their conservation that has been raised for pollinators in general (Kearns & Inouye, 1997; Kearns, Inouye, & Waser, 1998; Kearns, 2001) and for pollinators of crops in particular (Allen-Wardell et al, 1998).

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Biological Control of Diptera Calliphoridae: A Review

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ABSTRACT

The Calliphoridae family (order: Diptera), also known as blowflies, has a holometabolous cycle which spans across three larval instars. During development, they appear vermiform and exhibit necrophagous habits. It has been suggested that these organisms are responsible for the development of myiasis and transmission of pathogens to humans and animals. Biological control refers to the regulation of the number of pests by their respective natural enemies. It is an event that occurs naturally in the environment or due to mass creation and subsequent release of the controller in the laboratory. The objective of this review was to study the main biological controllers of the Calliphoridae family through a bibliographical survey of the last 10 years (2007-2017). The use of biological controllers avoids damages to the environment. One form of biological control of blowflies is to use plant extracts, which provides a great diversity of sustainable species to choose from. Additionally, it poses no harm to human health. Parasitoids, predators, bacteria and entomopathogenic fungi have also been tested. Though the results are promising, further studies are necessary before implementation in the society.

Key words: Entomopathogenic microorganisms, Parasitoids, Predators, Plant extracts.

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INTRODUCTION

Biological control refers to the management of agricultural pests, transmitter or disease-causing insects using natural enemies such as predators, parasitoids, and microorganisms. This method of control is more suitable than chemical pesticides since it leaves no residue in nature and is harmless to human health, thus promoting sustainability and protection of the environment (Parra, 2002).

Entomopathogenic microorganisms form the basis of bio-insecticides or biological insecticides. The advantages of its use are: high specificity, low risk to environment and human health, lower induction of insect resistance to the compound, and increased possibility of reproduction of the microorganism in the environment. The main disadvantages include greater susceptibility to environmental conditions and shorter shelf life, which may be minimized through good formulations (Angelo, Vilas-Bôas, & Castro-Gómez, 2010).

Commonly used microorganisms are *Bacillus thuringiensis* (Berliner, 1915) (Bt), *Wolbachia pipientis* (Hertig, 1936), and *Beauveria bassiana* (Balsamo-Crivelli & Vuillemin, 1912), and the fungus *Metarhizium anisopliae* (Metchnikoff & Sorokin, 1883). The entomopathogenicity of Bt is due to the crystal proteins (Cry) produced during their sporulation. These Cry proteins interact with and paralyze the microvilli of an insect's digestive tract, leading to death by starvation, general paralysis of the muscles or by septicemia (Angelo et al, 2010). *W. pipientis* acts by reducing the longevity of the insect, thus preventing it from acting as a carrier for the transmission of diseases such as dengue, zika, chikungunya and malaria (Oliveira & Moreira, 2012).

Spores of *B. bassiana* penetrate the insect cuticle thereby gaining access to its internal organs. Upon infection, the bacteria release toxins that interfere with the development of the insect leading to death (Feijó et al, 2008). The effect of *M. anisopliae* is similar to that of *B. bassiana*, where the spores colonize the insect through hyphal growth, followed by release of toxins that lead to death of the host (Mahdi, 2015).

The Calliphoridae family of the order Diptera, popularly known as blowflies, cause serious damage to livestock, as well as human health. These necrophagous insects are vectors of several diseases and cause primary and secondary myiasis in humans and animals. In a study conducted by Hadi (2013), 15 species of intestinal parasites, eggs, cysts and oocytes were isolated and identified from two species of Calliphoridae (*Chrysomya albiceps* (Wiedemann, 1819) and *Chrysomya megacephala* (Fabricius, 1794)). Eight of these were identified as nematode eggs and two as protozoan cysts.

According to Sandeman et al (2014), several studies in the last 50 years have been directed toward the control of *Lucilia cuprina* (Wiedemann, 1830), a major infestation affecting the sheep industry in Australia. Although, the use of chemical insecticides resolved the problem initially, the insect has begun to show resistance to several of these products making it necessary to come up with newer and better control methods.

The objective of this work was to carry out a bibliographical survey of the articles published in the last 10 years on the biological control of Diptera: Calliphoridae blowflies that cause primary and secondary myiases, and transmission of pathogens to humans and animals.

MATERIALS AND METHODS

A bibliographic survey was carried out on the biological control of the Calliphoridae family of the order Diptera, using the key words (Calliphoridae, fungi and entomopathogenic bacteria, biological control, predators, parasitoids and plant extracts) of articles published in Portuguese and English in the last 10 years (2007-2017) through the following platforms: *Portal Capes*, Science direct and Google Scholar. The most relevant literature were referred for the development of the review.

RESULTS

Thirty-one relevant scientific papers were selected for the development of the review, of which twenty-seven were published between the period 2007 and 2017, and four were prior to 2007 (Table 1).

Table 1. Number of scientific papers related to Biology of Calliphoridae, Biological control, Entomopathogenic microorganisms, Predators and Parasitoids, totaling thirty-one papers.

Papers	**Number of papers**
Biology of Calliphoridae	**8**
Biological control	**1**
Entomopathogenic microorganisms	**8**
Predators	**1**
Parasitoids	**7**
Plant extracts	**6**
Total	**31**

DISCUSSION

Calliphoridae

From the Calliphoridae family, 1020 species have been recognized worldwide, of which around 100 are Neotropical. The thorax and abdomen of these insects show metallic coloration, usually blue, green, or black. The antenna is tri-segmented, having feathery edges and well-developed calypters (Carvalho & Ribeiro, 2000). The female insects rely on decomposing matter for their protein needs, required for both nutrition and oviposition (Chaiwong et al, 2012).

Blowflies show a holometabolous life cycle, with three larval instars. Female blowflies can deposit up to 2000 eggs throughout its life. The time required for development is temperature dependent and accelerated with increase in temperature. The larvae of the Calliphoridae family show vermiform appearance and necrophagous habits (Donato & Liria, 2016).

The economic importance of blowflies is varied because of its ability to infest vertebrates (myiasis). The *L. cuprina* species is responsible for estimated losses of up to $170 million per year in the sheep industry in Australia (Sanderman et al, 2014). Apart from damage to livestock, some species also cause myiasis in humans, such as *Cochliomyia hominivorax* (Coquerel, 1858) and *Chrysomya bezziana* (Villeneuve, 1914) which cause primary myiasis, and *Cochliomyia macellaria* (Fabricius, 1775) which cause secondary myiasis (Zumpt, 1965). Another problem caused by these blowflies is the transmission of pathogens that cause diseases in animals and humans. The genera *Cochliomyia* sp. and *Chrysomya* sp. are known to act as vectors of infectious bacteria (Maldonado & Centeno, 2003; Paraluppi, Vasconcelos, Aquino, Castellón, & Silva, 1996). Due to their necrophagous habits, microorganisms of the genus *Mycobacterium* sp. (Lehmann & Neumann, 1896) can stay in the insect, making it a vector. The bacterium that causes paratuberculosis in pigs, birds and cattle (*Mycobacterium avium*) have been isolated from blowflies in different experiments (Fischer et al, 2001).

Due to these characteristics, several studies have been aimed at the biological control of blowflies. Most of these studies are related to the use of entomopathogenic microorganisms as a means of biological control.

Biological control

Biological control is a naturally occurring event in which the number of animals belonging to a particular species is limited through the action of their respective natural enemies. Humans observed this natural phenomenon and began to use it to their advantage, by studying the relation between pests and their natural enemies, followed by mass creation and release of these enemies in order to control the pests (Parra, 2002).

Biological control can occur in two ways. First, without human interference (natural event) and second, human-mediated, either by manipulating or introducing organisms capable of controlling the pest. Biological control can be categorized into four types: (i) artificial control, where man interferes positively for the increase of the natural enemies; (ii) classical control, which occurs through importation and colonization of natural enemies for the control of exotic pests; (iii) natural control, which refers to the population of naturally occurring enemies found in the location; and (iv) applied control, which deals with the dissemination of natural enemies created or mass-produced in the laboratory (Parra, 2002).

On the basis of the relationship with natural enemies, a parasite can be defined as an organism that needs a host to complete its life cycle, but most of the time do not lead to host mortality. On the other hand, parasitoids need a host to complete only part of their developmental evolution; parasitoids emerging as adults from the hosts cause host mortality. Predators lead a free life and feed on pests, and entomopathogens are microorganisms (fungi, viruses, bacteria, nematodes, and protozoa) that are used to control insect pests (Parra, 2002).

Biological control of Calliphoridae

Entomopathogenic bacteria

Several works have been carried out using entomopathogenic bacteria against Diptera: Calliphoridae flies. These include the use of bacteria such as *Brevibacillus laterosporus*, *Bacillus thuringiensis* and *Wolbachia pipientis*. Studies by Carramaschi, Pereira, Queiroz, & Zahner, 2015; Carramaschi et al, 2017 made use of strains of *Brevibacillus laterosporus* that showed control activity against *Chrysomya megacephala*, where a 70% mortality of the larvae could be achieved at a concentration of 1×10^8 spores/g diet. The activity of this bacterium resembles that of Bt, where after the ingestion of the spores by the insect, released toxins interact with intestinal receptors, causing death of the insect by starvation, septicemia and paralysis of the organ. This alteration in the intestine was confirmed by transmission electron microscopy. Pessanha, Carramaschi, Mallet, Queiroz, & Zahner (2015) also used strains of *Brevibacillus laterosporus* to control larvae of *Lucilia cuprina*, describing results ranging from 29% to 54% mortality.

Using different commercial concentrations of *Bacillus thuringiensis israelensis*, Mehdi & Noshee (2015) determined mortality of *C. albiceps* second instar larvae as 30% and 63.33% for 100 and 2000 parts per million (ppm), respectively. Larvae treated with 1000 ppm of the bacterial formulation had a 30% mortality rate after 2 days and 72.96% after 12 days while adult mortality of 6.67 and 73.33% occurred when flies were treated with 100 and 200 ppm respectively, thus indicating the potential of *B. thuringiensis* for the control of *C. albiceps*.

Wolbachia bacteria are known to cause reproductive abnormalities in their hosts, such as cytoplasmic incompatibility, feminization, parthenogenesis, and lethality in males. According to Mingchay et al. (2014), endosymbiotic bacteria were detected in natural populations of Calliphoridae, Sarcophagidae and Muscidae flies in different regions of Thailand. *Wolbachia* supergroups A and B were identified by PCR, which can be used for future research on fly control programs.

The application of entomopathogenic bacteria against Calliphoridae presents efficient results of mortality in laboratory, manly with *B. thuringiensis* and *B. laterosporus*. However, we observed only commercial formulations of Bt in the studies that we reviewed here, which suggests that additional studies are necessary on bacteria that are useful.

Entomopathogenic fungi

Like the use of bacteria, fungi present themselves as an alternative in the control of the Calliphoridae family. The main species used are *Metarhizium anisopliae* and *Beauveria bassiana*.

According to Mahdi (2015), *Metarhizium anisopliae* was grown in rice, potato and wheat broths; its secondary metabolites were filtered and then applied under the larvae and adults of *Chrysomya albiceps*. The results showed that the potato filtrate was the most efficient, with 46.67% mortality in second instar larvae, while the rice and wheat filtrates obtained accumulated mortality of 36.67% for larvae and 40% for pupae. Adults treated with potato filtrate had a mortality rate of 66.67%, reaching 90% after one week.

The use of *Beauveria bassiana* on eggs, larvae and adults of *C. albiceps* demonstrated that the fungus does not present a great inhibition in the rate of hatching of eggs, which the authors explained as non-specificity to the host in addition to the physical barrier posed by the chorion. Third-instar larvae infected with the fungus did not present significant differences in relation to the control during pre-pupal and pupal periods, due to the salivary action of these immature developmental stages. However, the rate of emergence and longevity of adults was reduced with increasing concentration of the microorganism. Thus, the fungus presents potential for control of this fly but further studies focused on the standardization of inoculum concentration must be carried out to develop products that could be commercially viable (Feijó et al, 2008).

The re-isolation of *Beauveria bassiana*, after passage under eggs, larvae and adults of *C. albiceps*, was carried out and the cytological aspects of the fungus were studied. The results show that it did not present significant differences related to its behavior and cytological aspects, indicating its high degree of adaptability, exhibiting its preference for insect-pest control (Feijó, Lima, Alves, & Lima, 2007).

The evaluation of the pathogenic action of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus* on egg, larva and adult stages of *C. putoria* was performed and presented the following results. The fungi *B. bassiana* and *M. anisopliae* showed 100% mortality of the larvae at the concentration of 10^8 conidia/mL. The isolate JAB 07 of *B. bassiana* when inoculated into pupae significantly reduced the emergence of adults. In relation to adults, there were only survival reductions at the concentration of 10^8 conidia/mL of *M. anisopliae* and *B. bassiana* (Yoshida, 2007).

Similar to bacteria, fungi exhibit a good control of the blowflies, but we did not observe any commercial formulation, so the use of this form of biological control is not available commercially. Therefore, further studies are necessary to determine the efficiency of fungi when exposed to the environment.

Predators

Botteon, Neves, & Godoy (2016) used third instar larvae of *Chrysomya putoria* as diet for the predator widely used in biological control, *Podisus nigrispinus* (Hemiptera: Pentatomidae), concluding that these larvae serve as a diet for predator breeding. This result indicates that biological control by means of this predator presents potential to be used on a large-scale.

The use of predators on Calliphoridae is scarce in literature. Our survey showed only one piece of evidence regarding the usage of *P. nigrispinus*, although it did not confirm that *P. nigrispinus* would predate the maggots. Thus, predators remain less explored with regard to different forms of biological control of Calliphoridae.

Parasitoids

The use of parasitoids is one of the most recognized forms of biological control. Several studies show the use of *Nasonia vitripennis* in relation to the control of blow flies.

Mello, Borja, & Aguiar-Coelho (2009) evaluated the exposure of different dosages of *Nasonia vitripennis* parasitoid females to the host *Chrysomya megacephala*. It was observed that parasitoids per host tended to greatly decrease with higher dosages of *N. vitripennis*, and thus, higher rates of parasitism were found in treatments 3:1 and 5:1, and an increase in the percentage of pupae was not observed with increase in the number of females per host, which is justified by superparasitism.

The exposure of *Nasonia vitripennis* to immature *Chrysomya megacephala* at different exposure times- 24, 48, 72 and 96 hours showed that in 72 hours, the development period of *C. megacephala* increased, and consequently the number of viable pupae decreased with the increased exposure time to the parasitoid (Mello, Sabagh, & Aguiar-Coelho, 2007; Mello et al, 2009; Mello, Borja, & Coelho, 2010). Similar results were described for *Cochliomyia macellaria* (Barbosa, Couri, & Coelho, 2008).

Mello (2012) evaluated the parasitoid *Nasonia vitripennis* on pupae of *Chrysomya megacephala* at different pupation depths. The following depths were used: from 0 cm to 5 cm with intervals of 0.5 cm and two exposure times, 48 hours and 72 hours. The results showed that the parasitism rate decreased as the depth increased and parasitism up to 2 cm and 3 cm were observed at 72 and 48 h, respectively. These results demonstrate that for effective biological control, the pupation habit of each insect is extremely important.

Research related to new parasitoid species of Calliphoridae has also been carried out in the last 10 years.

Chin et al (2009) reported that the parasitoids *Exoristobia philippinensis* (Hymenoptera: Encyrtidae) and larvae of *Ophyra spinigera* (Diptera: Muscidae) on pupae of *Chrysomya rufifacies* were collected from carcasses of monkeys in Malaysia. Marchiori & Miranda (2011) collected synanthropic Diptera from chicken feces and observed natural parasitoids of these insects. The parasitism rate was 28.4%. The following species were identified: *Muscidifurax raptorellus, Nasonia vitripennis, Pachycrepoideus vindemmiae, Spalangia cameroni, S. drosophilae, S. endius, S. nigra, S. nigroaenea, Spalangia* sp. (Hymenoptera: Pteromalidae) and *Tachinaephagus zealandicus* (Encyrtidae), with the *P. vindemmiae* species having the highest incidence. These data serve as a basis for future assessments of biological control of the Calliphoridae family by parasitoids.

The use of parasitoids is widely studied and have high efficiency against different insects. *Nasonia vitripennis* is the principal parasitoid which is used as a biological control of blowflies and presents good results. Other species have been focus of research, thus, indicating promise in this area.

Plant extracts

Another form of control of blowflies is the use of plant extracts, an area that presents a great variety of species to be used and great potential for a sustainable and harmless control of human health.

Banumathi et al (2017) used plant extracts for the control of second instar larvae of *Lucilia sericata*. The extraction was carried out using ethanol and the *Lobelia leschenaultiana* species at 60 mg/L (LC50 = 3.4 mg/L) obtained 100% control. In this way, the researchers selected this extract for the formulation of ZnO nanoparticles for use against the insect.

The evaluation of the crude extract of leaves of the plant *Pouteria sapota* (Jacq.) H.E. Moore & Stearn, Sapotaceae, altered the post-embryonic development of *C. putoria*. In the concentrations of 5, 10 and 25% of the extract, there was a decrease in the pupal period and the period between hatching and emergence of adults, as well as the decrease in weight when compared to the control. The 5% extract showed lower viability for the insect (47.5%) (Carriço et al, 2014).

The evaluation of the lyophilized latex activity of *Parahancornia amapa* (Huber) Ducke (Apocynaceae) under the post embryonic development of *Chrysomya megacephala* showed a change in insect development. At a concentration of 1% and 3% of latex, there was a change in the insect cycle, being the viability of 53% in 3% of latex, indicating that high concentrations of *P. amapa* latex present control potential for the species in question (Mendonça, Lima, Albuquerque, Carvalho, & Queiroz, 2011).

Abdel-Shafy, El-Khateeb, Soliman, & Abdel-Aziz (2009) evaluated the viability of control of 4 extracts of plants, extracted by different solvents, under third instar larvae of *Chrysomya albiceps*. Results showed that all extracts were effective in the control, presenting deformed pupae and further histological analyzes identified alterations in intestinal epithelial cells. Plant preparations of *A. herba-alba* and *A. monosperma* were considered more promising for insect control.

Khater & Khater (2009) observed the insecticidal effect of *Trigonella foenum-graecum*, *Apium graveolens, Raphanus sativus* and *Brassica campestris* against third-instar larvae of *Lucilia sericata*, and determined that these plants cause abnormalities in larvae, pupae and adults, as well as an imbalance in sexes, interfering in the medium term during the reproduction of these flies.

Nogueira, Mello, Kato, & Cabral (2009) determined the effect of tetrahydrofuran lignin grandisin against *C. megacephala* larvae. This component resulted in changes in the rates of development. The observed changes were 30% for eggs, 38% for larvae besides reducing their weight. These results confirmed the control exerted over populations of *C. megacephala* by tetrahydrofuran lignin grandisin.

In the last 10 years, there has been extensive research involving alternative forms to the chemical control of Calliphoridae family, recognized for causing serious damage to livestock and human health. We highlight the research involving entomopathogenic microorganisms, parasitoids and plant extracts that demonstrate great controlling potential. However, the studies are still under study and are restricted to the laboratory. Therefore, further studies are required to evaluate viable formulations of these agents of biological control when exposed to diverse environmental conditions.

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Determination of Some Structural Features of the Mud Nest Materials of *Sceliphron curvatum* (Smith, 1870), (Hymenoptera: Sphecidae) in Turkey

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ABSTRACT

The purpose of this paper is to identify the nest materials, some physical characteristics and the elemental composition of the nest of *Sceliphron curvatum* (Smith, 1870). The nest surfaces were observed with a stereomicroscope and scanning electron microscope (SEM). In the inner surface of the *S. curvatum* nest of the fibers in the mud-ball nest varied between 427 nm and 6300 nm, respectively and averaged 1465.730 nm. The thickness of the fibers in the outer and the inner surface section of the mud nest were between 14 nm and 1430 nm, respectively and averaged 629.373 nm. The nitrogen concentration was 3.94%, the percentages of the saliva, soil and the water absorption capacity were calculated to be 4.848%, 95.152%, and 18.51%, respectively. However, the amount of the elements carbon (C), hydrogen (H), sulphur (S) and nitrogen (N) in the nest was found to have a value of 3.38, 1.526, 0.020 and 0.38 weight % respectively. The colors of the individual mud pots are composed of building materials of different origin and with different shades of almost white over light brown to greyish black with long dark and light brown lines. We have found that the ratios and amount of physical characteristics, elemental composition and the fibers in the mud-ball of the nest of *S. curvatum* change with soil structure in which nest is built.

Key words: Sceliphron curvatum, nest, SEM.

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INTRODUCTION

The guild of mud-daubing spider-hunting sphecid wasps of the subfamily Sceliphrinae is represented with two genera in Europe, *Sceliphron* (Klug, 1801) currently comprising 10 native and 3 established exotic species (Bitsch, 2010). *Sceliphron* is a genus of Hymenoptera of the Sphecidae family of wasps, commonly referred to as mud daubers. The European fauna of the genus *Sceliphron* includes 4 native species, three of them being relatively widespread in southern and to a lesser extent in central and/or eastern Europe*: S. destillatorium* (Illiger, 1807; Pádr, 1989), *S. madraspatanum* (Fabricius, 1781), and *S. spirifex* (L., 1758); the fourth species, *S. funestrum* (Kohl, 1918), is distributed only in some peripheral Aegean Islands (and also further east, in Asiatic Turkey) (Vecht & Breugel, 1968; Hensen, 1987; Schmid-Egger, 2005). During the second half of the 20th century, two exotic species have become regular and widely represented members of the European fauna, the American *S. caementarium* (Drury, 1773) and Asian (Bogusch, Liška, Lukáš, & Dudich, 2005). Turkish fauna is characterized by large, but usually not clearly perceived biodiversity, which is in the easiest way expressed by the number of recorded taxa. These include the eventual impoverishment, but also the appearance of migrant species, together with potential invasive ones, which may in one way or another threaten native species *Sceliphron curvatum* (Smith, 1870), as documented in numerous recent reports and reviews (Pagliano, Scaramozzino, & Strumia, 2000a, b). This species looks very striking, as its body is 30 to 40 mm, (between 15 to 25 mm) long and colored black and yellow. It is known as a rare species nesting around small towns and villages and building nests of mud on rocks houses and but also very often indoor on piles of books, clothes or pieces of furniture (Gepp & Bregant, 1986; Bogusch, Straka, & Srba, 2004). Black and yellow mud daubers primarily prey on relatively small, colorful spiders, such as crab spiders (and related groups), orb weavers and some jumping spiders. Like all *Sceliphron* species, *S. curvatum* is not aggressive unless threatened. They usually find them in and around vegetation. *S. curvatum* appeared in Europe in the 1970s (Vecht, 1984), most likely having been carried over from its natural habitat in Central Asia to Turkey. This is the first Turkish record of *S. curvatum*, which was made by Gülmez & Can (2015).

The aim of this work is to present the current state of knowledge concerning the distribution of *S. curvatum* localities and determination of some properties of nests in Turkey. There are some new and very limited faunistic studies about *S. curvatum* Turkey. *S. curvatum* has been distributed only in the Black Sea region, not the other parts of Turkey so far (Gülmez & Can, 2015). However, new record of *S. curvatum* was made by us from the Ordu district. The mud-ball nesting behavior of the *S. curvatum* is little known. This is the first study about the nesting behavior of *S. curvatum* in Turkey and it will be the base for future studies.

MATERIALS AND METHODS

Nest collection

All the mud nest materials of *Sceliphron curvatum* (Smith, 1870) (Hymenoptera: Sphecidae) were collected during May to July 2015 from Perşembe district in Ordu, located in the Eastern Black Sea region of Turkey at an altitude of 27 m. In this area, which is usually warm and rainy, and with forest trees, a lot of flowers prevail. Larvae, pupae, spiders that larvae use as food and eggs were removed from the mud nests. Approximately 20 mud nest of *S. curvatum* were gathered. The mud nests were observed, and the mud nests were removed from curtain. The collected material is deposited in the Microbiology Research Laboratory, in Ordu University, Ordu, Turkey.

Observation of fine surface structure

Tittle parts from the mud nest were watched with a Leica Stereo Zoom S8 APO stereomicroscope (Leica Microsystems GmbH-Wetzlar, Germany) and scanning electron microscope (SEM) (Hitachi SU1510, Hitachi High-Technologies Co., Japan). Small piece separated from mud nest materials of *S. curvatum* for electron microscopy shots were secured with double-sided carbon tape and glued on. Fixed samples were coated with 10-30 mA one min gold-palladium (SEM coating system, sputter). Review and SEM shooting in was conducted at 5-15 kV voltage. 5 kV images were placed in this article.

Percentage of plant material and oral secretion

The dried clear mud on rocks nest piece was planned carefully. Mud nest piece was engrossed in 0.5 N KOH solutions, and held at 70°C for 3-4 hours. After oral self-secreted materials were dissolved and mud nest piece unconnected, mud nest piece ingredients were filtrated and separated from secretion. The mud nest material was washed with sterile water and dried in 250°C in a hot plate for 10 minutes. It was weighed with filter paper. Then percentage proportion of mud material and oral self-secreted materials were estimated as in the following formula: self-secreted (%) = $(k_1/k_2) \times 100$, (where k_1= dried weight of sample before process, k_2 = dried weight of sample after process) It was slightly modified (Yamane et al, 1998).

Absorbance

The larvae, pupas and eggs of the mud nest of *S. curvatum* were removed from the mud nest and small pieces were weighed. Each fragment was weighed after immersion in water for 30 s (Curtis, Aponte, & Stamp, 2005). The absorption capacity, given in percentage, was estimated using the following formula: Absorption capacity (%) = $[(m_2-m_1)/m_1] \times 100$ m_1= dried weight of sample before the process; m_2 = dried weight of sample after 30 s.

Elemental analysis

Elemental analysis of the mud nest of *S. curvatum* samples were determined for carbon, hydrogen, nitrogen, oxygen and sulphur atoms with an elemental analysis instrument of Elementar-Vario MICRO Cube (VARIO Co.).

RESULTS

Observation of surface under light microscope

Investigation of the surface of both inside and outside of mud nest under light microscope on the mud-ball nest of *S. curvatum.* There were grey clear and dark brown and dark yellow lines. While it was especially bright saliva silk inside, soil particles were of different color and size in outside surface because they are small and they are used in small quantities. The saliva shone like varnish in inside surface and the soil particle was short and thick (Fig. 1 A, B, C, Table 3).

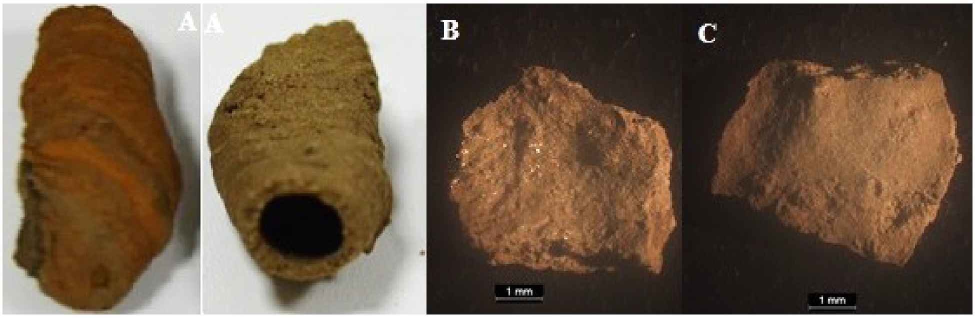

Fig. 1. a. *Sceliphron curvatum* mud nest removed from window curtain. Surface of mud nest in stereomicroscope. b. Inward facing of *S. curvatum* mud nest (1×1.25); c. Outward facing of *S. curvatum* mud nest (1×1.25).

Observation of surface under SEM

We first define that *S. curvatum* uses three kinds of nest material in mud ball nest. A SEM observation revealed that low amount of organic matter, tiny vegetable chips, plant hairs and a large amount of soil (ca 1465.730 nm, 14 nm) were used as a major mud- ball nest material in all parts of the surface of both inside and outside of mud nest (Fig. 3 A, B). In addition to the above plant fibers, *S. curvatum* also used moist wet soil particles and clay. On the inside surface of this mud material, there were several plant hairs (6.30-1.19 µm in width) that were regularly oriented parallel to each other. On the outside surface of mud nest, there were only a few very small plant fiber 449 nm in width (Fig. 2 A, B, C, D). From this appearance, such materials, especially the presence of larger plant fibers in the interior keeps the mud-ball nest strong. *S. curvatum,* three parts including fiber, inorganic material soil and the oral secretions were observed both on the surface. The plant fibers were hairs and tiny fiber scrapings. There were inorganic and organic materials, especially soil, oral secretions between the fibers. It was observed that the particles of mud nest material were not water-soluble and not separated easily from each other.

EDX Analysis

S. curvatum: Oxygen (O), aluminum (Al), silicium (Si), potassium (K), iron (Fe), magnesium (Mg), calcium (Ca), sodium (Na), nitrogen (N) and carbon (C) were determined in the sludge particle of the mud-ball nest wall with EDX analysis. O, Si, Al, and C were the major elements. Fe and N were higher than the other inorganic elements. Normally, it does not exist in nature. However, in almost all rocks, sand, clay and soil are found in silicates (SiO_2) or as SiO_2 with other elements such as O and Al, Mg, Ca, Fe, Na, K. The mud of the nest was soil. The other inorganic elements were mixed in the mud. K, Ca, and Na were in very low concentrations. Fe was found as a magnetic mineral (Fig. 3). *Sceliphron curvatum*: O, C, Si and Al were the major elements. Ca, Fe, K and N were in trace amounts. The concentrations of elements are shown in Table 4. EDX spectra are shown in Fig. 4.

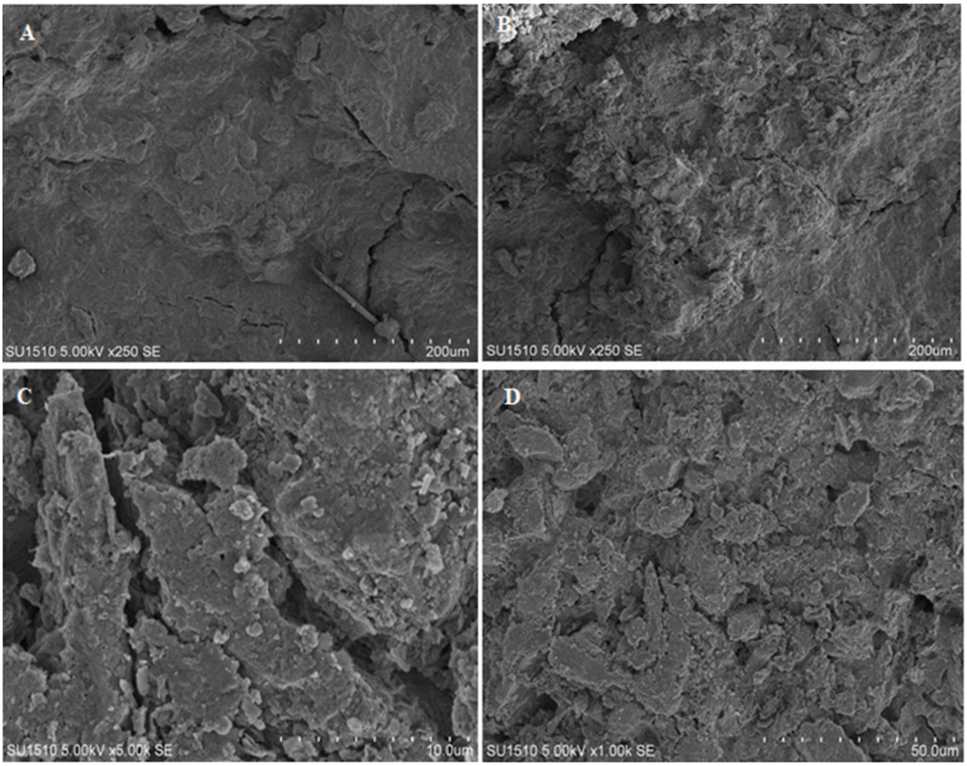

Fig. 2. Surface of the mud nest in SEM. a. The inner surface section; b. The outer surface of the section of the mud nest; c. Longitudinal section of mud nest; d. Cross section of the mud nest.

Percentages of plant material and oral secretion

Oral secretion was mainly used for connecting tiny plant fibers, mud and other nest materials (Fig. 1). It was found by a SEM observation and a stereomicroscope (Leica S8 APO) that very small amounts of oral secretion were used for mud ball nest construction, compared with that used in on the wasps nest. While the coating of oral secretion was seen more on the inside surface of the mud in nest, the coating of oral secretion was seen less on the outside surface of the mud in nest. The percentages of the soil and saliva in the *S. curvatum* mud-ball nest were calculated as 4.848% and 95.152%, respectively (Fig. 2). A, B, C and D are a SEM micrograph taken at a high magnification on the both surface of the mud-ball nest.

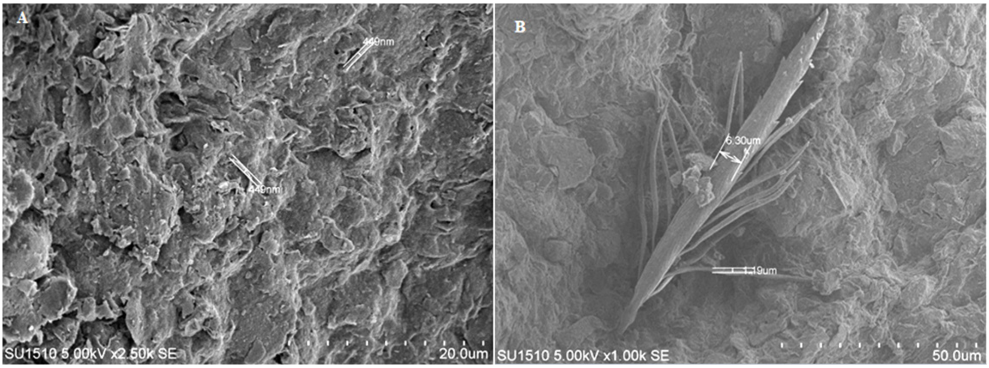

Fig. 3. Surface of the *S. curvatum* mud nest in SEM. a. Longitudinal section view of the fiber samples in the nest from outer surface of mud nest; b. Longitudinal section view of the fiber samples in the mud nest from inner surface of the mud nest.

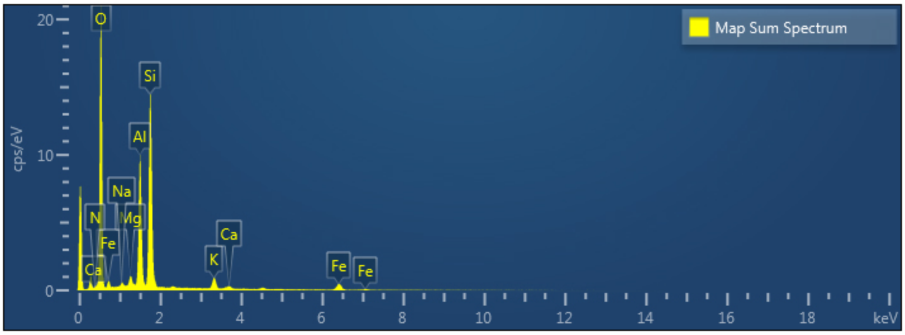

Fig. 4. EDX spectra of elements embedded in the wall mud nest: *S. curvatum*.

Absorption capacity and pH

While the water absorption capacity of the mud nest particles of *S. curvatum* was calculated as 18.51%, pH in water and in 0.5 N KOH were calculated 8.4 and 12.4 respectively. Some measured values of the *S. curvatum* of the mud-ball nest are shown in Table 2.

DISCUSSION

There are individual pots, which are composed of building materials of different origin and with different shades of almost white over light brown to greyish black. *S. curvatum* preferred to use fine clay, rarely interspersed with recognizable sand and pebble shares for mud-ball nest. *S. curvatum* uses three kinds of nest material, i.e., small vegetable chips, much of soil (sand, clay) and petty quantity of plant hairs and/or inorganic particles (Egger, 1974; Gepp & Bregant, 1986). According to Wenzel (1991), wasps of genus *Polybia* utilize a great variety of nest materials. In addition to the above nest materials, some *Polybia* species use long woody fibers like *Polistes* (Wenzel, 1991), but in the case of *P. paulista*, woody fibers were never found in the nests. However the plant fibers of *D. sylvestris* and *D. media* nests were long and thin (Bagriacik, 2013 a; b). Matsuura (1991) has reported that the structure of the envelope of a *Vespa* nest might be ball, bowl- or flask-shaped. The *Vespa* genus collects rotten wood, the dead parts of live trees and inorganic materials as nest materials (Spradbery, 1973; Matsuura, 1991). Matsuura (1991) has reported that the structure of the envelope of a *Vespa* nest might be ball, bowl- or flask-shaped. The *Vespa* collects rotten wood, the dead parts of live trees and inorganic materials as nest materials (Spradbery, 1973; Matsuura, 1991). The nests which were found in this study had no envelopes around the combs. There is only mud-ball cell. Short small plant fiber scrapings were found in the nests. However, contamination with inorganic particles has never been detected during SEM observations of nest material, which consists only of long woody fibers, in two Japanese *Polistes* genus wasps, *P. chinensis* (Kudô, Yamane, & Yamamoto, 1998) and *P. riparius* (Yamane et al, 1998). The fibers of the inside of mud nest were thicker than that of outside nest. We cannot only conclude that *P. paulista* regularly collects mud and/or inorganic particles in the field, because a possibility that such a material contaminated the plant materials is not excluded. The plant fibers are needed to keep sand and gravel granules together in mud -ball nest. However, contamination with inorganic particles has never been detected during SEM observations of nest material, which consists only of long woody fibres, in two Japanese *Polistes* wasps, *P. chinensis* (Kudô et al, 1998). Two subgenera of *Polybia*, *Pedothoeca* and *Furnariana*, rely solely on mud for their nest building (Wenzel, 1991). *P. paulista* belongs to another subgenus, *Myrapetra*, but there is still a possibility that this species partially depends on some inorganic materials for its nest building. Similarly *Sceliphron curvatum* and Sphecidae use soil when they make their nest. The oral secretions were seen as a thin membrane and such as the spider web in the inner region by under light microscope. The oral secretion in the mud nest of *S. curvatum* in the both regions was richer than that on the inside. The sand and clay in the mud-ball nest were spare and glued irregularly. We found from a SEM observation that *S. curvatum* uses only a small amount of oral secretion for the construction and maintenance of their nests. However, some researchers said that until this time there was no saliva secretion. Accordingly there are only certain moisture levels of the substrate to form the balls, also their transport and suitable for pottery (Gepp & Bregant, 1986). The nitrogen content of a small amount of mud nest was investigated in as much as nitrogen can serve as an index of the amount of oral secretion in all wild bee nests. The nitrogen content of the nest of *Polybia paulista* was 1.59-2.14%. *P. paulista* uses a small amount of oral secretion for nest construction. The nitrogen content of the *Vespa analis* nest was 1.1-2.0%, *V. simillima* 0.9-2.0%, and *V. crabro* 2.5% (Kudô et al, 2001). In general, the nitrogen content in most *Polistes* nests varies considerably (Espelie & Himmelsbach, 1990; Singer, Halldorson, Lear,

& Andrusiak), however the variation is not much elevated in the nests of *P. paulista* and *Vespa* species. Major dissimilarity in nitrogen content between distinct parts of the mud-ball nest in most polistine wasps can be seen because of differences in the frequency of licking different areas with oral secretion. In other words, these wasps would smear different nest parts with different amounts of the secretion. The nitrogen concentration in the *V. orientalis* nest was 18.75%, *V. crabro* nest 27.93% (Bagriacik, 2011). We found that the nitrogen concentration in the *S. curvatum* mud nest was 4.848%. We found this nitrogen concentration is less than that of the other species. The amount of oral secretion was equal to the amount of plant fibers, but the amount of soil was higher than the amount of oral secretion and plant fibers in the *S. curvatum* mud nest. The amount of saliva was less than the amount of fiber and soil in the mud nest, the shape of the mud nest depends on the sticky materials in the soil. There were sand, red soil and garden soil as nest materials in the *S. curvatum* mud nest from Turkey. The soil particles were composed mainly of O, Fe, Ti, Si, C, and Al with traces of Ca, K, Na and Mg according to the EDX spectra (Ishay, Riabinin, Kozhevnikov, van der Want, & Stokroos, 2003; Ishay et al, 2008). In this study, the concentration of O, Si, C and Fe in the mud nests of *S. curvatum* species was higher than the other elements (Table 4). The soil in the nest of *S. curvatum* was sand and fine gravel. The sand was collected from the garden by the female *S. curvatum.* There was a small amount of C, H, N, O and S atoms elemental in the mud-ball nest piece of *S. curvatum* (Table 1). Mud-ball nest strength is highly dependent on its moisture content. There is a relationship between the water absorbency and low moisture content of the mud nest. The processing of sludge dough affects the sticking ability of the fibers and sand, the absorbency of nest mud and its durability (Basil-Edwardes, 1921; Vecht, 1961; 1984; Biermann, 1993). The saliva protects the nest from rain and other weather conditions (Kudô et al, 2001). In particular anthropogenic substances are preferred as underground for the planting of clay pots: porozell, bakelite, paper (books, wallpaper), rare bricks and artificial marble. Somehow, *S. curvatum* has managed in selection processes to ensure the required rain protection through the selection of the storage substrates. The reason for the preference of anthropogenic substrates becomes evident by a test: If the pots are sufficiently wetted with water or immersed completely in water, they break down into pulpy constituents within a few seconds. The soil nests of *S. curvatum* exposed to rain and droplet, were under threat from open skies (Vecht, 1984). In this study, the mud-ball nest wall of *S. curvatum* was very fragile. When it was touched, the mud nest was broken. This is likely because the moisture content of the mud-ball nest we analyzed was very low, 18.51% and flexible plant fibers were not preferred by female *S. curvatum*. The saliva was not effective on the water permeability of mud-ball nest because the mud nest has not included dry dense fibers and soil which is hydrophilic.

Table 1. Element analysis of the mud nest of *S. curvatum* samples.

	Area				**Weight %**			
Elements	N	C	H	S	N	C	H	S
Nest sample	186.5	787	2158.5	55	0.38	3.38	1.526	0.020

Table 2. The thickness of the fibers of the mud nest (nanometer).

	N**10**	***S. curvatum***	
		Outer surface of nest	**Inner surface of nest**
Thickness (nm)	Min. Max. Average	14 nm 1430 nm 629.373 nm	427 nm 6300 nm 1465.730 nm

Table 3. Values of parameters of nests of *S. curvatum* mud nest within the area at the final stage of their development.

Some parameters for mud nest	Dimensions of small mud nest cells	Dimensions of medium mud nest cells	Dimensions of large mud nest cells
N	10	10	10
Nest weight ±SE (mg)	866.6±0.060	935.3±0.059	1100.20±0.032
Nest internal volume±SE(µl)	850.35±0.076	890.44±0.063	1020.12±0.042
Nest diameter±SE (cm)	0.634±0.054	0.765±0.032	0.825±0.065
Nest height±SE (cm)	1.910±0.041	2.104±0.092	2.458±0.012

Table 4. Elements and their concentration % in a fragment according to EDX analysis.

Element	O	Al	Si	K	Fe	Mg	Ca	Na	N	C	Total
Weight %	53.08	9.80	16.04	1.77	3.99	0.75	0.47	0.37	3.94	9.79	100.00

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New Records for the Caddisfly (Insecta: Trichoptera) Fauna of Montenegro

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ABSTRACT

Adult caddisflies were collected sporadically in the Ulqin Municipality and Çakorr Mountains in Montenegro during 2015 and 2016. Six species found during this investigation are first records for the caddisfly fauna of Montenegro: *Hydroptila sparsa* Curtis, 1834, *Hydropsyche modesta* Navàs, 1925, *Hydropsyche siltalai* Doehler, 1963, *Neureclipsis bimaculata* (Linnaeus, 1758), *Setodes punctatus* (Fabricius, 1793) and *Ernodes skipetarum* Malicky, 1986.

This study shows that this area is still poorly investigated and thus, more records are expected.

Key words: Aquatic insects, Balkans, rare species, biodiversity.

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INTRODUCTION

Montenegro is an important biodiversity hot-spot in Europe, characterized by a rich and diverse alpine, forest, freshwater and marine ecosystems. A large number of rare and important plant and animal species is influenced by its geographic position in the Balkan Peninsula, heterogeneity of habitats, favorable and unique geologic and climate conditions. However, as it is the case with other Balkan countries, there are several groups of organisms which are still under-investigated. This is true for aquatic insects in general and caddisflies as well. First information on caddisflies of Montenegro date back to thirties and fifties of last century, carried out by the famous entomologist Radovanović (1935, 1953). During the seventies and eighties of the last century several other investigations on Balkan caddisflies contain fragmentary data about Montenegro as well, including several new species described from the area (Malicky, 1982, Marinković-Gospodnetić, 1975, 1980, 1981). There is only one detailed investigation related to the caddisflies of Montenegro, more precisely from Durmitor Mouontains, where 95 species were found (Krusnik, 1987). Several other scarce data about caddisflies of Montenegro were published during the last years with several new species described, such as *Agapetus kampos* Oláh, 2013 and *Drusus gombos* Oláh, 2013 (Oláh, 2010; Oláh & Kovács, 2013).

The goal of this paper is to contribute to the list of caddisflies of Montenegro.

MATERIAL AND METHODS

Adult caddisfly specimens were collected by entomological net, handpicking and ultraviolet light trap. The ultraviolet light trap follows Malicky (2004). The sampling was carried out during 2015 and 2016 in three sampling stations in Montenegro (Figure 1). Collected samples were preserved in 80 % ethanol. The specimens were identified by the first author using the determination keys from Malicky (2004) and Kumanski (1985, 1988). The collection is deposited at the Laboratory of Zoology of the Faculty of Mathematics and Natural Sciences, University of Prishtina, Republic of Kosovo.

The first sampling station (S1) (42.033105° N, 19.247907° E, 431 m asl) is located around the spring area of a tributary of the Buna River at Kaliman village on the northern side of the Ulqin town. Most of the water coming out from this spring goes through the pipes for Water Supply Company and only a small amount flows downwards. The second sampling station (S2) (41.979916° N, 19.376023° E, 29 m asl) is located at the Buna River in Shtodër village along the border with Albania. The third sampling station (S3) (42.687398° N, 20.066640° E, 1170 m asl) is located at a nameless stream in the Çakorr Mountains, few meters far away from the former border pass with the Republic of Kosovo.

Note: Female specimens of the genera *Hydropsyche* Pictet, 1834 and *Tinodes* Leach, 1815 are identified only up to the generic level due to the difficulties in identifying these genera up to the species level.

Fig. 1. The map of the three sampling stations in Montenegro: 1. S1 - Spring area of a stream in Kaliman village, 2. S2 - Buna River in Shtodër village, 3. S3 - Stream in Çakorr Mountains.

RESULTS AND DISCUSSION

During this investigation thirteen taxa in total were found belonging to the following families: Rhyacophilidae, Hydroptilidae, Hydropsychidae, Polycentropodidae, Psychomyiidae, Ecnomidae, Leptoceridae and Beraeidae. Six species are first records for the caddisfly fauna of Montenegro: *Hydroptila sparsa*, *Hydropsyche modesta*, *Hydropsyche siltalai*, *Neureclipsis bimaculata, Setodes punctatus* and *Ernodes skipetarum.*

Hydroptila sparsa is amongst the few hydroptilid species found in Montenegro. During this investigation it was collected only once with ultraviolet light trap at Station S2, which is located at the Buna River. It is the fourth species of the genus *Hydroptila* Dalman, 1819 and the fifth species of the family Hydroptilidae found in Montenegro. As in most of other Balkan countries, lower reaches of rivers in Montenegro are still not adequately investigated and further samplings in these segments of the rivers will reveal more species of this family of caddisflies.

Both species of the family Hydropsychidae found during this investigation are recorded for the first time from Montenegro. *Hydropsyche modesta* was previously reported from all countries in the Balkan Peninsula except Montenegro (Malicky, 2018). It has been sampled with ultraviolet light trap and entomological net, at station S2 in the Buna River and at station S3 in the Çakorr Mountains. The most interesting finding during this investigation is the species *Hydrospyche siltalai*, which was found at station S1 and is apparently the first finding for ecoregion 5 (Graf, Murphy, Dahl, Zamora-Muñoz, & López-Rodríguez, 2008). This species is distributed mainly in

Western Europe up to Slovenia and in Central Europe, mostly in Hungary and Romania. The occurrence of *H. siltalai* in Montenegro close to the border with Albania shows that the area of this species is larger than previously thought.

Setodes punctatus and *Neureclipsis bimaculata* are not found very frequently in the Balkan Peninsula. *Mystacides azureus* and *Ecnomus tenellus* are widespread species in Europe and Balkans, present almost in all countries surrounding Montenegro (Malicky, 2018).

Ernodes skipetarum was found in the Çakorr Mountains at a stream close to the former border pass with the Republic of Kosovo. Previously this species has been known from few localities in Kosovo and Albania (Chvojka, 1997; Ibrahimi et al, 2016a), all of them located in the Bjeshkët e Nemuna Mountains.

Recent studies in the Balkan Peninsula (e.g. Ibrahimi, Kučinić, Gashi & Grapci Kotori, 2014a, Ibrahimi et al, 2014b, 2015a, 2015b, 2016b; Kučinić et al, 2013; Oláh, Ibrahimi, & Kovács, 2013; Previšič et al, 2014; Vitecek et al, 2015), which is known as an important hotspot of caddisfly biodiversity in Europe, show that there are still poorly investigated areas. This investigation with several first findings is a result of few days collecting effort in Montenegro and shows that this country is also under-investigated. The current documented number of known caddisfly species in Montenegro is only 137 species.

Systematic list of caddisflies collected at three stations (S1, S2, S3) in Montenegro during 2015 and 2016. Species new to the fauna of Montenegro are indicated by an asterisk.*

EN - Entomological net; UV - Ultraviolet light trap

Family Rhyacophilidae

Rhyacophila fasciata Hagen, 1859

S2 Buna River in Shtodër village: 25.10.2015. 2♂♂ (EN). S3 Stream in the Çakorr Mountains: 13.10.2016. 5♂♂, 3♀♀ (EN). S3 Stream in the Çakorr Mountains: 22.11.2016. 2♂♂, 2♀♀ (EN).

Rhyacophila balcanica Radovanovic, 1953

S3 Stream in the Çakorr Mountains: 13.10.2016. 1♂ (EN).

Family Hydroptilidae

Hydroptila sparsa Curtis, 1834 *

S2 Buna River in Shtodër village: 25.08.2015. 5♂♂, 3♀♀ (UV).

Family Hydropsychidae

Hydropsyche spp. females.

S1 Tributary of Buna River in Kaliman village: 24.08.2015. 2♀♀ (UV). S2 Buna River in Shtodër village: 24.08.2015. 2♀♀ (UV). S2 Buna River in Shtodër village: 24.08.2015. 2♀♀ (EN). S3 Stram in the Çakorr Mountains: 13.10.2016. 3♀♀ (EN).

Hydropsyche modesta Navàs, 1925 *

S2 Buna River in Shtodër village: 24.08.2015. 2♂♂ (UV). S3 Stream in the Çakorr Mountains: 13.10.2016. 1♂ (EN).

Hydropsyche siltalai Doehler, 1963 *

S1 Tributary of Buna River in Kaliman village: 24.08.2015. 4♂♂ (UV). S2 Buna River in Shtodër village: 20.07.2015. 1♂ (EN).

Family Polycentropodidae

Neureclipsis bimaculata (Linnaeus, 1758) *

S2 Buna River in Shtodër village: 25.08.2015. 2♂♂ (EN). S2 Buna River in Shtodër village: 13.07.2016. 4♂♂ (EN). S2 Buna River in Shtodër village: 14.07.2016. 2♂♂ (UV).

Family Psychomyiidae

Psychomyiia pusilla (Fabricius, 1781)

S3 Stream in the Çakorr Mountains: 22.11.2016. 2♂♂.

Tinodes spp. females.

S1 Tributary of Buna River in Kaliman village: 24.08.2015. 1♀ (UV).

Family Ecnomidae

Ecnomus tenellus (Rambur, 1842)

S2 Buna River in Shtodër village: 25.08.2015. 2♂♂ (UV). S2 Buna River in Shtodër village: 14.07.2016. 3♂♂ (UV).

Family Leptoceridae

Mystacides azureus (Linnaeus, 1761)

S2 Buna River in Shtodër village: 25.08.2015. 3♂♂, 1♀ (UV).

Setodes punctatus (Fabricius, 1793) *

S2 Buna River in Shtodër village: 25.08.2015. 2♂♂ (EN).

Family Beraeidae

Ernodes skipetarum Malicky, 1986*

S3 Stream in the Çakorr Mountains: 23.06.2016. 2♂♂ (EN).

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On the Ceutorhynchinae (Coleoptera: Curculionidae) Fauna of Turkish Thrace, with Additional Records for Turkey

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ABSTRACT

This study is based on specimens of the subfamily Ceutorhynchinae Gistel, 1848 collected in Turkish Thrace by the first author in 2016 and 2017. *Amalorrhynchus melanarius* (Stephens, 1831) and *Parethelcus pollinarius* (Forster, 1771) are new records as genera and species for the Ceutorhynchinae fauna of Turkey. *Ceutorhynchus duvali* C. Brisout de Barneville, 1883 and *Nedyus quadrimaculatus* (Linnaeus, 1758), both already indicated from Anatolia, are recorded for the first time from Turkish Thrace. A list of all ceutorhynchines occurring in European Turkey is included. The synonymy: *Microplontus rugulosus* (Herbst, 1795) [= *M. melanostigma* (Marsham, 1802); syn. rev.]. is re-established.

Key words: Curculionidae, Ceutorhynchinae, Turkey, new records.

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INTRODUCTION

Turkish Thrace is the region of Turkey situated in Europe. It includes the entire provincial borders of Edirne, Kırklareli and Tekirdağ, and partly those of İstanbul and Çanakkale. The fauna of the region is relatively poorly known due to the presence of only few mountains with their highest points between 1000 and 700 m in contrast to the high mountains in Anatolia. These include the Istranca Mountains in the north of the region with Mahya peak at 1031 m of elevation and the Ganos and Koru Mountains in the south, with Uçakbaşı peak at 920 m of elevation and with Kızılpınar peak at 725 m of elevation, respectively (Dönmez, 1990). Therefore, the region has not been paid special attention by entomologists who visited mostly Anatolian Turkey in the past.

Evaluating the suprageneric rank, given the continuous debate and the different conclusions about the higher systematic of Curculionoidea, is beyond the scope of this paper. However, in this paper, we have not followed the hyper-lumping so-called phylogenetic arrangement of Curculionoidea proposed by Oberprieler, Anderson, & Marvaldi (2013) and accepted by Alonso-Zarazaga et al (2017). We have considered here Ceutorhynchinae Gistel, 1848 in its traditional subfamilial rank, in the deep belief that phylogeny-focused taxonomy is weakly supported by supposed relationships, which change according to the ongoing accumulation and evaluation of data, and are based on past historical events that are difficult to trace.

Ceutorhynchinae is currently represented in Turkey by 262 species belonging to five tribes (Gültekin, 2014; Aydın & Hacet, 2016a; Alonso-Zarazaga et al, 2017; Korotyaev, Gültekin, & Colonnelli, 2017). Of these, 35 species are known only from Turkey (Gültekin, 2014; Alonso-Zarazaga et al, 2017; Korotyaev et al, 2017). The majority, namely 247 species in 37 genera, belong to the tribe Ceutorhynchini Gistel, 1848; while Phytobiini Gistel, 1848 contains 11 species in five genera, Mononychini LeConte, 1876 contains two species of *Mononychus* Germar, 1823, and Hypurini A. Schultze, 1902 and Scleropterini A. Schultze, 1902 include a single species of *Anthypurinus* Colonnelli, 1979 and *Tapinotus* Schoenherr, 1826, respectively (Alonso-Zarazaga et al, 2017).

Alongside new data from Turkish Thrace, it is reported for the first time the occurrence in Turkey of two species, one of *Amalorrhynchus* Reitter, 1913 and the other of *Parethelcus* Wagner, 1943, both genera previously unknown from Turkey.

MATERIAL AND METHODS

Adults were collected from plants using a sweeping net and an aspirator during fieldwork in Turkish Thrace in 2016 and 2017. Collected specimens were killed with ethyl acetate and then stuck dry at the tip of triangular labels. Labelled specimens have been preserved in the Zoological Museum of the Biology Department of Trakya University, Edirne, Turkey.

Locality data, including place, date, coordinates and elevation for each species are given below, and localities are shown in Fig. 1. In the result section, the distribution of species recorded for the first time in the Turkish Thrace region and in Turkey are given in alphabetical order according to country.

Collecting localities

1. Çanakkale province: between Behramlı and Alçıtepe, 137 m, 40°06'19''N, 26°14'05''E, 12.05.2016. 2. Edirne province: Süloğlu-Demirhanlı, 123 m, 41°41'45''N, 26°43'34''E, 15.05.2016. 3. Edirne province: Uzunköprü-Ömerbey, 50 m, 41°15'40''N, 26°50'13''E, 16.05.2016. 4. Edirne province: Meriç-Kadıdondurma, 46 m, 41°10'39''N, 26°21'38''E, 18.05.2016. 5. Edirne province: between Lalapaşa and Hacıdanişment, 224 m, 41°51'01''N, 26°45'09''E, 7.06.2016. 6. Edirne province: Trakya University, Balkan Campus, 43 m, 41°38'44''N, 26°37'21''E, 16.05.2017. 7. Edirne province: Doğanköy, 333 m, 41°56'39''N, 26°42'02''E, 7.06.2017. 8. Edirne province: Lalapaşa (Demirköy-Doğanköy road), exit of Demirköy, 297 m, 41°55'06''N, 26°40'41''E, 7.06.2016. 9. Edirne province: Hıdırağa, 80 m, 41°44'22''N, 26°40'02''E, 7.06.2017. 10. İstanbul province: Çatalca, between Danamandıra and İhsaniye, 224 m, 41°18'26''N, 28°19'26''E, 11.06.2016. 11. İstanbul province: Arnavutköy-Durusu (Durusu lake), 7 m, 41°19'06''N, 28°40'33''E, 12.06.2016. 12. İstanbul province: Çatalca-Hisarbeyli (Durusu lake), 6 m, 41°22'37''N, 28°27'37''E, 12.06.2016. 13. İstanbul province: Silivri-Küçüksinekli, 237 m, 41°13'56''N, 28°09'55''E, 13.06.2016. 14. Kırklareli province: Çukurpınar, 509 m, 41°49'00''N, 27°28'01''E, 19.05.2016. 15. Kırklareli province: Center-Beypınar, 567 m, 41°47'47''N, 27°30'34''E, 6.06.2016. 16. Kırklareli province: Babaeski, 78 m, 41°25'27''N, 27°07'23''E, 9.06.2016. 17. Tekirdağ province: Malkara-Doğanköy, 286 m, 41°04'27''N, 26°49'05''E, 14.07.2016. 18. Tekirdağ province: Muratlı-Hanoğlu, 93 m, 41°11'56''N, 27°21'36''E, 17.07.2016.

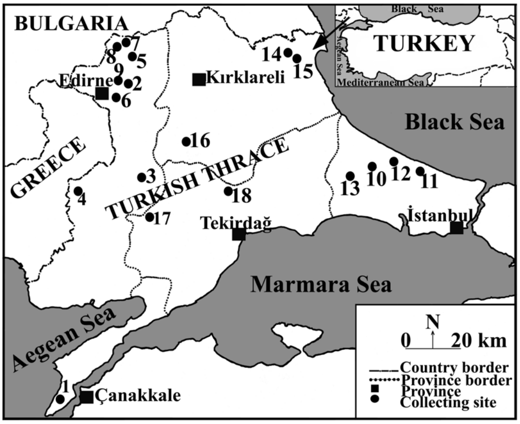

Fig. 1. Sampling localities of the study material in Turkish Thrace. The numbers correspond to the localities (see above).

RESULTS

MONONYCHINI LeConte, 1876

Mononychus punctumalbum (Herbst, 1784)

Material examined: Kırklareli province: Çukurpınar, 509 m, 41°49'00''N 27°28'01''E, 19.05.2016, 1 ♀.

CEUTORHYNCHINI Gistel, 1848

Amalorrhynchus melanarius (Stephens, 1831)

Distribution: Armenia, Austria, Belarus, Belgium, Bulgaria, northwestern territory of China, the Czech Republic, Denmark, France, Germany, Great Britain, Hungary, Italy, Kazakhstan, Lithuania, Luxembourg, Moldavia, the Netherlands, Poland, Romania, Russia (European Russia and Far East), eastern and western Siberia, Slovakia, Spain, Sweden, Switzerland, Ukraine. Nearctic Region (Alonso-Zarazaga et al, 2017; Tamutis, Tamutė, & Ferenca, 2011). New record for Turkey.

Material examined: İstanbul province: Arnavutköy-Durusu (Durusu lake), 7 m, 41°19’06’’N, 28°40’33’’E, 12.06.2016, 3 ♂♂, 2 ♀♀.

Short description: Length of body 1.8- 2.5 mm, integument black. Pronotum with small, shortly oval greyish scales. Similar scales present on elytra, and condensed in the form of a sutural stripe. Antennal funiculus six-segmented. Tarsal claws and femora edentate (Morris, 2008).

Note: *Amalorrhynchus* is currently composed of only two Palaearctic species. *Amalorrhynchus lukjanovitshi* Korotyaev, 1980 has a relatively narrow range in western Siberia and Far East of Russia, whereas the other species, *A. melanarius*, has a wide Holarctic distribution (Alonso-Zarazaga et al, 2017). To date, this species has never been recorded in Turkey. The above-mentioned Turkish specimens of *A. melanarius* were collected at the shore of a lake. The host plants of *A. melanarius* are the Brassicaceae *Nasturtium officinale* R. Br. and *Rorippa amphibia* (L.) Bess. (Colonnelli, 2004). Of these, *N. officinale* is known from Edirne, İstanbul, Kırklareli and Tekirdağ, while *R. amphibia* is known from Edirne and Tekirdağ in Turkish Thrace (Anonymous, 2014a, 2014b; TÜBİVES, 2018).

Ceutorhynchus duvali C. Brisout de Barneville, 1883

Distribution: Albania, Bosnia Herzegovina, France, Greece, Iran, Italy, Spain, Asiatic Turkey (Alonso-Zarazaga et al, 2017). New record for Turkish Thrace.

Material examined: Edirne province: Lalapaşa (Demirköy-Doğanköy road), exit of Demirköy, 297 m, 41°55’06’’N, 26°40’41’’E, 07.06.2016, 1 ♀.

Note: In addition to being a new record for Turkish Thrace, the finding of this species in the region is the easternmost record in Europe, although the range of *C. duvali* extends into Anatolian Turkey and Iran. According to Colonnelli (2004), host plants of this species are the Brassicaceae *Bunias erucago* L. and *Cakile maritima* Scop., both plants observed in Edirne (Anonymous, 2014a).

Ceutorhynchus obstrictus (Marsham, 1802)

Material examined: İstanbul province: Çatalca between Danamandıra and İhsaniye, 224 m, 41°18’26’’N, 28°19’26’’E, 11.06.2016, 3 ♂♂, 2 ♀♀; Arnavutköy-Durusu (Durusu lake), 7 m, 41°19’06’’N, 28°40’33’’E, 12.06.2016, 1 ♀.

Ceutorhynchus pallidactylus **(Marsham, 1802)**

Material examined: Edirne province: Doğanköy, 333 m, 41°56’39’’N, 26°42’02’’E, 07.06.2017, 2 ♂♂, 4♀♀. İstanbul province: Çatalca between Danamandıra and İhsaniye, 224 m, 41°18’26’’N, 28°19’26’’E, 11.06.2016, 1 ♀; Silivri-Küçüksinekli, 237 m, 41°13’56’’N, 28°09’55’’E, 13.06.2016, 1 ♀. Kırklareli province: Babaeski, 78 m, 41°25’27’’N, 27°07’23’’E, 9.06.2016, 1 ♀.

Ceutorhynchus picitarsis **Gyllenhal, 1837**

Material examined: İstanbul province: Çatalca between Danamandıra and İhsaniye, 224 m, 41°18’26’’N, 28°19’26’’E, 11.06.2016, 1 ♂.

Ceutorhynchus sulcicollis **(Paykull, 1800)**

Material examined: Edirne province: Doğanköy, 333 m, 41°56’39’’N, 26°42’02’’E, 07.06.2017, 1 ♀.

Ceutorhynchus viridipennis **C. Brisout de Barneville, 1869**

Material examined: Çanakkale province: between Behramlı and Alçıtepe, 137 m, 40°06’19’’N, 26°14’05’’E, 12.05.2016, 1 ♀. Edirne province: between Lalapaşa and Hacıdanişment, 224 m, 41°51’01’’N, 26°45’09’’E, 07.06.2016, 1 ♂.

Glocianus distinctus **(C. Brisout de Barneville, 1870)**

Material examined: İstanbul province: Çatalca-Hisarbeyli (Durusu lake), 6 m, 41°22’37’’N, 28°27’37’’E, 12.06.2016, 1 ♂. Tekirdağ province: Malkara-Doğanköy, 286 m, 41°04’27’’N, 26°49’05’’E, 14.07.2016, 1 ♂; Muratlı-Hanoğlu, 93 m, 41°11’56’’N, 27°21’36’’E, 17.07.2016, 1 ♂.

Hadroplontus trimaculatus **(Fabricius, 1775)**

Material examined: Edirne province: Meriç-Kadıdondurma, 46 m, 41°10’39’’N, 26°21’38’’E, 18.05.2016, 1 ♂.

Microplontus rugulosus **(Herbst, 1795)**

Material examined: Edirne province: Uzunköprü-Ömerbey, 50 m, 41°15’40’’N, 26°50’13’’E, 16.05.2016, 1 ♀; Trakya University, Balkan Campus, 43 m, 41°38’44’’N, 26°37’21’’E, 16.05.2017, 2 ♀♀. Kırklareli province: Center-Beypınar, 567 m, 41°47’47’’N, 27°30’34’’E, 06.06.2016, 1 ♀.

Note: Recently Wanat & Mokrzycki (2018), contrary to the opinions of Colonnelli (2004, 2013) and Alonso-Zarazaga et al (2017), resurrected the name *M. melanostigma* (Marsham, 1802) from its synonymy with *M. rugulosus* (Herbst, 1795), based on the possibility of morphologically distinguishing individuals collected on different plants in central Europe, following the opinion of Morris & Barclay (2015). The second author of this note studied approximately 5,000 specimens of the greatly variable and relatively polyphagous *M. rugulosus* collected over a span of around 180 years in several countries. It is seen that unequivocal separation of more than a morphological species in the observed specimens is impossible, although the form “melanostigma” is much more common (though mixed with the typical form) in North Africa, western Palaearctic Asia and in the southern regions of Europe than in central and northern regions. Central and northern European authors may over-estimate tiny differences of widespread common phytophagous species by considering morphological and molecular variation to be results of evolution - see for example, the incorrect resurrection of *Phyllobius vespertinus* (Fabricius, 1792) from synonymy with *P. pyri* (Linnaeus, 1758) and of *Otiorhychus smreczynskii* Cmoluch, 1968 from that of *O. rotundus* Marseul, 1872 by the same authors (Wanat & Mokrzycki, 2018). Hence, we re-establish the synonymy: *Microplontus rugulosus* (Herbst, 1795) [= *M. melanostigma* (Marsham, 1802); syn. rev.].

Mogulones beckeri **(A. Schultze, 1900)**

Material examined: Edirne province: Trakya University, Balkan Campus, 43 m, 41°38’44’’N, 26°37’21’’E, 16.05.2017, 5 ♂♂, 3 ♀♀.

Mogulones geographicus **(Goeze, 1777)**

Material examined: Edirne province: Süloğlu-Demirhanlı, 123 m, 41°41’45’’N, 26°43’34’’E, 15.05.2016, 2 ♂♂; Trakya University, Balkan Campus, 43 m, 41°38’44’’N, 26°37’21’’E, 16.05.2017, 8 ♂♂, 6 ♀♀; Lalapaşa (Demirköy-Doğanköy road), exit of Demirköy, 297 m, 41°55’06’’N, 26°40’41’’E, 07.06.2016, 6 ♂♂, 6 ♀♀.

Nedyus quadrimaculatus **(Linnaeus, 1758)**

Distribution: Algeria, Armenia, Austria, Azerbaijan, Belarus, Belgium, Bosnia Herzegovina, Bulgaria, Croatia, the Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Ireland, Italy, Japan, Kazakhstan, Mongolia, Latvia, Lithuania, Luxembourg, Moldavia, Montenegro, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Asiatic Turkey, Ukraine (Alonso-Zarazaga et al, 2017). New record for Turkish Thrace.

Material examined: Edirne province: Doğanköy, 333 m, 41°56’39’’N, 26°42’02’’E, 07.06.2017, 3 ♂♂, 1 ♀.

Note: *Nedyus quadrimaculatus*, a common species found in many areas across of the Palaearctic region (Alonso-Zarazaga et al, 2017) and living on nettles, is the member with the widest distribution of this genus, represented by three species in the world in the present (Colonnelli, 2004). This species has been reported from Asian Turkey (Alonso-Zarazaga et al, 2017), and this is the first record of its presence in Turkish Thrace.

Parethelcus pollinarius **(Forster, 1771)**

Distribution: Algeria, Austria, Belgium, Bulgaria, the Czech Republic, Denmark, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Kazakhstan, Latvia, Luxembourg, Moldavia, Morocco, the Netherlands, Norway, Poland, Portugal, Romania, European Russia and western Siberia, Serbia, Slovakia, Spain, Sweden, Ukraine (Alonso-Zarazaga et al, 2017). New record for Turkey.

Material examined:Edirne province: Hıdırağa, 80 m, 41°44’22’’N, 26°40’02’’E, 07.06.2017, 1 ♂, 1 ♀.

Short description: Body length 3.0-4.0 mm, integument blackish, covered by small pale appressed scales. Antennae, except antennal club and tarsi, reddish, tibiae and femora black. Antenna in female inserted at about 2/5 of rostral length. Pronotum wider than long, with sharp lateral tubercles. Humeri quite strongly protruding, flat intervals tuberculate at the level of elytral declivity with a patch of white scales (Morris, 2008).

Note: *Parethelcus* is represented by two species worldwide (Alonso-Zarazaga et al, 2017), namely *P. dentipes* (Israelson, 1980) from the Canary Islands and *P. pollinarius* which has the Western Palaearctic distribution detailed above. Colonnelli (2004) reported that *Urtica pilulifera* L. and *U. dioica* L. are host plants of this last species. The second plant is known from the Edirne province (Anonymous, 2014a). Here, we give evidence of its presence in Anatolia, a region in which the species has never been reported before: Niğde province: Aladağlar, Sarımemetler, 1751 m, 37°46’04”N 35°05’41”E, 01.07.2016, 1 ♀. Note that *U. dioica* has also been observed in Niğde province (TÜBİVES, 2018). This is the first record from both European and Asian Turkey.

Prisistus obsoletus **(Germar, 1823)**

Material examined: Edirne province: between Lalapaşa and Hacıdanişment, 224 m, 41°51’01’’N, 26°45’09’’E, 07.06.2016, 1 ♀.

PHYTOBIINI Gistel, 1848

Rhinoncus leucostigma **(Marsham, 1802)**

Material examined: Edirne province: Trakya University, Balkan Campus, 43 m, 41°38’44’’N, 26°37’21’’E, 16.05.2017, 1 ♀.

COMMENTS

The above records and also distributional data given by Lodos, Önder, Pehlivan, & Atalay (1978), Aydın & Hacet (2016a, 2016b), Alonso-Zarazaga et al (2017) and Alonso-Zarazaga (2018) showed that the number of Ceutorhynchinae recorded in European Turkey has reached 54 species in three tribes. These have been detailed in the following alphabetical list, where an asterisk indicates a new record for Turkish Thrace, and two asterisks mark new records for Turkey:

Amalorrhynchus melanarius (Stephens, 1831) **, *Amalus scortillum* (Herbst, 1795), *Calosirus terminatus* (Herbst, 1795), *Ceutorhynchus assimilis* (Paykull, 1792), *Ceutorhynchus atomus* Boheman, 1845,*Ceutorhynchus chalybaeus* Germar, 1823, *Ceutorhynchus chlorophanus* Rouget, 1858, *Ceutorhynchus contractus* (Marsham, 1802), *Ceutorhynchus duvali* C. Brisout de Barneville, 1869 *, *Ceutorhynchus erysimi* (Fabricius, 1787), *Ceutorhynchus fallax* Boheman, 1845, *Ceutorhynchus hirtulus* Germar, 1823, *Ceutorhynchus nanus* Gyllenhal, 1837, *Ceutorhynchus obstrictus* (Marsham, 1802), *Ceutorhynchus pallidactylus* (Marsham, 1802), *Ceutorhynchus picitarsis* Gyllenhal, 1837,*Ceutorhynchus posthumus* Germar, 1823, *Ceutorhynchus pyrrhorhynchus* (Marsham, 1802), *Ceutorhynchus sulcicollis* (Paykull, 1800), *Ceutorhynchus turbatus* A. Schultze, 1903, *Ceutorhynchus typhae* (Herbst, 1795), *Ceutorhynchus viridipennis* C. Brisout de Barneville, 1869, *Coeliastes lamii* (Fabricius, 1792), *Ethelcus denticulatus* (Schrank, 1781), *Glocianus albovittatus* (Germar, 1823), *Glocianus distinctus* (C. Brisout de Barneville, 1870), *Glocianus fennicus* (Faust, 1895), *Glocianus maculaalba* (Herbst, 1795), *Glocianus moelleri* (C. G. Thomson, 1868), *Glocianus ragusae* (C. Brisout de Barneville, 1884), *Hadroplontus trimaculatus* (Fabricius, 1775), *Microplontus rugulosus* (Herbst, 1795), *Mogulones beckeri* (A. Schultze, 1900), *Mogulones euphorbiae* (C. Brisout de Barneville, 1866), *Mogulones geographicus* (Goeze, 1777), *Mogulones korbi* (A. Schultze, 1901), *Mononychus punctumalbum* (Herbst, 1784), *Nedyus quadrimaculatus* (Linnaeus, 1758)*, *Oprohinus consputus* (Germar, 1823), *Oprohinus suturalis* (Fabricius, 1775), *Parethelcus pollinarius* (Forster, 1771)**, *Prisistus obsoletus* (Germar, 1823), *Ranunculiphilus italicus* (C. Brisout de Barneville, 1869), *Ranunculiphilus faeculentus* (Gyllenhal, 1837), *Rhinoncus leucostigma* (Marsham, 1802), *Rhinoncus perpendicularis* (Reich, 1797), *Sirocalodes depressicollis* (Gyllenhal, 1813), *Sirocalodes mixtus* (Mulsant & Rey, 1859), *Stenocarus cardui* (Herbst, 1784), *Trichosirocalus horridus* (Panzer, 1801), *Trichosirocalus troglodytes* (Fabricius, 1787), *Trichosirocalus urens* (Gyllenhal, 1837), *Zacladus asperatus* (Gyllenhal, 1837), *Zacladus exiguus* (Olivier, 1807).

All the above species but *Ceutorhynchus pyrrhorhynchus*, *Glocianus moelleri*, *Glocianus ragusae* and the newly recorded one in this study *Amalorrhynchus melanarius* are also known from Asian Turkey. This number is quite low, and surely future field surveys will increase our knowledge about the distribution of this weevil subfamily in Turkish Thrace.

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The Distribution of Gomphocerinae Taxa in the Black Sea Region of Turkey: The Role of Vegetations and Elevations

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ABSTRACT

In the conducting the present study, the Gomphocerinae specimens were collected from June to September between 2003 and 2017 according to factors such as the Black Sea Region's topography, its vegetation and altitudes. A total of 207 different localities (including 70 main localities) from 25 provinces in region (7 of them from neighbour to region provinces) were visited. Approximately 5991 adult sample belonging to Gomphocerinae subfamily were collected from the Black Sea region. The collected samples comprised 53 Gomphocerinae taxa, belonging to 17 genera. The total number of Gomphocerinae taxa in the study area reached to 59 with six species from the literature. According to data from this study and other literature, the Black sea region ranks first among the geographical regions of Turkey with regard to the presence of the Gomphocerinae genera and its species number. It has been determined that some 17 species in area belong to Europe (Boreal) sub-region in Palaearctic. The data reveal that the Gomphocerinae taxa prefers mostly altitudes ranging between 600 and 2250 meters in *Castanetum zone*, *Broad-leaf deciduous forest zone,* and *subalpine meadows* in this elevational range. According to all data from this study, the Gomphocerinae taxa prefer the Black Sea Region as refugia.

Key words: Orthoptera, Gomphocerinae, zoogeographical zones, vegetation zones, Black Sea Region, Anatolia.

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INTRODUCTION

Anatolia is represented during the glacial periods one of the most important refugees for various species in western Asia and southern Europe along with Spain, Italy, Greece, Balkans and Caucasus (Hewitt, 1996). Anatolia plays the role of a land bridge for the distribution of fauna elements from east to west and from north to south; as part of the Palaearctic region, it contains a high proportion of endemic plant and animal species (Akman, 1993; Demirsoy, 2002; Atalay, 2015).

The following factors constitute the reasons for the high rate of endemism in Anatolia: (i) acting as a bridge for the exchange of fauna between Eurasia and Africa; (ii) having contacts with different land masses and sometimes the loss of this connection at different geological times; and (iii) showing topographical and climatically large changes in a very short distance, by which bringing biotope diversity along with species diversity (Demirsoy, 1997; 2002; Çıplak, 2008), and being in the "gene center" position for many taxa (Hewit, 1996; 2000; Çıplak, 2008). In addition, Anatolia acts as "refugia" for many species through the Pliocene and Pleistocene glacial periods in the last 2.4 MY, especially for those forms that prefer colder climates and for many species in interglacial periods; it also acts as a bridge during the migration from south to north for the warm climate preferring taxa (Demirsoy, 1977; 2002; Çıplak, 2004; 2008; Mol & Zeybekoğlu, 2013). The phytogeographical and zoogeographical studies should be evaluated together in order to understand the reasons for the richness of biodiversity in the region, the formation of fauna, and how the area is affected. There are few essential studies for the investigation of Anatolia biogeography (Uvarov, 1921; 1934; Weidner, 1969; Demirsoy, 1977; 2002; Çıplak, Demirsoy, & Bozcuk, 1993, 1996; Çıplak & Demirsoy, 1996; Hewitt, 1996; 2000; Çıplak, 2004; 2008).

Gomphocerinae (Orthoptera: Acrididae) includes 1738 taxa belonging to 192 genera in Holoarctic region (Cigliano, Braun, Eades, & Otte*,* 2018). The number of the Gomphocerinae taxa shows a variety in southern refugia, namely are 65 taxa in Spain, 56 in France, 42 in Italy, 45 in Greece, and 98 taxa in Turkey (Bei-Bienko and Mistshenko, 1951; Harz, 1975; Demirsoy, 1977, 2002; Mol & Zeybekoğlu, 2013; Ünal, 2015: Cigliano et al*,* 2018). Literature reports that Gomphocerinae taxa prefer mostly the cold climate of the northern hemisphere, and cold and ever green vegetation in the central and northern parts of Europe and Asia (Bei-Bienko and Mistshenko, 1951; Harz, 1975; Demirsoy, 1977). Considering the ecological preferences of the Gomphocerinae subfamily, it appears that the Black Sea region and partly the Eastern Anatolia region of Anatolia are the fit areas where this demand can be answered. Especially the Black Sea region is known for its temperate rainforest ecosystem whereas most of the area is covered by coniferous or broadleaf forests and subalpine meadows which develop in the temperate zone and receive heavy rainfall (Şensoy, Demircan, Ulupinar, & Balta, 2008; Atalay, 2015).

This study aims to contribute to the zoogeography of Turkey by revealing the role of the vegetation and altitude role in the distribution of the Gomphocerinae taxa in the Black Sea region.

MATERIALS AND METHODS

The specimens were collected from June to September between 2003 and 2017 by taking in consideration factors such as the Black Sea Region's topography, its vegetation and altitudes. A total of 207 different localities (including 70 main localities which were visited more than two times) from 25 provinces in region (7 of them from neighbouring provinces the region) were visited and the field trip was performed (Fig. 1). Gomphocerinae taxa were sampled with an insect net and the most commonly used method to determine relative abundance and to estimate the grasshopper species composition (Gardiner, Hill, & Chesmore, 2005). Samples were collected using the insect net during daytime on the feeding habitats. Most of the collected samples were identified by using the morphological characters (Uvarov, 1934; Bei-Bienko & Mistschenko, 1951; Karabağ, 1953, 1957, 1959, 1963; Harz, 1975; Demirsoy, 1977; Salman, 1978; Soltani, 1978; Willemse, 1985). The songs of some species, especially those belonging to the genera *Stenobothrus* and *Chorthippus,* were recorded by different song recorder equipments (Sony tape recorder, Tascam DR-100 and Tascam HD-P2). The acoustic characters of the species were compared to those from the literature (Ragge & Reynolds, 1998; Savitsky, 2007; Vedenina & Helversen, 2009; Şirin, Helversen, & Çıplak, 2010b; Şirin, Mol, & Çıplak, 2011; Şirin, Mol, & Akyıldız, 2014; Mol, 2012) in order to be identificated. The altitudinal distribution and the vegetation zones preference of the taxa collected by the authors and reported in previous studies (Salman, 1978; Ünal, 1999, 2008, 2010) were demonstrated according to the related literature (Atalay, 2015). Atalay (2015) analyzed and described in detail the vegetation zones of the Black Sea region; we simplified these zones and compared them with the grasshopper distribution in the area. The generally vegetation zones of the Black Sea region are grouped into 5 main vegetation zones, the Maquis zone (mostly between 0-200 m), *Castanetum* zone (mostly between 200-600 m), the Broad-leaf deciduous forest zone (mostly between 600-1200 m), the Conifer forests zone (mostly between 1200-2000 m), and the Subalpine meadows (mostly over the 2000 m) (Figs. 2, 3).

According to Uvarov (1921), the distribution of the Palaearctic region Orthoptera taxa is divided into 4 sub-regions, and this view is particularly widely used in the description of the structure of the fauna of Anatolia and the Caucasus. These sub-regions are: (1) Boreal sub-region (Central and Northern Europe): (2) Step sub-region (Angara: Caspian vicinity, Central and Northern Asia): (3) sub-region Mediterranean; and (4) Eremial sub-region (Syrio Eremial, Irano-Turanian and African origin ones). In this study, we followed Uvarov (1921) sub-regions description for Gomphocerinae subfamily members.

RESULTS AND DISCUSSION

Approximately 5991 adult samples belonging to Gomphocerinae subfamily were collected from the Black Sea region (Table 1). The collected samples comprised 53 Gomphocerinae taxa, belonging to 17 genera (Table 1) could not collect six Gomphocerinae species (Table 1), which are mentioned in literature (Salman, 1978; Ünal, 1999, 2008).

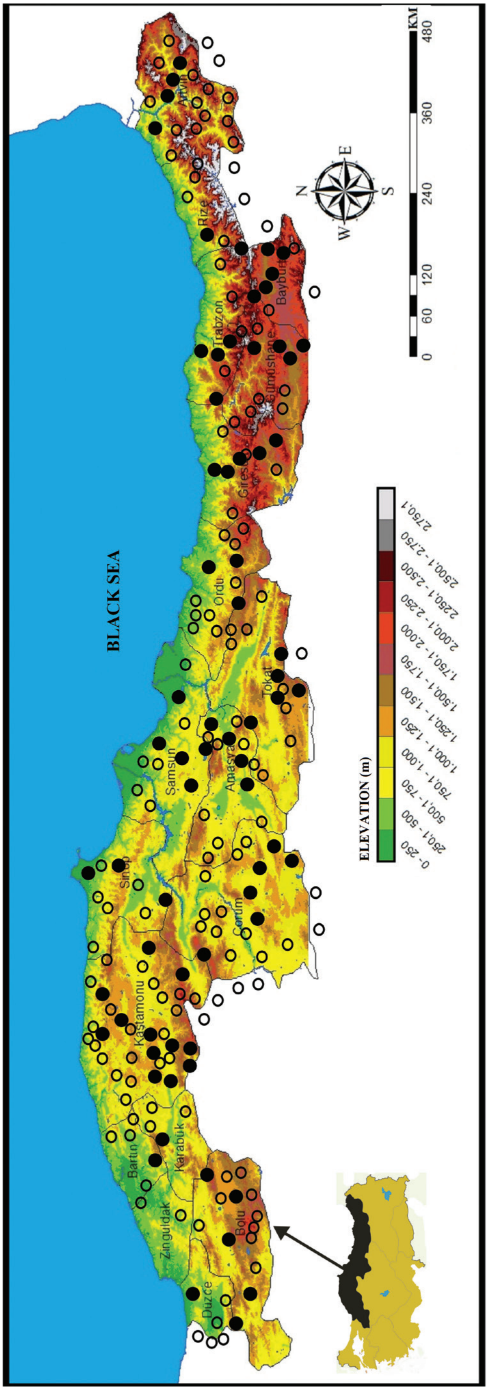

Fig. 1. The localities visited to collect Gomphocerinae taxa in Black Sea Region of Turkey (filled circles represent the main localities which were visited by the researchers more than two times and small circles represent the only one time visited locality).

Fig. 2. The photographs of the general vegetation zones of the Black Sea region A: Maquis zone, B: Castanetum zone, C: Broad-leaf deciduous forest zone, D: Conifer forests zone and E1-E2: Subalpine meadows.

The total number of Gomphocerinae taxa in the study area reached 59 with six species from the literature. According to data from this study and the literature, the Black sea region ranks first among the geographical regions of Turkey with the Gomphocerinae genera and species number (Figs. 4 A-B). The genus *Chorthippus* diversity was first ranked (15 species, representing 25.43% of the total grasshoppers' species richness) in study area. This genus was followed by Stenobothrus (11 species, representing 18.64% of the total grasshoppers' species richness) in the study area. Our data are consistent with the data from previous studies reporting that the most diverse genera in the Gomphocerinae members are *Chorthippus* with 25 taxa and *Stenobothrus* with 16 taxa in Anatolia (Demirsoy, 2002; Ünal, 2015). These two genera cover 45% of the Gomphocerinae fauna of the Blacksea region and these data correspond to the assumptions that *Chorthippus* and Stenobothrus are the mountainous forms which dispersed to Anatolia from northern territories during cold periods of glacial cycles (Uvarov, 1921; Demirsoy, 1977). The *Omocestus* and *Dociostaurus* are represented by six and five species respectively, in the sampling area (Table 1); these are also known from Anatolia with 9 species for Dociostaurus and 7 species for Omocestus (Demirsoy, 2002; Ünal, 2015). The *Gomphocerus*, *Euchorthippus*, and *Ramburiella* were represented by four, three and two species, respectively, in the Black Sea region. The other genera in the area are *Duroniella*, *Ptygippu*s, *Euthystira*, *Arcyptera*, *Pararcyptera*, *Eremippus*, *Stauroderus*, *Pseudochorthippus*, *Aeropedellus*, *Dasyhippus*, *Rammeihippus*, and *Myrmeleotettix*, each being represented by one species (Table 1).

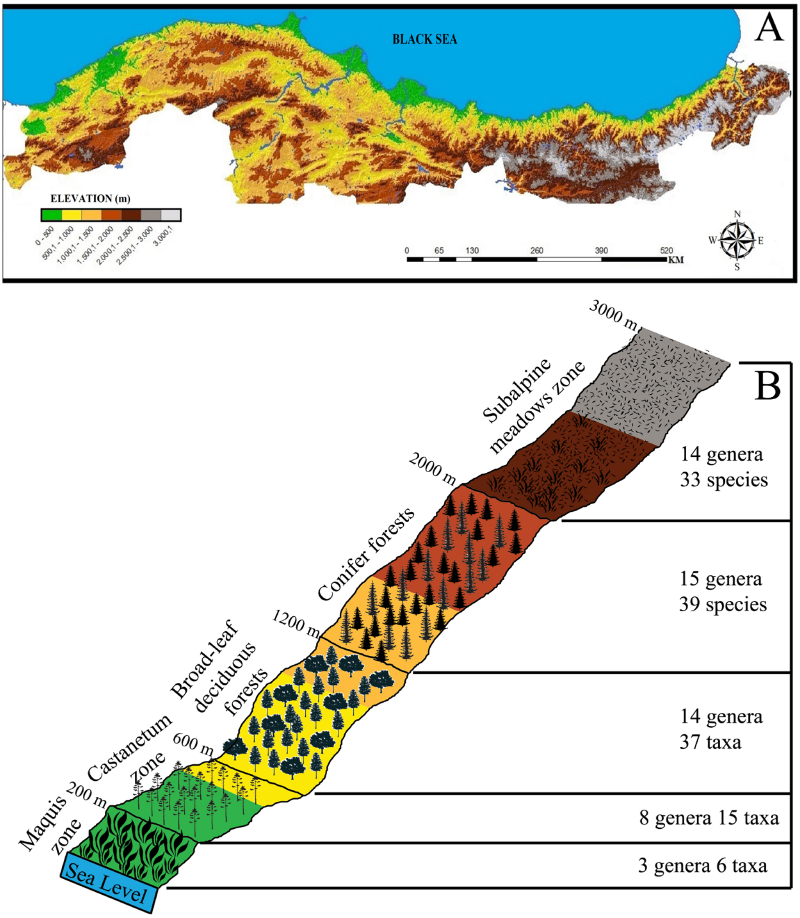

Fig. 3. A: The map of study area with elevational gradient. B: Vegetation zones of the study area according to altitudinal level and distribution of Gomphocerinae taxa in each zones.

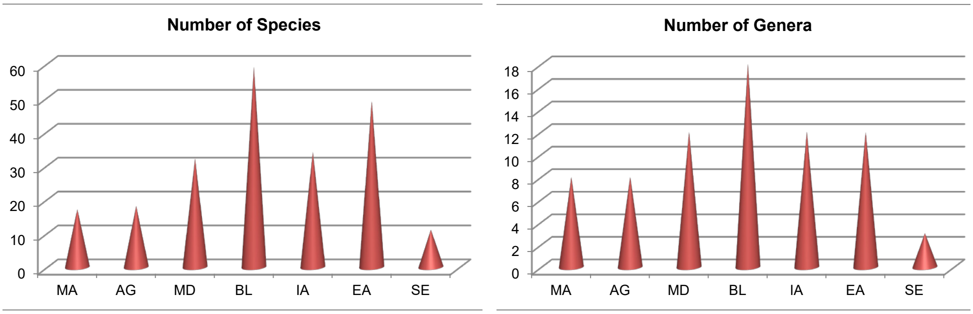

Fig. 4. The number of species (A) and genera (B) of Gomphocerinae in the geographical regions of Turkey (Ma: Marmara region, AG: Aegean region, MD: Mediterranean region, BL: Black Sea region, IA: Inner Anatolia region, EA: Eastern Anatolia region, SE: South-eastern Anatolia region).

The identified 59 Gomphocerinae taxa in the Black Sea Region were classified according to Uvarov (1921) description. The sub-regions and the numbers of the taxa belong to 17 Europe (Boreal), 16 Angara, 14 Europea-Angara, 6 Mediterranean, 4 Eremial (Iran-Turan) and 2 Eremiyal (Syrio), respectively (Table 1). Within these taxa, it was determined that 8 taxa from the sub-region of Angara, 4 taxa from the sub-region of Europe (Boreal), 1 taxon from the sub-region of Mediterranean, and 1 taxon from the sub-region of Eremial (Iran-Turan) are endemic. The number of taxa found from Black Sea region, which belongs to Europe (Boreal), European-Angara and Angara sub-regions, is 47. This number constitutes about 80% of the Gomphocerinae subfamily in the whole area (Table 1).

When we consider the altitudinal distribution of the Gomphocerinae species in the Black Sea region, the species *Pseudochorthippus parallelus* and *Chorthippus macrocerus* seem to be widespread in the altitudinal scale. However, it seems that 15 species show a very narrow distribution in altitudinal scale and they are found only in one vegetation zone of the area (Table 1). Although we are not able to measure statistically the significant proportions of the collected species, it seems that the most abundant species in the study area are *Dociostaurus brevicollis*, *Notostaurus anatolicus*, *Stenobothrus lineatus*, *Omocestus haemorrhoidalis haemorrhoidalis*, *Stauroderus scalaris*, *Pseudochorthippus parallelus parallelus*, *Chorthippus dichrous*, *Chorthippus apricarius apricarius*, and *Chorthippus macrocerus*.

The collected species *Eremippus zeybekoglui, Stenobothrus weidneri, S. selmae, Chorthippus aktaci, C. ilkazi, G. armeniacus dimorphus, G. sibiricus hemipterus, G. sibiricus acutus, Aeropedellus turcicus,* and *Rammeihippus turcicus* are endemic to the Black Sea region, whereas *Dasyhippus uvarovi* and *Omocestus nanus* are the endemic species for Turkey (Demirsoy, 1977, 2002; Mol & Zeybekoglu, 2013). The distribution of these taxa according to vegetation zones are 1 species in *Castanetum* zone, 4 species in Broad-leaf deciduous forest zone, 9 species in Conifer forests zone, and 7 species in Subalpine meadows zone (Table 1, Fig. 5).

Table 1. The list of all taxa were collected in between 2003-2017 years in Black Sea region and the information about the specimen number, vegetation zones, Endemism, altitudinal distribution range and sub-regions detail belongs to all identified taxa from Black Sea region.

Taxa	**Maquis**	**Castanetum**	**Broad-leaf deciduous forest**	**Conifer forests**	**Subalpine meadows**	**Endemism**	**Altitude**	**Sub-Regions**
1-*Duroniella laticornis* (Krauss, 1909)	-	-	15	-	-		800	Mediterranean
2-*Ptygippus brachypterus* Mistshenko, 1951*	-	-	-	-	+		2460	Angara
3-*Euthystira brachyptera* (Ocskay, 1826)*	-	-	-	+	-		1550-1600	Europe (Boreal)
4-*Arcyptera fusca fusca* (Pallas, 1773)	-	-	3	11	9		865-2460	Angara
5-*Pararcyptera labiata* (Brulle, 1832)	-	-	22	44	2		1200-2460	Mediterranean
6-*Ramburiella turcomana* (Fis, de Wald.,1846)	-	-	6	-	-		865-1160	Mediterranean
7-*Ramburiella bolivari* (Kuthy, 1907)	-	4	-	-	-		600	Mediterranean
8-*Dociostaurus brevicollis* (Eversmann, 1848)	-	-	188	341	71		730-2460	Mediterranean
9-*Dociostaurus tartarus* Stschelkanovzev, 1921	-	3	-	-	-		600	Eremial (Iran-Turan)
10-*Dociostaurus genei* (Ocskay, 1832)*	-	+	-	-	-		600	Eremial (Iran-Turan)
11-*Dociostaurus moraccanus* (Thunberg, 1815)	-	-	3	-	-		1275	Eremial (Syrio)
12-*Dociostaurus haunesteini* Bolivar, 1893	-	-	-	3	-		1680-1790	Eremial (Iran-Turan)
13-*Notostaurus anatolicus* (Krauss, 1896)	-	-	150	156	-		625-1955	Eremial (Syrio)
14-*Eremippus zeybekoglui* Mol, 2012	-	1	-	-	-	E	600	Eremial (Iran-Turan)
15-*Stenobothrus weidneri* (Demirsoy , 1977)	-	-	9	44	18	E	1725-2302	Angara
16-*Stenobothrus werneri* Adelung, 1907	-	-	10	63	58		1300-2460	Angara
17-*Stenobothrus sviridenkoi* Ramme, 1930	-	-	5	15	6		1400-2460	Angara
18-*Stenobothrus lineatus* (Panzer, 1796)	-	3	41	181	48		350-2400	Europe-Angara
19-*Stenobothrus fischeri* (Eversmann, 1848)	-	-	4	4	-		865-1350	Europe-Angara
20-*Stenobothrus nigrogeniculatus* Krauss, 1896	-	-	16	38	19		865-2460	Europe-Angara
21-*Stenobothrus zubowskyi* Bolivar, 1899	-	-	53	20	37		865-2460	Europe-Angara
22-*Stenobothrus mirame* Dirsh, 1931*	-	-	-	+	-		733-1825	Europe (Boreal)
23-*Stenobothrus graecus* Ramme, 1926	-	7	21	47	-		650-2460	Europe (Boreal)
24-*Stenobothrus selmae* Unal, 1999*	-	-	-	+	-	E	1950	Europe (Boreal)
25-*Stenobothrus nigromaculatus* (Herrich-Sch., 1840)	-	-	-	3	13		1920-2035	Europe-Angara
26-*Omocestus nanus* Uvarov, 1934	-	-	13	62	11	E	1650-2460	Angara
27-*Omocestus ventralis* (Zetterstedt, 1821)	24	18	71	-	-		200-1450	Europe (Boreal)
28-*Omocestus minutus* (Brulle, 1832)	-	-	20	-	-		850-1100	Europe (Boreal)
29-*Omocestus h. haemorhoidalis* (Charpentier, 1825)	-	-	20	250	22		865-2460	Europe (Boreal)
30-*Omocestus h. ciscaucasicus* Mistshenko, 1951	-	-	-	-	23		2100-2460	Angara
31-*Omocestus petraeus* (Brisuot de Barn.,1855)	-	9	56	72	-		650-1800	Europe-Angara
32-*Stauroderus scalaris* (Fisch. de Wald., 1846)	-	-	61	245	44		740-2460	Europe-Angara

Table 1. Continued.

Taxa	**Maquis**	**Castanetum**	**Broad-leaf deciduous forest**	**Conifer forests**	**Subalpine meadows**	**Endemism**	**Altitude**	**Sub-Regions**
33-*Euchorthippus pulvinatus* (Fischer de Wald.846)	-	-	91	63	2		650-2250	Europe-Angara
34-*Euchorthippus declivus* (Brisuot de Barn.,1855)	-	13	-	-	-		600	Europe (Boreal)
35-*Euchorthippus transcaucasicus* Tarb., 1930*	-	-	+	-	-		1350	Angara
36-*Pseudochorthippus parallelus* (Zetterstedt, 1821)	38	30	172	291	12		100-2250	Europe (Boreal)
37-*Chorthippus karelini* (Uvarov, 1910)	-	-	6	9	3		1000-1860	Europe (Boreal)
38-*Chorthippus labaumei* Ramme, 1926	-	-	-	-	15		2400-2460	Angara
39-*Chorthippus loratus* (Fisch. de Wald., 1846)	45	29	91	-	-		100-1650	Europe-Angara
40-*Chorthippus dorsatus* (Zetterstedt, 1821)	-	-	43	-	-		1050	Europe (Boreal)
41-*Chorthippus dichrous* (Eversmann, 1859)	-	11	82	139	76		600-2460	Europe-Angara
42-*Chorthippus a. apricarius* (Linnaeus, 1758)	-	-	48	121	89		900-2250	Europe (Boreal)
43-*Chorthippus apricarius major* Plynov, 1914	-	-	-	28	98		1800-2450	Angara
44-*Chorthippus bornhalmi* Harz, 1971	6	7	22	71	-		200-1920	Europe (Boreal)
45-*Chorthippus aktaci* Unal, 2010*	-	-	-	+	-	E	1550-2000	Europe (Boreal)
46-*Chorthippus vagans* (Eversmann, 1859)	-	-	42	93	13		700-2460	Europe-Angara
47-*Chorthippus macrocerus* (Fisc. de Wald., 1846)	13	39	236	92	12		200-2460	Europe-Angara
48-*Chorthippus demokidovi* (Ramme, 1930)	-	-	-	-	25		2360-2460	Europe (Boreal)
49-*Chorthippus ilkazi* Uvarov, 1934	-	-	27	26	-	E	900-1920	Europe (Boreal)
50-*Chorthippus biguttulus euhedickei* Helversen, 1989	2	2	109	-	-		100-1300	Europe (Boreal)
51-*Chorthippus mollis* (Charpentier, 1825)	-	-	81	76	9		630-2250	Europe-Angara
52-*Gomphocerus transcaucasicus* Mists., 1951	-	-	2	7	51		2100-2450	Angara
53-*Gomphocerus sibiricus acutus* Karabağ, 1957	-	-	-	7	8	E	2000-2323	Angara
54-*Gomphocerus armeniacus dimorphus* Karabağ, 1953	-	-	-	-	16	E	2460	Angara
55-*Gomphocerus sibiricus hemipterus* Karabağ, 1953	-	-	-	6	11	E	1800-2300	Angara
56-*Aeropedellus turcicus* Karabağ, 1959	-	-	-	15	32	E	2150	Angara
57-*Dasyhippus uvarovi* Karabağ, 1953	-	-	11	-	-	E	1180	Mediterranean
58-*Rammeihippus turcicus* (Ramme, 1939)	-	-	-	186	12	E	1200-2035	Angara
59-*Myrmeleotettix maculatus* (Thunberg, 1815)	-	-	-	14	129		2000-2630	Europe-Angara
Total 59 taxa	**6**	**15**	**37**	**39**	**33**	**12**	**100-2640**	**Boreal, Angara, Mediterranean, Eremial**

*These species were not collected in this study.

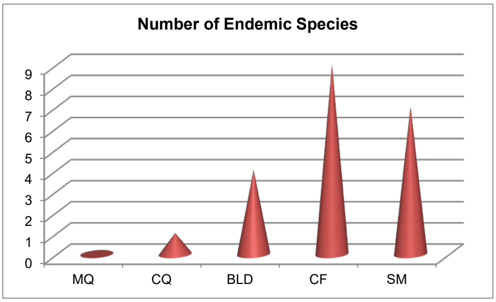

Fig. 5. The number of endemic species in the vegetation zones of the Black Sea region (MQ: Maquis zone, CA: Castanetum zone, BLD: Broad-leaf deciduous forest zone, CF: Conifer forests zone, SM: Subalpine meadows).

Among the collected 59 taxa, which show the distribution in the study area, 6 taxa belong to 3 genera in *Maquis zone*, 15 taxa belong to 8 genera in *Castanetum zone*, 37 taxa belong to 14 genera in *Broad-leaf deciduous forest zone*, 39 taxa belongs to 15 genera in *Conifer forests zone,* and 33 taxa belong to 14 genera in *Subalpine meadows* (Figs. 3, 6A,B). When the altitude increases from the *Maquis* zone to the subalpine zone, there is also an increase in the number of species (Table 1). In *Maquis zone*, minimum number of species was collected, whereas in the *Conifer forests zone* the greatest number of species was collected. According to the data of this study, Gomphocerinae taxa prefer mostly altitudes ranging between 600 and 2250 meters (Fig. 3). The specimens belonging to *C. loratus* and *O. ventralis* species were collected from the lowest locality (Samsun, Atakum, 100 m); those of *M. maculatus maculatus* were collected from the highest locality (Ovit Mountain, 2650 m) of research area. All the species collected from this locality have a northern origin (Demirsoy, 2002). There is only one study about the species richness and the relative abundance of the different families of the Caelifera in Akdaglar Mountain range, which is a part of Taurus mountains in southern part of Anatolia. It was reported that Gomphocerinae is dominant between 1000 and 2000 m with 13 species (Şirin, Eren, & Çıplak, 2010a). Taurus Mountains (including Akdaglar Range) of southern Anatolia are considered as a hotspot for Anatolian endemics (Çıplak & Demirsoy, 1995; 1996; Çıplak, 2003) and include 32 Gomphoceriane species, of which eight are endemic (Mol, 2001; Demirsoy, 2002; Çıplak, Mol, Şirin, Zeybekoğlu, & Taylan, 2005; Şirin et al*,* 2010b; Şirin et al, 2011). Only five of these endemic species are known from the southern part of Anatolia (Çıplak et al, 2005; Demirsoy, 2002, Şirin et al, 2010a, 2011). According to our study, the Black Sea region contains 12 endemic species and only 10 of them are known from this region. The data obtained in this study reveal (i) the species richness of the subfamily in the area; (ii) the local endemic species number; (iii) the distributional range in altitudinal scale; and (iv) the vegetational composition and climate, clearly supporting the idea that Gomphocerinae taxa prefers Black Sea region as refugia.

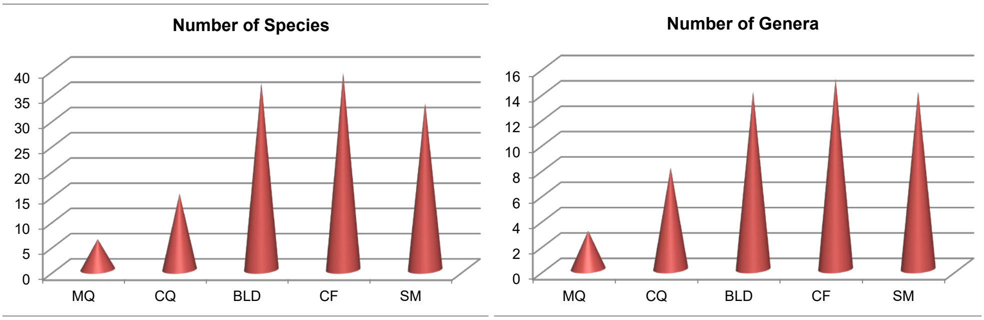

Fig. 6. The number of species (A) and genera (B) in the vegetation zones of the Black Sea region (MQ: Maquis zone, CA: Castanetum zone, BLD: Broad-leaf deciduous forest zone, CF: Conifer forests zone, SM: Subalpine meadows).

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Distribution and Conservation Status of the European Red Wood Ant Species *Formica pratensis* Retzius, 1783 (Hymenoptera, Formicidae) in (European) Turkey

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ABSTRACT

The European Red Wood ant species *Formica pratensis* Retzius, 1783 (Hymenoptera: Formicidae) is a mound building ant species distributed in a number of European countries and is listed in IUCN Red List of Threatened species with near-threatened status. The distributional range of the species in Turkey covers only the Thrace Region. In the present study, we performed a three-stepped (inventory, monitoring and conservation) study to determine the current distribution of the species in the region and to propose a national red list status for the species. During the inventory and monitoring studies, a total of 340 localities were inspected in the region thoroughly for the presence of the colonies and a total of 89 colonies in 48 localities were recorded of which 20 died during the study period. The extent of occurrence (EOO) and area of occupancy (AOO) of the species were measured. According to the evaluation of the distributional data, *F. pratensis* was assessed as Vulnerable (VU) in Thrace Region in Turkey following the criteria B1b (i, ii, iii, iv) due to the small AOO and EOO. The possible threats acting on the species were also discussed.

Key words: Formica pratensis, European Red Wood ant, species conservation, monitoring, IUCN red list.

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INTRODUCTION

Biological diversity, or biodiversity, refers to the variety of all forms of life on earth and is usually considered at three different levels: genetic diversity, species diversity and ecosystem diversity (Vellend & Geber, 2005; Gugerli et al, 2008). Species diversity within a geographical area can be measured in terms of species richness, species abundance and taxonomic or phylogenetic diversity (Gotelli & Chao, 2013). Ants constitute a diverse group of invertebrates and about 15.363 described species and subspecies account for less than 1% of all described insect species (Bolton, 2018). However, they are considered keystone species in the ecosystem and therefore have been a subject of a number of biodiversity and conservation studies (Alonso, 2000; Robinson, 2001; Hughes, 2006; Mabelis 2007; Seppä, 2008; Bution, Tango, & Caetano, 2010; Dekoninck, Hendrickx, Grootaert, & Maelfait, 2010). They are also valuable indicators for measuring environmental change and ecosystem functioning (Andersen & Majer, 2004; Underwood & Fisher, 2006). According to Davic (2003), species can be put in different categories as “keystone species”, “key species”, “intraguild competitors/predators” and “ecosystem engineers”. Red wood ants, which fall into several of these categories, prey on a wide range of insects including destructive ones, provide habitats for many organisms in their nests and are important food sources for birds. Therefore red wood ants are the target organisms of conservation actions in Europe (Mabelis, 2007).

The current ant fauna of Turkey includes 367 taxa (Kiran & Karaman, 2012; Kiran, Lapeva-Gjonova, & Aksoy, 2017; Karaman, Kiran, Aksoy, & Çamlıtepe, 2017; Csősz, Salata, & Borowiec, 2018; Steiner et al, 2018) and the genus *Formica* L. is represented by 17 species, one of which is *Formica pratensis* Retzius 1783, commonly known as European red wood ants or the black-backed meadow ants. *Formica pratensis* is distributed only in European part of Turkey (Çamlıtepe, 1987; Aktaç, 1987; Aras, 1989; Aktaç, Aras, & Camlitepe, 1994; Lapeva-Gjonova & Kiran, 2012; Karaman & Kiran, 2018; Wagner, Karaman, Aksoy, & Kiran, 2018) while the closely related species, the southern wood ant *F. rufa* L. 1761 is distributed exclusively in the Anatolian part of the country (Schulz & Sanetra, 2002; Kıran & Aktaç, 2006; Kıran, Aksoy, & Camlitepe, 2009). *Formica pratensis* is a mound building ant with a distributional range covering a number of countries, i.e. Albania; Andorra; Austria; Belarus; Belgium; Bosnia and Hercegovina; Britain; Bulgaria; Channel Is.; Croatia; Czech Rep.; Denmark; Estonia; Finland; France: mainland; Georgia; Germany; Greece; mainland; Hungary; Italy: mainland; Latvia; Lithuania; Luxembourg; Macedonia; Moldova; Montenegro; Netherlands; Norway; Poland; Portugal; Romania; Russia; Serbia; Slovakia; Slovenia; Spain: mainland; Sweden; Switzerland; Turkey; Ukraine (Borowiec, 2014). Although the species is widespread in Europe it is declining across the whole of its range due to fragmentation of suitable habitats, general lack of appropriate habitat management, deforestation, urbanization and intensive agricultural activities (Gyllenstrand & Seppä 2003; Domisch, Finér, & Jurgensen, 2005; Dekoninck et al, 2010). *Formica pratensis,* along with *F. aquilonia* Yarrow, 1955, *F. lugubris* Zetterstedt, 1838, *F. polyctena* Foerster, 1850 and *F. rufa* is included in the 1983 IUCN Invertebrate Red Data Book (Wells, Pyle, & Collins, 1983) and one of the 149 ant species listed in the 2008 Red

List (IUCN, 2008). The near-threatened status of *F. pratensis* in the IUCN Red List of Threatened species is based on old records, hence, the need of an update about the status of the species is noted in the list. *Formica pratensis* is considered to be extinct in Britain because no record has been given since 1988 (Nicholson, 1997). It is vulnerable in Belgium (Dekoninck, Vankerkhoven, & Maelfait, 2003; Dekoninck, Maelfait, Vankerkhoven, & Grootaert, 2005) and is protected with legislations in Netherlands, Germany and Hungary (Tartally, 2009). Kiss & Kóbori (2010) reported that the number of *F. pratensis* colonies in Hoia, Romania was 50 in 2004 but decreased to 8 in 2010. Benedek & Kobori (2014) reported presence of more than 100 *F. pratensis* nests in a limited area in Fânaţele Clujului Nature Reserve, Romania and highlighted that a dramatic decline in the number of colonies occurred from autumn 2004 to spring 2005. While Lapeva-Gjonova, Antonova, Radchenko, & Atasanova (2010) presents the distribution of *F. pratensis* in Bulgaria, they stated that the distribution is poorly known, so an update on its protection status is necessary in Bulgaria. The species is also known to occur in the neighboring country Greece. The records given by Legakis (2011) are based on former records given in Agosti & Collingwood (1987) but Bračko, Kiran, Karaman, Salata, & Borowiec, (2016) recorded the species from Greece Thrace 2016, and recently Borowiec & Salata (2018) from Thessaly region of Greece.

The earliest records of *F. pratensis* in Turkey were given by Forel (1906) and Donisthorpe (1950) at an altitude above 1900 meters from Bursa-Uludağ and Bitlis Nazik Lake, respectively. Since *F. pratensis* is distributed at lower altitudes with an average of 900 meters, it is clear that this collected material belongs to another *Formica* species. Moreover, *F. pratensis* has not yet been recorded so far in faunal studies of Anatolian part of Turkey (Aktaç, 1976, 1987; Kıran & Aktaç, 2006; Kıran et al, 2009; Kıran & Karaman, 2012).

Although ant fauna in Turkey is represented with a comparatively high number of taxa (Kıran & Karaman, 2012), studies on the fauna generally focused on sampling-identification-record based species lists and no long-term study was performed on a particular species. The evaluation of former and current available records of *F. pratensis* in Turkish Thrace led us to start a national conservation study for the species considering the rarity of its colonies in the region and the dramatic loss of all formerly recorded colonies. The rarity, in particular, increases the importance of *F. pratensis* for the preservation of species diversity and ecological processes which in turn raises the need of an effective and recognizable conservation status. Prior to our study, *F. pratensis* in Turkish Thrace was represented with 18 colonies which were recorded during field studies dating back to late 80s in only 8 localities in Arpaç, Avarız, Bakışlar, Doğanköy and Ortakçı villages in Edirne and Yeniceköy, Koruköy and Kula villages in Kırklareli provinces. However, ongoing monitoring studies have given us an impression that all colonies died in the past either due to natural or anthropogenic reasons, one of which, and most probably the leading one, is the increasingly continuing industrial activities in the region.

The process of identifying and listing threatened species is inevitably a dynamic and iterative process needing revisions, additions and updates to the list which will help researchers to determine which species may warrant listing, delisting or status change. In the present study we evaluated the former and most recent distributional data of *F. pratensis* in Thrace region of Turkey and proposed a national red list status for the species. A distribution map for the species was constructed and the possible threats that the species faces in the region were also discussed in a framework of a conservation action plan.

MATERIAL AND METHODS

Study area

The study area, Turkish Thrace, is most probably the south-easternmost border of distributional range of *F. pratensis* in Europe. It covers an area of 23.485 km^2 and provides suitable habitats and habitat patches for the species particularly in the northern and southern woodland parts separated from each other with a wide anthropogenic steppe area in the central part of the region. *Bromus* sp. (cheatgrass) and *Festuca* sp. (fescue) pastures and scattered *Paliurus spina-christii* Mill. (Jaruselam thorn) patches can also be observed in the steppic area. The highest elevation of the region is 1031 m asl. in Mahya Tepe in the north and 924 m asl. in Ganos Mountain in the south (Dönmez, 1990).

The northern mountainous region is covered by dry and humid forest areas. *Fagus orientalis* Lipsky. (beech), *Ilex colchica* Pojark. (Black Sea holly) and *Rhododendron ponticum* L. (rhododendron) are typical vegetation members of the humid forests. The beech forests include *Acer campestre* L. (field maple), *Cornus mas* L. (cornelian cherry), *Corylus avellana* L. (common hazel), *Mespilus germanica* L. (Medlar) and *Prunus spinosa* L. (blackthorn) and *Sorbus torminalis* (L.) Crantz (wild service tree). *Quercus cerris* L. (Turkey oak), *Q. frainetto* Ten. (Hungarian oak), *Q. infectoria* Olivier (Aleppo oak), *Q. petraea* (Matt.) Liebl. (sessile oak), *Q. pubescens* Willd. (downy oak) and *Q. robur* L. (common oak) are members of the deciduous dry forests. *Acer campestre*, *Fraxinus ornus* L. (Manna ash)*, Sorbus* sp., *Pyrus elaeagnifolia* Pall. (silver sail), *Tilia platyphyllos* Scop. (large-leaved lime), *T. tomentosa* Moench (silver linden) are other trees seen occasionally in oak forests. The southern mountainous region is characterized with *Pinus brutia* Ten. (red pine) and maquis vegetation. Scrubby/heathland type areas are also common in both north and southern parts.

Data collection

The field studies were performed from 2012 to 2015 in a total of 340 localities throughout Thrace Region. The localities were selected in a manner that ensured a homogenous sampling of the region. The main field studies were performed from May to October of each year in three simultaneous steps as i) inventory and habitat characterization, ii) monitoring and iii) conservation of *F. pratensis* colonies. The first step included determination of the distributional range of the species in the region. Habitats providing suitable conditions for colonization of *F. pratensis* were visited and visually inspected thoroughly for the presence of colonies. The localities where the species had formerly been recorded were also visited. Colonies were determined either by their visual identifications in vegetation poor environments or by following the inbound journals of foraging individuals in vegetation dense environments, who eventually helped us to pinpoint the colonies. The locations of some of the colonies were reported to us by local residents. The coordinates and habitat details/types of all determined colonies were recorded. Social structures (polydomy - monodymy) of the colonies were also recorded by determining presence of inter-nest traffic between sympatric colonies. The monitoring of recorded colonies was performed whenever possible during the study. The conservation step was achieved by enclosing the colonies vulnerable to disturbance with a wire net when they were first found in the field. A short description and biological importance of the species with its visuals were printed on UV resistant Plexiglas plates and fixed on visible parts of the nests. The local people in all localities, irrespective of presence or absence of *F. pratensis*, were given short briefings on the species on site with the live material or using photographs, videos, slides and leaflets. All possible factors that may have caused disturbance on and/or extinction of the colonies were evaluated and listed.

Data analysis

The obtained GIS data of *F. pratensis* was mapped and analyzed using QGIS 2.0.1. The total study area was divided into 5x5 km^2 UTM grid cells and the evaluation was based on the number of colonies per grid cell and on the total number of grid cells with colony records. The extent of occurrence (EOO) and area of occupancy (AOO) of *F. pratensis* in the study region were measured with the help of Google Earth and GE Path using the convex hull method for EOO and 2x2 km^2 grid method for AOO. Area of occupancy is defined as the area within the total range (and hence within EOO) that is currently occupied by the species and excludes unsuitable and unoccupied habitat (IUCN, 2017). Based on the distribution pattern of *F. pratensis* and its habitat choice in the region, the species was assigned one of the categories given in Table 1 (see Dekoninck et al, 2003 for details), all which agree with the criteria and categories developed by the IUCN (IUCN, 2001; see also Binot, Bless, Boye, Gruttke, & Pretscher, 1998; Maelfait, Baert, Janssen, & Alderweireldt, 1998).

RESULTS

Inventory and monitoring studies in the study region revealed presence of 89 colonies in 48 localities scattered in 47 UTM grids (5x5 km^2, Fig. 1). However, 20 of these colonies died at one stage of the study period (see Fig. 2 for the localities of the lost colonies). A total of 70 colonies were enclosed with wire nets and 45% of the dead colonies were those enclosed with a net. Vast majority of the colonies (74%) were determined in the northern woodland parts and some (26%) in areas to the south of the northern distribution range with typical steppe characteristics. No colony was determined in the southern parts of the region. Most of the localities were represented with only one colony and some with multiple sympatric colonies ranging from 2 to 9 in number. The planimetric distance between sympatric colonies ranged from 22 meters to more or less 1000 meters.

Table 1. IUCN categories. Stenotopic species (ST): a species found in only one habitat type, Almost stenotopic species (AST): species present in only two or three habitat types; Moderate stenotopic (MST): species present in four or five different habitat types, with no discernable preference for any one, Eurytopic species (EU): species found in six or more habitat types.

IUCN category	Assignment requisites
Critical (CR)	Species with few recent observations, that became very rare due to a drastic reduction of their preferred habitat or living in highly threatened habitats, which are stenotopic and were found in less than five UTM 5x5 km^2 and less than 10 records.
Endangered (EN)	Species that became rare because of the extensive deterioration and destruction of their habitat, which are at least moderately stenotopic (ST, AST or MST), which were found in 5-10 UTM 5x5 km^2 and for which we have less than 15 records.
Vulnerable (VU)	Species which became quite uncommon or with a restricted distribution in Thrace Region, which are at least moderately stenotopic (ST, AST or MST) and which were found in 10 to 40 UTM 5x5 km^2
Indeterminate (IN)	Species assumed to be threatened, but for which there is not enough information to decide which of the preceding categories is appropriate.
Not Threatened (NT)	Common and widespread, eurytopic (EU) species that are not currently threatened.

The area of occupancy (AOO) and the extent of occurrence (EOO) were measured as 280 km^2 and 6380 km^2, respectively (Fig. 2). Based on the different habitat types where the colonies were recorded, two separate habitat preference were evident as woodland (mostly open forest areas with sun exposure) and scrubby-heathland making *F. pratensis* in the region an AST species in terms of habitat preference (see Table 1). The evaluation of distribution of the species and the different types of habitats preferred led us to assess *F. pratensis* as Vulnerable (VU) in Thrace Region following the criteria B1b (i, ii, iii, iv) due to the small AOO and EOO.

The threats that *F. pratensis* face in the region were evaluated and they were placed in two categories as human related and natural threats. Human related activities which were observed to have a direct effect on viability of the colonies included increasing rate of industrialization, insecticide usage, urbanization, road construction and increasing number of mining activities mostly taking place in the northern parts of the region where most of *F. pratensis* colonies were recorded. Habitat fragmentation is another human related threat factor posing on the species by dividing the distributional range of the species into several habitat patches, which eventually reduces dispersal rate of the species in the region. Natural threats were listed as deaths of colonies due to natural aging and physical destroying of colonies by boars and foxes. Locals reported that they saw boars and foxes feeding on the brood of *F. pratensis.* All colonies except one in Balaban village were determined to be monodomous meaning that they were most probably governed by one reproductive queen.

The conservation studies such as protection nets, information plates, face to face briefings, leaflets, posters etc. greatly contributed to awareness-raising activities. A number of news appeared in regional and national newspapers which also led to an apparent increase in the popularity of the species in the region. Moreover, the locations of some of the colonies were reported to us by local people who paid special attention to the species after being informed about its presence and importance in the region.

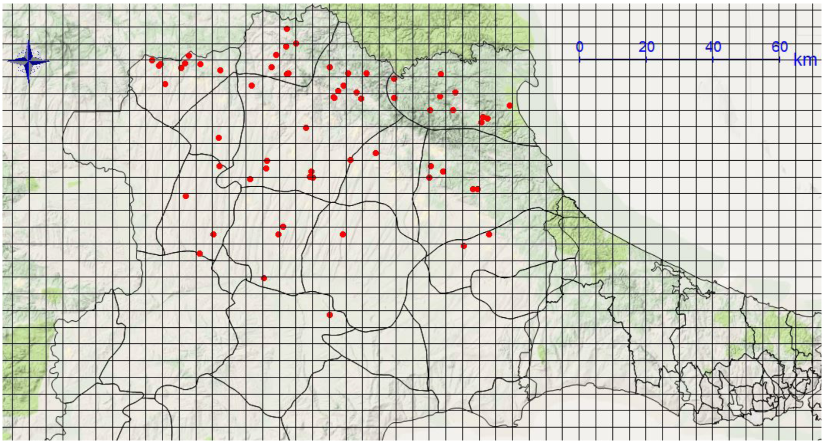

Fig. 1. The map showing distributional range of *F. pratensis* in Thrace Region. All recorded colonies were shown in the map. Localities with one or more colony deaths were shown in Fig. 2. Solid red circles denote the localities where one or more colonies were recorded during the study.

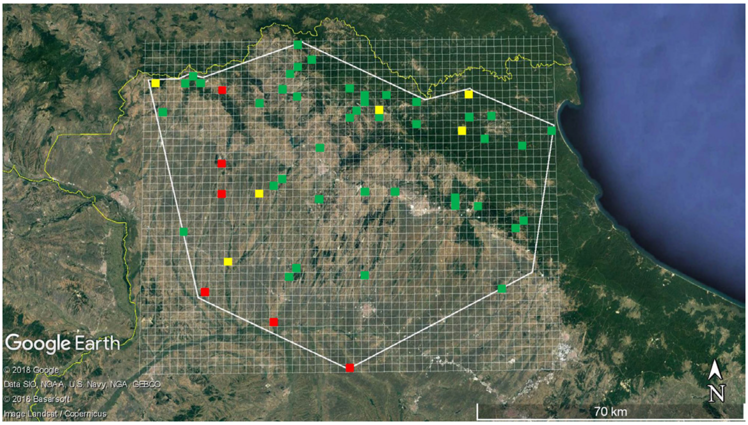

Fig. 2. The map showing EOO and AOO of *F. pratensis* in Turkish Thrace. The white polygon shows the borders of the obtained EOO and colored squares denote grids inside the EOO containing localities with a colony record (AOO). Red squares show the localities where all recorded colonies died, yellow squares show the localities where some of the colonies died and green squares show the localities where no colony loss occurred.

DISCUSSION

A vulnerable species is one which has been categorized by the IUCN as likely to become endangered unless the circumstances that are threatening its survival and reproduction improve (IUCN, 2001). As revealed by current distributional data, *F. pratensis* has a vulnerable status in Turkish Thrace pointing out the importance of planning and implementation of active and urgent conservation strategies for the species in the region. A successful conservation study should be based on a thorough knowledge of the factors that cause the vulnerability of the species.

Formica pratensis has a very restricted area of occupancy (AOO) (280 km^2) in Turkish Thrace within the relatively large extent of occurrence (EOO). In other words, the substantially larger EOO than AOO means that occurrences spread over a large area (Fig. 2). The difference between the two areas of occupancies and the loss of all earlier records of the colonies has indicated a clear tendency of declination of the species in the region. As reported in IUCN (2017), a species with a smaller AOO is likely to have a higher risk of extinction not only because of its smaller population sizes but also because of the threats to its restricted habitat are likely to reduce its habitat more rapidly to an area that cannot support a viable population.

When the present distributional data of *F. pratensis* in Turkish Thrace is considered, the study area seems to potentially support two separate meta-populations, one in the northern woodland parts and the second in the northern parts of the central steppic area. The rarity of the species and the low number of suitable habitats in the latter shows that the central steppe area is the weak point of the dispersal corridor and can explain the non-existence of the species in the southern parts of the region. Moreover, the number of colonies died during the study is higher outside the northern woodland parts showing how the species is vulnerable to extinction here. As far as we know, Turkish Thrace is the southeasternmost border of the range of the species in Europe. As one moves from the core to the periphery of a species' geographical range, populations occupy less favorable habitats and exhibit lower and more variable densities (Channel & Lomolino, 2000). Keeping in mind this conclusion, one may expect a wide range of or a relatively better distribution in the neighboring Bulgaria and Greece, but this is not the case. The mountainous southwestern parts of Bulgaria is located to the north of Turkish Thrace and a few *F. pratensis* colonies were reported in this region (Lapeva-Gjanova et al, 2010) but the picture is clearer for the western parts of the country where more colonies were recorded. In Greece, although the species was reported from only 3 localities in neighboring Greek Thrace, it is with a more northern distribution and is rarely found in other parts of the country (Bračko et al, 2016). Borowiec & Salata (2012) reported distributional range of *F. pratensis* in Greece as Greek Macedonia to the west of Greek Thrace. On the other hand, Borowiec & Salata (2018) recorded the species from Thessaly region of Greece. These data shows that the species in the two neighboring countries is also rare and more detailed studies are urgent.

The evaluation of the data about the possible threats on *F. pratensis* showed that low representation of the species in the study region is an inevitable outcome of activities mainly related with human actions leading to habitat fragmentation and physical disturbances of colonies. It is also clear that the lack of public awareness about the biological and ecological importance of the species also favors disturbance and loss of the colonies. Bution et al (2010) reviewed the main risks that ants have been facing to maintain their communities and he reported that habitat fragmentation by human activities greatly influenced distributional patterns of ants which in turn led to fluctuations in ecosystem dynamics. The researchers also proposed that ants could be used as bioindicator organisms in monitoring of ecosystem dynamics and highlighted the importance of ants in conservation studies. Ants can be very sensitive to habitat transformation and disturbance, and for this reason they have already been extensively used as indicator species (Hoffmann & Andersen, 2003). Studies related with wood ants reported that human agricultural activities, industrialization, recreation and habitat fragmentation are, alone or in combination with each other, the main threats of wood ant colonies (Robinson, 2001; Mabelis, 2007; Bernasconi, Maeder, Cherix, & Pamilo, 2005; Mäki-Petäys, Zakharov, Viljakainen, Corander, & Pamilo, 2005; Dekoninck et al, 2010). For instance, Kiss & Kobori (2010) reported that the number of *F. pratensis* mounds in Hoia forests in Romania was 50 in 2004 and decreased to 8 in 2010 due to human destructions. A similar decrease was observed in Fanatele Clujului where more than 100 mounds decreased to 15 in a relatively short time period of monitoring.

There are a number of factors which affect the habitat or niche requirements of wood ants, including food supply, microclimate, competition from other ant species, type of social organization of the colony and dispersal ability. As in the case of many other ant species, red wood ants rely on honeydew as the primary sugar source (Rosengren & Sundström, 1991). Therefore, availability of aphid bearing trees is one of the important factors in selection of nest location. They also prefer sunny hotspots to ensure temperature conditions for optimal brood development. Canopy close and clearfelling in mature and old growth stands are among the reasons of declination of wood ants in dense forests by reducing sunlight exposure and leading to loss of ants' primary food source and their orientation ability which is based on visual cues (Rosengren & Pamilo, 1978). We recorded almost all nests in open forest areas, meadows or along borders of agricultural fields all which provide optimum sunlight conditions and vegetation with enough aphid source. However, such preference clearly brings the species close to human activities which is the main cause of loss of some of the existing colonies and prevention of dispersal. It is therefore a necessity to take strict measurements for protection and facilitate dispersal and viability of the species in the region. Red wood ants (*Formica* spp.) have been the targets of conservation action in Europe mainly to preserve the essential services they provide to their ecosystem, e.g. heavy predation on a wide range of insects including destructive ones, dispersing seeds of many myrmechorous plants such as *Viola* spp., providing habitat in their nests for a multitude of organisms (e.g. over 30 beetle species), and as an important food source for birds (especially woodpeckers) and other animals (Mabelis, 2007).

The social structure of *F. pratensis* colonies in our study region can be considered as another factor that may have played role in low level of spreading of the species in the region. *Formica pratensis* can form polygyne colonies with several functional queens which allow polydomy (Seifert, 1996), but monogyny is frequent (Rosengren, Sundström, & Fortelius, 1993). We determined that all colonies in the study region were monodomous colonies except one polydomous nest determined in Balaban village with two interconnected colonies. Polydomy allows colonies to create new nests without going through the high-risk bottleneck of single-queen nest foundation (Robinson, 2014). Risk spreading is one of the ways used by polydomous systems for survival. For instance, if local conditions change, the inhabitants of a nest that becomes unsuitable can relocate to other more successful nests (McGlynn, 2012) or ants can isolate pathogens or parasites by cutting off contact with an infected nest (Ugelvig & Cremer, 2012). Although single large monodomous nests promote homeostasis and increase chances of survival, monodomy in our case can be a potential factor that may affect dispersal of colonies negatively in the region. A colony of a species that accepts many queens, like *F. polyctena*, has a lower probability of going extinct than a colony of a species that accepts just one or only a few queens, like *F. rufa* (Mabelis, 1986). Moreover, a species which has many queens per nest can disperse by means of budding, during which workers transport several queens from the mother nest to newly built daughter nests. This is a much safer means of dispersal than trying to colonize an area by means of flying queens (Rosengren & Pamilo, 1983; Rosengren et al, 1993). The presence of multiple nests, i.e. 9 nests in Ahmetler village, in a single locality is interesting since one can ask how these sympatric colonies achieved to disperse while most others in other localities were not successful even though most were very similar in terms of their ecological conditions. Although these individual nests showed no sign of inter-nest traffic among each other proving monodomy, we do not know precisely that they are individually founded colonies.

In conclusion, the available data on distribution of *F. pratensis* in Thrace region points out the importance of protection of the species which can be achieved by conservation and sustainable management of the biotopes in which they occur. *Formica pratensis*, a keystone and vulnerable species, deserves to be a target species to be monitored and conserved. The protection of the species is a challenging task requiring involvement of not only scientists but also local people. The preparation of a national red list of invertebrates, which are paid less attention compared to vertebrates and plants in conservation studies, is urgently needed to reveal a more clear picture of the biodiversity which in turn will lead to more robust and well planned conservation studies for those species needing protection.

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Synergistic Effects of Some Secondary Compounds Combined with Some Heavy Metals on *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) Larvae

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ABSTRACT

In this study, the effects of phenolic compounds such as tannic acid and gallic acid, and heavy metals such as iron, zinc, copper, nickel and cobalt on the last larval stage of the first generation of *H. cunea* were investigated. The larvae were collected from mulberry and hazelnut trees in the district of Çarşamba, Samsun in 2016. 18 foods in total were prepared; two foods by adding 5% concentration of tannic acid and gallic acid to the artificial diet (dry weight), 5 foods by preparing nickel, zinc, copper, cobalt and iron solutions prepared at 8% concentrations, 10 foods by adding 8% concentrations of heavy metal solutions to 5% concentrations of tannic acid and gallic acid separately, and a control diet. Compared to those fed with control food, the consumption amount of the larvae whose diet contained iron, zinc and copper decreased, whereas it increased in the ones fed with nickel and cobalt containing foods. Pupae weight of the larvae fed with tannic acid and metals added(other than nickel) to it has decreased compared to the larvae in control group. While the amount of pupa protein was the lowest in larvae fed with the food in which both gallic acid and zinc used, it was highest in larvae fed with food containing gallic acid. The pupa lipid amount of the larvae fed with foods supplemented with iron, zinc, copper and cobalt were lower than the control group larvae. The longest development time was observed in the larvae in food groups where iron was added to food alone and tannic acid and iron were added together. The results in this study have shown that the addition of secondary compounds and heavy metals together to the artificial food content may increase toxicity of compounds (synergism) or reduce it (antagonism).

Key words: Hyphantria cunea, artificial diet, secondary compound, heavy metals, feeding, synergistic effect.

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INTRODUCTION

Plants produce proteins and secondary compounds that are toxic or repellent for herbivores or specialized morphological structures to protect themselves against herbivore attacks (Usha Rani & Jyothsna, 2010; War, Paulraj, War, & Ignacimuthu, 2011a; b). Through secondary compounds they produce, plants influence host plant preference of insects, thus their survival and reproduction success, which is direct defense against herbivores, or by attracting species that are natural enemies of pests, which is indirect defense mechanism against herbivores (Dudareva, Negre, Nagegowda, & Orlova, 2006; Howe & Jander, 2008; Arimura, Matsui, & Takabayashi, 2009).

Tannins, which are amassed in many plants especially in woody ones (Bernays, Driver, & Bilgener, 1989; Peters & Constabel, 2002) against herbivory, demonstrate their effects by binding many proteins. Their effects on majority of herbivores from insects to mammals have been reported (Bernays et al,1989). Tannins have a variety of biochemical activities ranging from beneficial antioxidants to harmful prooxidants and toxins (Barbehenn & Constabel, 2011). Gallic acid is a phenolic substance with low molecular weight. Gallic acid is synthesized by shikimic acid pathway directly, not by phenylalanine pathway. It is known that this phytochemical has characteristics of being antioxidant, antibacterial, anti-inflammatory, anti-mutagenic, and chemical preservative (Birošová, Mikulásová, & Vaverková, 2005; Kim et al, 2006; Kang, Oh, Kang, Hong, & Choi, 2008; Giftson, Jayanthi, & Nalini, 2009).

All metals, in spite of being naturally present on the earth's crust and many of them being necessary for cells (i.e., copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn)), are toxic at high concentrations for living organisms (Yang, Feng, He, & Stoffella, 2005). Elemental defense hypothesis asserts that excessive metal accumulation at least affects some herbivores/pathogens in habitats negatively, and thus, acts as a defense mechanism evolved in hyperaccumulators against some natural enemies such as herbivores and pathogens. There are two different metabolic pathways which the defenses are successful. One of them is that the excessive toxicity of plant tissues containing metals (Boyd & Martens, 1994; Martens & Boyd, 1994), that is, consuming plant material causes death. Another one is the repellent way, which means the less consumption of plant tissue that has high levels of metals (Boyd & Martens, 1998). The first difference between elemental defense and organic plant defenses is that elemental defenses are acquired from soil, but not synthesized by plants. The second difference is elements cannot be degraded chemically; therefore, herbivore defense mechanism is blocked. The third one is that when metabolism considered, organic plant mechanisms are more costly than elemental defenses for plants (Boyd, 1998). It was shown in the previous studies that thrips were deterred by the cadmium and snails, locusts and caterpillars were deterred by the zinc in *Noccaea caerulescens* (formely *Thlaspi caerulescens*) (Pollard & Baker, 1997; Behmer et al, 2005; Jiang, Ma, Zhao, & McGrath, 2005), aphids by the selenium in *Brassica juncea* (Hanson, Lindblom, Loeffler, & Pilon-Smits, 2004), locusts by the arsenic in *Pteris vittata* (Rathinasabapathi et al, 2007), and snails by the nickel in *Streptanthus polygaloides* (Boyd & Jhee, 2005).

The fall webworm (*Hyphantria cunea* Drury), a native North American moth species, has become an invasive pest in Europe and Asia (Tadić, 1963; Yang, Wang, Wei, Qu, & Qiao, 2008).The fall webworm (*Hyphantria cunea* Drury) is a moth species native to North America but an invasive pest in Europe and Asia (Tadić, 1963; Yang et al, 2008). *H. cunea* causes serious damage to agricultural and forest lands. The plants that cause damage include fruit trees with economic importance such as hazelnuts, plums and apples.

In this study, the purpose was to show the effect of some heavy metals combined with secondary compounds on the last instar of first generation of *H. cunea*, which is a polyphagous species, exotic to Turkey, and causes severe damages to agriculture and forest areas in the north of Turkey.

MATERIALS AND METHODS

Obtaining the larvae

H. cunea larvae were collected during the field studies conducted at different times in the borders of Çarşamba district of the City of Samsun in Turkey in 2016.

Contents of artificial diet

To feed the larvae, the artificial diet developed by Yamamoto (1969) was used as the control diet. The content of Yamamoto's artificial diet is wheat germ, casein as the protein, saccharose as the carbohydrate, torula yeast, vitamin mixture, salt mixture, cholesterol, sorbic acid, methyl paraben, linseed oil, agar, and water. Diets were prepared by adding the secondary compounds such as tannic acid (TA) and gallic acid (GA), and heavy metals such as nickel (Ni), zinc (Zn), copper (Cu), cobalt (Co), and iron (Fe) to the diet at specific concentration suitable for the purpose of this research. Tannic acid and gallic acid were added into artificial diet at 5% concentration of dry weight (1.036 g). We decided on these concentrations of secondary compounds by reference to our previous work (Yanar, Topkara, & Gömeç, 2016). 5 diets were provided by preparing nickel, zinc, copper, cobalt, and iron solutions. Stock solutions were obtained by weighing 1.576 mg of heavy metals. 10 diets were prepared by adding heavy metals solutions into tannic acid and gallic acid's 5% concentrations. In total 18 diets, one of which is control diet, were prepared (Table 1).

Feeding experiments

The last instar larvae were put in plastic cups one by one, 30 larvae in each cup, then feeding experiment in which the larvae were fed every other day was started. In these feeding experiments, new diet was weighed on 0.001 precision scale and given to the larvae, and the remaining diets were dried in incubator, their dry weights were measured. In addition, weight changes of the larvae were noted every other day, and this process was continued until the larvae became pupae.

Table 1. Diet types and diet contents.

Diet Types	Diet Contents		Diet Types	Diet Contents
A	Control Diet (CD)		K	CD+ 5% TA+ Zn solution
B	CD+ 5% TA		L	CD+ 5% TA+ Cu solution
C	CD+ 5% GA		M	CD+ 5% TA+ Ni solution
D	CD+ Fe solution		N	CD+ 5% TA+ Co solution
E	CD+ Zn solution		O	CD+ 5% GA+ Fe solution
F	CD+ Cu solution		P	CD+ 5% GA+ Zn solution
G	CD+ Ni solution		R	CD+ 5% GA+ Cu solution
H	CD+ Co solution		S	CD+ 5% GA+ Ni solution
J	CD+ 5% TA+ Fe solution		T	CD+ 5% GA+ Co solution

Pupal lipid and protein analysis

The pupae at the end of the feeding experiments were placed in incubator at 45^{o}C to dry. Then, in order to determine the lipid amount, they were kept in chloroform for 24 hours and this process was repeated three times. They were put into the incubator and redried. When this process was finished, the weights of the pupae without lipid were calculated (Simpson, 1983). The determination of nitrogen in pupae was carried out with semi-micro Kjeldahl method and Kjeltec Auto 1030 analyzer (Tecator, Sweden) (Kacar & İnal, 2008). The nitrogen amounts calculated at the end of this process were multiplied by 6.25 constant and the percentage of the protein amounts was found (Monk, 1987). Each treatment was repeated three times.

Statistical analysis

In the study, whether total diet consumption amounts of *H. cunea* larvae, the pupal weights, lipid and protein amounts of the pupae and their development times are statistically different from each other or not were determined by ANOVA with Dunnett's post-hoc test. SPSS Statistics 21 was used for these tests.

RESULTS AND DISCUSSION

Total consumption amount

It was found that, when compared with the control group, adding tannic acid into the diet reduced the total diet consumption amount, whereas gallic acid increased this amount. Although tannic acid and gallic acid are phenolic compounds, their effects are different. This result is similar to the affirmation that the ecological activities of phenolic compounds depend on their chemical structures (Barbehenn & Constabel, 2011). Tannins are astringent bitter polyphenols and therefore act as feeding repellents against many pests (War et al, 2012). The reason why total diet consumption amount declines might be due to direct repellent feature of tannic acid (Simpson & Raubenheimer,

2001) (Table 2). In a study (Sorvari, Rantala, Rantala, Hakkarainen, & Eeva, 2007), conducted between the local and translocated colonies of *Formica aquilonia* living in a soil contaminated with heavy metal, the former showed higher heavy metal levels than the latter, but the encapsulation response was similar between the two groups. A study with *Epirrita autumnata* (van Ooik, Pausio, & Rantala, 2008) indicated that moderate amount of Ni and Cu in the diet of moth larvae increased their encapsulation rate, but a large amount of Cu caused the immune function to decrease. Dubovskiy, Grizanova, Ershova, Rantala, & Glupov (2011) showed *Galleria mellonella* larvae fed a low dose of nickel had significantly higher GST, phenoloxidase activity and encapsulation responses than controls fed on a nickel-free diet. When iron was added to the diets of *Orchesella cincta*, it caused a significant reduction in consumption, especially at high concentrations (Nottrot, Joosse, & van Straalen, 1987). This shows that heavy metals have different effects. In this study, iron, zinc, and copper added into diet have negative effects on consumption.

Many insects can usually tolerate the great amounts of tannins (Barbehenn & Constabel, 2011). The consumption amount of the larvae fed on the diet containing tannic acid (B diet) was lower than that of control group. In the groups where tannic acid and metals were put together, the consumption amount was seen to increase compared to the groups in which only metals used. As a result, while tannic acid caused a decrease in the consumption amount of the larvae (Hemming & Lindroth, 1995; Becker & Makkar, 1999; Hemming & Lindroth, 2000; Simpson & Raubenheimer, 2001), it increased the consumption amount when used together with the metals. Gallic acid scavenges free radicals due to its anti-inflammatory effect (Kim et al, 2006). Thus, it reduces the negative effect of some metals. The consumption amount of the larvae fed on C diet containing gallic acid was higher than that of control group. When this secondary compound used together with metals, it caused an increase in the consumption amount of all the diet groups (except S). As a result of this experiment it was determined that consumption amounts of all the diet groups were statistically different than that of control group.

Pupal weight

While adding tannic acid into the diet decreased the weights of the pupae compared to the control group, gallic acid addition increased the weights. In a study of Simpson & Raubenheimer (2001) on *Locusta migratoria*, they found that in the pupae whose grasshoppers had been fed on diet containing tannic acid, increase in the amount of tannic acid caused a decrease in the pupal weight. Yanar et al (2016) found that the pupal weights of *H. cunea* larvae fed on diet containing tannic acid were less than the larvae which became pupae by feeding on other diet groups. Reduction in pupal weight can affect the fecundity of their imagoes (Honek, 1993). Being heavier due to feeding with the diet containing gallic acid can positively affect the fecundity.

In a study conducted by van Ooik, Rantala, & Saloniemi (2007), when the moths of *E. autumnata* were fed on metal (sulphur, copper, nickel, cadmium, lead, iron and zinc) contaminated leaves, their pupal weights went down compared with the ones that fed on control leaves. In an study with *Apis mellifera* (Di, Hladun, Zhang, Liu,

& Trumble, 2016), it was reported that pupal weights of larvae fed in artificial diets containing different concentrations of copper decreased compared to the control group. In our study, when iron, zinc, and copper added to the diet, pupal weights were recorded to be decreased. Pupal weight of the larvae fed on B diet containing tannic acid was lower than that of control group. Pupal weights of the groups whose diet contained tannic acid and iron, copper, and nickel together were higher than the groups where only these metals were used. It can be said that tannic acid increased the effects of these metals by dominating them. On the other hand, when tannic acid was used together with zinc and cobalt, pupal weights were lower than the groups where only these metals were used. When gallic was used together with iron and copper, it caused an increase in the pupal weight, whereas combining it with zinc and nickel caused a decrease in the weight. It did not change the effect which cobalt would cause itself. In accordance with these results, we can see three effects (positive, negative, neutral) of gallic acid on pupal weight. The group containing cobalt (H group) and the group in which gallic acid and cobalt used together (T group) were statistically same as control group.

Pupal protein amount

It was found that when tannic acid was added to the diet, this reduced the pupal protein amount compared to control group, whereas adding gallic acid into the diet increased this amount. Simpson & Raubenheimer (2001) found that *L. migratoria* feeding on diet containing tannic acid had higher pupal protein amount when compared with the ones that were fed with the diet which did not contain this compound, thus this finding is opposite of our data. Stored proteins that are transferred from larval to imago stage can have important function in herbivore insects especially in the imagoes due to limited nitrogen consumption (Hahn, 2005). Therefore, gallic acid in diets can be advantageous for *H. cunea* to store protein.

It was found that when the heavy metals, except cobalt, were added into diet, pupal protein amounts lessened compared to the ones in the control group. Even though Ni, K, Ca, Zn, and Fe are important cofactors of functional proteins (Dadd, 1985), in this study, pupal protein amounts of groups containing iron, zinc, and nickel (D, E, and G) were less than that of control group.

Pupal protein amounts in all the groups whose diet contained tannic acid and metals, except M group, were lower than the control. Negative effects of tannins in insects may depend on high tannin concentration (Aerts, Barry, & McNabb, 1999). Protein amounts of the groups whose diet contained both gallic acid and the metals were lower than that of control group, except for T diet group. All the groups, except G, H, and T diet groups, were statistically different than control group.

Pupal lipid amount

Storage function of insect’s fat body is essential for the survival of holometabolous insects. Throughout the larval feeding stages, energy reserves are accumulated to be used along metamorphosis as well as for new imagoes. Insects need to accumulate

Table 2. Total diet consumption, pupal weight, pupal protein and lipid amount and development time of *H. cunea* in the feeding experiment.

	Diet types	**Total diet consumption (mg)**	**Pupal weight (mg)**	**Pupal protein amount (mg)**	**Pupal lipid amount (mg)**	**Development time (day)**
Average ± standard error	A	365.6±0.4	58.0±1.0	27.1±0.4	17.3±0.2	3.0±0.0
	B	310.8±0.4	40.9±0.6	21.4±0.3	12.4±0.2	3.4±0.1
	C	420.5±0.5	63.7±1.1	34.8±0.6	18.1±0.3	3.0±0.0
	D	322.7±0.4	31.8±0.2	17.2±0.1	7.9±0.5	3.8±0.0
	E	333.4±0.4	43.7±0.9	22.5±0.4	11.7±0.2	3.0±0.0
	F	278.3±0.3	29.3±0.2	16.9±0.1	7.7±0.1	3.2±0.1
	G	375.7±0.5	63.2±1.0	25.8±0.4	17.5±0.2	2.8±0.1
	H	369.0±0.5	60.9±1.0	27.6±0.4	15.7±0.2	3.3±0.1
	J	343.8±0.4	37.5±0.9	19.7±0.4	8.3±0.2	3.8±0.0
	K	346.9±0.4	40.9±0.5	17.9±0.2	17.1±0.2	3.2±0.1
	L	340.3±0.4	44.2±0.9	23.1±0.4	11.6±0.2	3.1±0.0
	M	381.0±0.5	65.0±1.0	30.0±0.4	20.3±0.2	3.2±0.1
	N	388.1±0.5	42.5±0.9	24.1±0.5	9.4±0.2	3.3±0.1
	O	373.8±0.5	39.5±0.9	18.3±0.4	17.9±0.2	3.2±0.1
	P	383.4±0.4	34.0±0.9	15.5±0.4	13.7±0.2	3.2±0.1
	R	356.5±0.5	42.6±0.9	20.0±0.4	16.6±0.2	2.8±0.0
	S	346.7±0.4	41.2±0.9	18.9±0.4	16.4±0.2	3.0±0.0
	T	389.6±0.5	60.2±1.0	27.6±0.4	21.7±0.2	2.9±0.0
ANOVA	df	269	269	269	269	269
	F	4365.5	169.6	142.5	279.4	8.5
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Dunnet test	B < 0.001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 G < 0.001 H < 0.001 J < 0.001 K < 0.001 L < 0.001 M < 0.001 N < 0.001 O < 0.001 P < 0.001 R < 0.001 S < 0.001 T < 0.001	B < 0.001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 G < 0.001 J < 0.001 K < 0.001 L < 0.001 M < 0.001 N < 0.001 O < 0.001 P < 0.001 R < 0.001 S < 0.001	B < 0.001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 J < 0.001 K < 0.001 L < 0.001 M < 0.001 N < 0.001 O < 0.001 P < 0.001 R < 0.001 S < 0.001	B < 0.001 D < 0.001 E < 0.001 F < 0.001 H < 0.001 J < 0.001 L < 0.001 M < 0.001 N < 0.001 P < 0.001 T < 0.001	D < 0.001 J < 0.001

*Statistically significant means according to Dunnet's Multiple Range Test ($P<0.05$)

at least a small portion of stored nutrients in order to survive during metamorphosis (Mirth & Riddiford, 2007). Lipids that are used at imago stage are met from the lipids stored along growth phases before reaching imago (Giron & Casas, 2003). While having lower lipid amount in pupae of the larvae due to feeding on the diet (B) containing tannic acid can be disadvantageous for species, having higher lipid amount as a result of feeding on diet containing gallic acid (C) can be advantageous.

Adding iron, zinc, copper, and cobalt into diet caused a decline in pupal lipid amount. The level of nutrient reserves accumulated in insect fat body regulates some important points in the life of insects such as insect growth rate, timing of metamorphosis, and egg growth (Mirth & Riddiford, 2007). In the diet groups where metals and secondary compounds are used together, except N and S, increase in the lipid amount due to secondary compounds is important with respect to this matter. Lipid amounts of the groups, except C, G, K, O, R, and S diets, were found to be statistically different than that of control group.

Development time

Behavior, growth, and development of some insects are affected by plant flavonoids (Simmonds, 2001, 2003; Simmonds & Stevenson, 2001). It was found that in the group fed with tannic acid containing diet (B), development time was longer than that of control group, whereas this period did not change in the group whose diet contained gallic acid (C). Sublethal doses of metals cause the growth rate of herbivores to decrease (Williams, 1999), thus prolonging the development time. It was seen that when iron, copper, and cobalt were added into the diet, it took longer for the larvae to develop compared to the ones in the control group. Boyd & Moar (1999) found that nickel concentrations in the leaves up to 93 мg/g prolonged reaching pupae stage in *Spodoptera exigua* larvae. In our study, development time of the group whose diet contained nickel was shorter than that of control group.

A study carried out by Jhee, Boyd, & Eubanks (2006) showed that when the amount of tannic acid and nickel added to artificial diet increased, the survival percentage and the percentage of becoming pupae in *Plutella xylostella* larvae went down. In our study, when tannic acid and nickel used together, development time was found to be prolonger compared to the group in whose diet only nickel is used. This finding shows that the synergistic effect is different. In the groups where iron and zinc are used together with gallic acid development time was slower than that of control group. Extension in development time causes an increase in the possibility of encountering with natural enemies while feeding or looking for food (Bernays, 1997), or with the extension in growth and feeding the risk of preying/parasitism increases (Moran & Hamilton, 1980; Loader & Damman, 1991; Benrey & Denno, 1997), and these are true for this species, as for many others. Of all the diet groups, D and J groups were found to be statistically different from the control group.

Since plants provide the basis of many terrestrial food chains, secondary compounds and metals in plants play important roles for the livings at higher levels of food chain. These compounds can be repellent or toxic against the herbivores.

When secondary compounds and heavy metals are used together, they show different effects on larvae. When these compounds are combined, these combinations can cause a more complex influence on an herbivore. Since compounds can interact and affect each other, their combined effect becomes different than their individual effect. Findings in our study prove that the interaction can increase the toxicity of such combined compounds (synergism), or decrease it (antagonism).

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An Annotated Catalogue of the Iranian Attelabidae (Coleoptera: Curculionoidea)

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ABSTRACT

An overview of Iranian Attelabidae (Coleoptera: Curculionoidea) is given. In total, 30 species within 12 genera are listed as belonging to the fauna of Iran (three species of Attelabinae, and 27 species of Rhynchitinae). Two Rhynchitinae species, *Temnocerus coeruleus* (Fabricius, 1798) and *Mecorhis* (*Pseudomechoris*) *aethiops* Bach, 1854 are new records for the fauna of Iran.

Key words: Curculionoidea, Attelabidae, checklist, host plants, distribution, Palaearctic Region, Iran.

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INTRODUCTION

The family Attelabidae Billberg, 1820, comprises a group of curculionoid beetles of about 2500 described species in 150 genera worldwide (Skuhrovec & Kresl, 2014). Some authors consider Rhynchitidae Gistel, 1856 as a distinct family (Legalov, 2003, 2007, 2018; Alonso-Zarazaga, 2011) whereas others treat it as belonging to Attelabidae as subfamily (Kuschel, 1995; Oberprieler, Marvaldi, & Anderson, 2007; Bouchard, et al., 2011; Skuhrovec & Kresl 2014; Alonso-Zarazaga et al, 2017). We follow here the taxonomic arrangement by Alonso-Zarazaga et al (2017).

Attelabidae, commonly named leaf-rolling weevils, morphologically differ from other Curculionoidea mainly in their straight antennae with relatively short scape, and ventrites with similar form and structure in combination with non-geniculate antennae (Legalov, 2004, 2018; Urban, 2012a; Riedel, 2014). They are found in all zoogeographic regions, excluding New Zealand and Pacific islands, with the notable exception of a single species occurring in New Caledonia (Riedel, 2014). In addition to those making various leaf rolls for their larvae, other species damage fruits or vegetative parts of plants inside which their larvae will develop (Legalov, 2007). Females of leaf-rollers show sophisticated behaviour in caring for offsprings. Their are laying eggs in self-made “nests” inside rolled up leaves in the form of compact, thimble-shaped leaf rolls, that often remain hanging on the tree by a narrow strip of leaf tissue, rolls where larvae develop. Other members of this weevil family deposit eggs in fruit and buds. In general, these beetles are too scarce to cause serious damage to trees (Craighead, 1950; Urban, 2012b), but some are important pests in agriculture, fruit growing and viticulture. Also, species developing in young shoots or leaf rolls can be occasionally harmful (Urban, 2012a, b; Riedel 2014).

Adults of the subfamily Attelabinae are characterized by claws connate, mandibles without teeth on external edge, tibiae serrate on inner margin and with obvious mucro on outer apex, ventrites 1-4 fused, rostrum short, body usually glabrous or with appressed setae (Legalov, 2007, 2018). Almost all Attelabinae roll leaves in order to provide protection and food for their larvae (Zuppa, Osella, & Biondi, 1994; Legalov, 2005).

Adults of the large subfamily Rhynchitinae are characterized by rostrum long, mandibles with teeth at external edge and absence of labrum, claws free from base, tibiae not serrate on inner margin and lacking obvious mucro on outer apex, distinct elytral epipleura, only ventrites 1 and 2 fused, body usually with erect and/or adpressed setae (Bright, 1993; Legalov, 2007, 2018; Riedel, Santos Rolo, Cecilia, & van de Camp, 2012). These beetles lay their eggs in young buds, fruits, or seeds of trees which are then destroyed by the larvae. Some species feed on blossoms or foliage, and some others skillfully cut leaves to build an apparatus called “cradle” for breeding (Sawada, 1993). A peculiar behaviour is shown by a few species (e.g., *Lasiorhynchites sericeus* (Herbst, 1797), members of *Pterocolus* Say) which enter the leaf rolls of *Attelabus* spp., destroying the *Attelabus* egg, and then oviposit his own egg inside the roll (Bright, 1993).

Iran forms a large part of the Iranian plateau and covers an area of 1,623,779 km². Climatologically, Iran is a predominantly arid and semi-arid country, but the northern slopes of the Alburz ranges and the Caspian lowland receive from 800 to 2000 mm annual rainfall, making them the most humid parts of the country. The Dasht-e Kavir and Dasht-e Lut deserts are the driest areas with an annual precipitation of less than 150 mm. The highlands receive from 250 to 800 mm (Zehzad, Kiabi, & Madjnoonian, 2002).

The aim of this work is to update the catalogue of Iranian Attelabidae which was published by Legalov, Ghahari, Arzanov, Yu (2010).

MATERIAL AND METHODS

The published data on the distribution of the family Attelabidae in Iran are summarized by province. Subfamilies, tribes, genera and species are listed alphabetically. The following data are included in the catalogue for each species: valid name, published Iranian records by province in alphabetical order with relevant references in chronological order, precise data of newly studied materials, general distribution by country at a world scale, comments when appropriate, published host plants in Iran when available. Taxonomy and nomenclature are according to Alonso-Zarazaga et al (2017), distribution data were taken from Alonso-Zarazaga (2011) and Alonso-Zarazaga et al (2017). When accurate data about local distribution in Iran are lacking in a quoted reference, the mere mention “Iran” is used. The provinces of Iran are shown in Fig. 1.

Fig. 1. Map of Iran with provincial boundaries.

RESULTS

Totally 30 species of Attelabidae within two subfamilies, Attelabinae (3 species) and Rhynchitinae (27 species), comprising 12 genera *Aletinus* (2 species), *Attelabus* (3), *Byctiscus* (2), *Deporaus* (2), *Eurostauletes* (1), *Involvulus* (4), *Lasiorhynchites* (1), *Mecorhis* (2), *Neocoenorrhinus* (3), *Rhynchites* (7), *Tatianaerhynchites* (1) and *Temnocerus* (2) are listed as the fauna of Iran. *Temnocerus coeruleus* (Fabricius, 1798) and *Mecorhis* (*Pseudomechoris*) *aethiops* Bach, 1854 (both Rhynchitinae) are new records for Iran. The list of species is given below alphabetically with distribution data and host plants in Iran.

Family Attelabidae Billberg, 1820

Subfamily Attelabinae Billberg, 1820

Tribe Attelabini Billberg, 1820

Genus *Attelabus* Linnaeus, 1758

Attelabus chalybaeus K. Daniel & J. Daniel, 1898

Distribution in Iran: East Azarbaijan (Radjabi, 1991; Modarres Awal, 1997, 2012), Mazandaran (Hoffmann, 1968, as *Attelabus cyanellus* Voss, 1925; Legalov et al, 2010), Northern provinces (Farahbakhsh, 1961; Modarres Awal, 1997).

General distribution: Azerbaijan, Iran, Turkmenistan.

Comments: It is clear that the indication by Hoffmann (1968) of *A. cyanellus* Voss, 1925 from Iran (Mazandaran province) was due to a misidentification with the similar *A. chalybaeus*. In both revisions by Legalov (2003, 2007) and by Alonso-Zarazaga et al (2017), *A. cyanellus* is reported only from the East of Palaearctic (China, Mongolia, East Siberia, Far East of Russia), whereas only *A. chalybaeus* is recorded from Mazandaran (Legalov et al, 2010).

Host plants in Iran: *Crataegus aronia* (L.) DC. (Rosaceae) (Farahbakhsh 1961; Modarres Awal, 1997, 2012), *Malus* (Rosaceae) (Radjabi, 1991; Modarres Awal, 1997, 2012); occasionally on *Quercus* spp. (Fagaceae) (Hoffmann, 1968, as *A. chalybaeus*).

Attelabus nitens (Scopoli, 1763)

Distribution in Iran: East Azarbaijan (Borumand, 1998; Sadaghian, Nikdel, & Dordaei, 2000; Kamangar & Abaii, 2002; Nikdel, Sadaghian, & Dordaei, 2002; Legalov et al, 2010; Modarres Awal, 2012), Mazandaran (Hoffmann, 1968), Northern and northwestern provinces (Farahbakhsh 1961; Modarres Awal, 1997, 2012 both as *Attelabus curculionoides* (Linnaeus, 1767)).

General distribution: Albania, Armenia, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Israel, Italy, Latvia, Lithuania, Luxembourg, Macedonia, Moldavia, Montenegro, the Netherlands, Poland, Portugal, Romania, European part of Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Turkey, Turkmenistan, Ukraine.

Host plants in Iran: *Acer* sp. (Sapindaceae), *Alnus* sp. (Betulaceae), *Castanea sativa* Mill. (Fagaceae), *Salix* sp. (Salicaceae) (Farahbakhsh, 1961; Modarres Awal 1997, 2012), *Quercus* spp. (Fagaceae) (Farahbakhsh 1961; Hoffmann 1968; Modarres Awal 1997, 2012; Nikdel et al, 2002).

***Attelabus sulcifrons* (Argod-Vallon, 1895)**

Distribution in Iran: West Azarbaijan (Samin, Háva, & Kubisz, 2016).

General distribution: Armenia, Bulgaria, Georgia, Greece, Macedonia, Syria, Turkey (Alonso-Zarazaga et al, 2017), Iran (Samin et al, 2016).

Subfamily Rhynchitinae Gistel, 1848

Tribe Auletini Desbrochers des Loges, 1908

Genus *Aletinus* Desbrochers des Loges, 1908

Subgenus *Heterauletes* Voss, 1933

***Aletinus* (*Heterauletes*) *akinini* (Faust, 1885)**

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

General distribution: Afghanistan, China, Iran, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan.

***Aletinus* (*Heterauletes*) *constrictus* (Reitter, 1891)**

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

General distribution: Armenia, Azerbaijan, Georgia, Iran, Turkey, Turkmenistan, Uzbekistan.

Genus *Eurostauletes* Voss, 1933

***Eurostauletes procerus* (Reitter, 1901)**

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

General distribution: Afghanistan, Iran, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan.

Tribe Byctiscini Voss, 1923

Genus *Byctiscus* Thomson, 1859

***Byctiscus betulae* (Linnaeus, 1758)**

Distribution in Iran: East Azarbaijan (Radjabi, 1991; Modarres Awal 1997, 2012), Markazi, Tehran, Zanjan (Modarres Awal, 1997, 2012), Northern provinces (Farahbakhsh 1961; Modarres Awal, 1997, 2012).

General distribution: Armenia, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Israel, Italy, Kazakhstan, Korea, Latvia, Lithuania, Luxembourg, Macedonia, Moldavia, Montenegro, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Turkey, Turkmenistan, Ukraine.

Host plants in Iran: *Cydonia*, *Malus domestica* (Borkh.), *Pirus*, *Prunus*, *Rosa*, *Sorbus* (Rosaceae) (Radjabi, 1991; Modarres Awal, 1997, 2012), *Betula* (Betulaceae), *Carpinus* (Betulaceae), *Castanea* (Fagaceae), *Fagus* (Fagaceae), *Populus* and *Salix* (Salicaceae), *Tilia* (Tiliaceae), *Ulmus glabra* Huds. (Ulmaceae), *Vitis* (Vitaceae) (Modarres Awal 1997, 2012).

***Byctiscus populi* (Linnaeus, 1758)**

Distribution in Iran: Iran (no locality cited) (Legalov, 2007; Legalov et al, 2010; Alonso-Zarazaga, 2011; Alonso-Zarazaga et al, 2017).

General distribution: Austria, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Italy, Japan, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Macedonia, Moldavia, Mongolia, the Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tajikistan, Turkey, Ukraine, Uzbekistan.

Tribe Deporaini Voss, 1929

Genus *Deporaus* Samuelle, 1819

Subgenus *Deporaus* Samouelle, 1819

***Deporaus* (*Deporaus*) *betulae* (Linnaeus, 1758)**

Distribution in Iran: Mazandaran (Sakenin et al, 2018).

General distribution: Algeria, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Ireland, Italy, Japan, Kazakhstan, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Moldavia, Mongolia, the Netherlands, North Korea, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine.

***Deporaus* (*Deporaus*) *podager* Desbrochers des Loges, 1889**

Distribution in Iran: Iran (no locality cited) (Legalov 2007; Legalov et al, 2010; Alonso-Zarazaga 2011).

General distribution: Iran, Turkey, Syria.

Tribe Rhynchitini Gistel, 1848

Genus *Involvulus* Schrank, 1798

Subgenus *Involvulus* Schrank, 1798

Involvulus (_Involvulus_) _cupreus_ (Linnaeus, 1758)

Distribution in Iran: Tehran and probably other Northern provinces (Farahbakhsh 1961, Radjabi 1991; Modarres Awal 1997, 2012 as *Rhynchites cupreus*), Iran (no locality cited) (Legalov, 2007; Legalov et al, 2010).

General distribution: Algeria, Austria, Belgium, Bulgaria, Belarus, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Hungary, Iran, Italy, Japan, Kazakhstan, Latvia, Lithuania, Luxembourg, Macedonia, Moldavia, Mongolia, the Netherlands, North Korea, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, South Korea, Spain, Sweden, Switzerland, Turkey, Ukraine.

Host plants in Iran: Cultivated Rosaceae of the genera *Malus* and *Prunus* (Farahbakhsh, 1961; Modarres Awal, 1997, 2012).

Subgenus *Teretriorhynchites* Voss, 1938

Involvulus (_Teretriorhynchites_) _icosandriae icosandriae_ (Scopoli, 1763)

Distribution in Iran: Mazandaran (Hoffmann, 1968 as *Rhynchites coeruleus* (DeGeer, 1775)), Iran (no locality cited) (Erol, 1994; Legalov et al, 2010 as *I.* (*Teretriorhynchites*) *coeruleus coeruleus*).

General distribution: Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, Estonia, France, Georgia, Germany, Great Britain, Hungary, Iran, Italy, Kazakhstan, Latvia, Lithuania, Luxembourg, Moldavia, the Netherlands, Poland, Romania, European Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine.

Involvulus (_Teretriorhynchites_) _icosandriae iranensis_ Legalov, 2004

Distribution in Iran: Mazandaran (Legalov 2004, Legalov et al, 2010 as *Teretriorhynchites* (*Teretriorhynchites*) *caeruleus iranensis*), Iran (no locality cited) (Alonso-Zarazaga 2011 as *I.* (*Teretriorhynchites*) *coeruleus iranensis*).

General distribution: Iran.

Involvulus (_Teretriorhynchites_) _pubescens_ (Fabricius, 1775)

Distribution in Iran: Iran (no locality cited) (Modarres Awal 1997, 2012 as *Rhynchites pubescens*; Legalov, 2007; Legalov et al, 2010 as *Teretriorhynchites* (*Aphlorhynchites*) *pubescens*).

New material examined: East Azarbaijan province, Sarab, 1800 m, 37°56’N 47°23’E, 3 exx, leg. Pad. Mal., 25.05.1999 (Biondi personal collection).

General distribution: Armenia, Austria, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, France, Georgia, Germany, Hungary, Iran, Italy, Kazakhstan, Kyrgyzstan, Latvia, Moldavia, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Switzerland, Turkey, Ukraine.

Genus *Lasiorhynchites* Jekel, 1860

Subgenus *Lasiorhynchites* Jekel, 1860

***Lasiorhynchites* (*Lasiorhynchites*) *cavifrons* (Gyllenhal, 1833)**

Distribution in Iran: Guilan (Sakenin et al, 2018).

General distribution: Albania, Algeria, Armenia, Austria, Azerbaijan, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Italy, Luxembourg, Macedonia, Moldavia, the Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, European Russia, Sweden, Switzerland, Turkey, Ukraine.

Host plants in Iran: *Quercus petraea* (Matt.) (Fagaceae) (Sakenin et al, 2018).

Genus *Mecorhis* Billberg, 1820

Subgenus *Mecorhis* Billberg, l820

***Mecorhis* (*Mecorhis*) *ungarica* (Herbst, 1783)**

Distribution in Iran: Iran (no locality cited) (Farahbakhsh, 1961 as *Rhynchites hungaricus*; Erol, 1994 as *Involvulus hungaricus*); Modarres Awal, 1997, 2012 as *Rhynchites* (*Homalorhychites*) *hungaricus*; Legalov et al, 2010).

General distribution: Albania, Armenia, Austria, Azerbaijan, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Georgia, Germany, Greece, Hungary, Iran, Iraq, Israel, Italy, Kazakhstan, Macedonia, Moldavia, Poland, Russia (South European Territory, West Siberia), Slovakia, Slovenia, Syria, Turkey, Ukraine.

Host plants in Iran: *Rosa canina* L. (Rosaceae) (Farahbakhsh 1961; Modarres Awal 1997, 2012).

Subgenus *Pseudomechoris* Legalov, 2003

***Mecorhis* (*Pseudomechoris*) *aethiops* Bach, 1854**

Material examined: Lorestan province, Shool-Abad, 33°18′N 49°19′E, 1 ex, leg., H. Ghahari, det. E. Colonelli, August 2009. New record for Iran.

General distribution: Albania, Armenia, Austria, Azerbaijan, Bosnia Herzegovina, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Iran, Italy, Macedonia, Moldavia, Poland, Romania, Russia (Central European Territory, South European Territory), Slovakia, Slovenia, Switzerland, Turkey, Ukraine.

Genus *Neocoenorrhinus* Voss, 1952

Subgenus *Neocoenorhinidius* Legalov, 2003

***Neocoenorrhinus* (*Neocoenirhinidius*) *pauxillus* (Germar, 1823)**

Distribution in Iran: Mazandaran (Hoffmann 1968 as *Rhynchites pauxillus*), Razavi Khorasan (Legalov et al, 2010), Iran (no locality cited) (Winkler 1930; Voss 1933;

Ter-Minassian, 1950 all as *Coenorrhinus* (*Coenorrhinus*) *pauxillus*; Modarres Awal 1997, 2012 as *Rhynchites pauxillus*).

General distribution: Armenia, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Italy, Kazakhstan, Latvia, Liechtenstein, Lithuania, Luxembourg, Moldavia, Montenegro, the Netherlands, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Turkmenistan, Ukraine, Oriental region.

Host plants in Iran: Several Rosaceae of the genera *Malus*, *Pirus*, *Prunus*, *Mespilus*, and *Crataegus* (Modarres Awal, 1997, 2012).

***Neocoenorrhinus* (*Neocoenorhinidius*) *pseudocribrum pseudocribrum* Legalov, 2002**

Distribution in Iran: Fars (Legalov & Fremuth, 2002; Legalov et al, 2010).

General distribution: Azerbaijan, Iran, Turkey.

Subgenus *Schoenitemnus* Legalov, 2003

***Neocoenorrhinus* (*Schoenitemnus*) *minutus* (Herbst, 1797)**

Distribution in Iran: Razavi Khorasan (Legalov et al, 2010 as *Schoenitemnus minutus*).

General distribution: Algeria, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, Estonia, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Italy, Lithuania, Luxembourg, Macedonia, Moldavia, Montenegro, the Netherlands, Norway, Poland, Portugal, Romania, European Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine.

Genus *Rhynchites* Schneider, 1791

Subgenus *Epirhynchites* Voss, 1969

***Rhynchites* (*Epirhynchites*) *auratus* (Scopoli, 1763)**

Distribution in Iran: Alborz (Borumand, 1959; Behdad, 1991; Sanaei & Seiedy 2017), East Azarbaijan, Sistan & Baluchestan (Borumand, 1998; Legalov et al, 2010 as *Epirhynchites* (*Tshernyshevinius*) *auratus*; Modarres, Awal 2012), Fars (Farahbakhsh 1961; Modarres Awal, 1997, 2012 as *R. auratus ferganensis* Nevskii, 1928; Borumand, 1998; Legalov et al, 2010), Hamadan (Modarres Awal, 1997, 2012, Radjabi, 1991), Isfahan, Zanjan, central and northern provinces (Modarres Awal 1997, 2012), Kerman (Behdad, 1991; Radjabi, 1991; Modarres Awal, 1997, 2012 as *R. auratus ferganensis*; Borumand, 1998; Legalov et al, 2010), Kordestan (Khanizad, Mansour, & Karimi, 2012), Markazi (Kolyaee, Dezianian, & Akbarzadeh Shoukat, 2004), Mazandaran (Borumand, 1998; Legalov et al, 2010 as *E.* (*Tshernyshevinius*) *auratus*), Qazvin (Farahbakhsh 1961; Behdad 1991; Radjabi, 1991), Razavi Khorasan (Radjabi, 1991; Modarres Awal, 1997, 2012; Kolyaee et al, 2004), Semnan (Dezianian, 2004, 2005), Tehran (Farahbakhsh, 1961; Behdad, 1991; Radjabi, 1991; Modarres Awal, 1997, 2012 as *R. auratus ferganensis*; Borumand,1998; Kolyaee et al, 2004; Legalov et al, 2010), West Azarbaijan (Kolyaee et al, 2004; Legalov et al, 2010), Iran (no locality cited) (Ter-Minassian, 1950; Erol 1994; Esmailii, Mirkarimi, Azmayesh Fard, 2006; Esmailii, 2011).

New material examined: Lorestan province, SE of Harsin, 1875 m, 1♀, leg. G. Sama, det. E. Colonnelli, 10.v.2008 (Colonnelli personal collection); Guilan province, Tootakabon Barehsar Road Views, 1125 m, 36°48'5.89''N 49°38'5.86''E, 1♀, leg. Jaroslav Dalihod, det. E. Colonnelli, 3.vi.2015 (Biondi personal collection); Mazandaran province, Mt. Elburz, Valiabad, 2100 m, 36°14'N 51°18'E, 1♂, leg. Pad. Mal., det. S. Biondi, 21.05.1999 (Biondi, personal collection).

General distribution: Afghanistan, Armenia, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, China, Croatia, Czech Republic, Estonia, France, Georgia, Germany, Greece, Hungary, Iran, Iraq, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Macedonia, Moldavia, the Netherlands, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Switzerland, Tajikistan, Turkey, Turkmenistan, Ukraine, Uzbekistan.

Host plants in Iran: Cultivated and wild Rosaceae of the genera *Prunus* (Farahbakhsh, 1961; Radjabi, 1991; Modarres Awal, 1997; Esmailii et al, 2006; Esmailii, 2011) and *Malus* (Radjabi, 1991; Modarres Awal, 1997).

***Rhynchites* (*Epirhynchites*) *giganteus* Schoenherr, 1832**

Distribution in Iran: Mazandaran (Hoffmann, 1968 as *Rhynchites versicolor*), Northern provinces (Farahbakhsh, 1961 as *Rhynchites giganteus* Krynicki, 1832; Modarres Awal 1997, 2012), Iran (no locality cited) (Radjabi, 1991; Erol, 1994; Modarres Awal, 1997, 2012 as *Rhynchites versicolor* Costa, 1839; Legalov, 2007, Legalov et al, 2010 as *Epirhynchites* (*Pyrorhynchites*) *giganteus* (Krynicky, 1832)).

New material examined: East Azarbaijan province, Kaleibar, 1600/1700 m, 38°52'N 47°0'E, 1♂, leg. Pad. Mal., det. S. Biondi, 26.05.1999 (Biondi, personal collection).

General distribution: Armenia, Austria, Azerbaijan, Bulgaria, China, Croatia, Czech Republic, France, Georgia, Greece, Hungary, Iran, Italy, Kazakhstan, Moldavia, Romania, European Russia, Slovakia, Slovenia, Turkey, Ukraine, Uzbekistan.

Comments: The indications of *Rhynchites* (*Epirhynchites*) *heros* (Roelofs, 1874) from Iran are erroneous (Biondi, personal communication), and indeed this eastern Palaearctic species is not quoted for Iran by Alonso-Zarazaga et al (2017). Most probably, the Iranian records of *R. heros* for Alborz (Borumand, 1959; Farahbakhsh 1961; Behdad 1991; Radjabi 1991), Mazandaran, Tehran (Radjabi 1991; Modarres Awal 1997, 2012), and for Iran (Esmailii, 2011) on the cultivated Rosaceae of the genus *Prunus* (Farahbakhsh 1961; Radjabi 1991; Modarres Awal 1997, 2012; Esmailii, 2011) belong to the closely related *R. giganteus*, instead.

Host plants in Iran: Cultivated Rosaceae of the genera *Malus*, *Pirus* and *Prunus* (Farahbakhsh, 1961; Radjabi, 1991; Modarres Awal, 1997, 2012) and *Crataegus* (Farahbakhsh, 1961; Modarres Awal, 1997, 2012).

***Rhynchites* (*Epirhynchites*) *lopatini* Ter-Minassian, 1968**

Distribution in Iran: Yazd (Zare Khormizi, Heidari Latibari, Khandehroo, Moravvej, Sadeghi Namaghi 2016).

General distribution: Iran (zare Khormizi, Heidari Latibari, Khandehroo, Moravvej, & Sadeghi Namaghi, 2016), Turkmenistan, Uzbekistan (Alonso-Zarazaga et al, 2017).

***Rhynchites* (*Epirhynchites*) *smyrnensis* (Desbrochers des Loges, 1869)**

Distribution in Iran: Tehran (Radjabi, 1991; Modarres Awal, 1997, 2012), Iran (no locality cited) (Erol, 1994; Legalov et al, 2010 as *Epirhynchites* (*Colonnellinius*) *smyrnensis*; Avgın & Colonnelli, 2011; Esmailii, 2011).

New material examined: Fars province, 7 Km E of Dasht-e Arzhan, 2050 m, 1♀, leg. D. Baiocchi, det. E. Colonnelli, 8.05.2016 (Colonnelli, personal collection); Markazi province, 35km E Borujerd - Ghale Samurkhan env., 2160 m, 1 ex, leg. J. Simandl, det. S. Biondi, 3.06.2016 (Biondi, personal collection).

General distribution: Greece, Iran, Israel, Jordan, Syria, Turkey, Turkmenistan.

Host plants in Iran: Cultivated Rosaceae of the genus *Malus* and *Prunus* (Modarres Awal 1997, 2012; Esmailii, 2011).

***Rhynchites* (*Epirhynchites*) *zaitzevi* Kieseritzky, 1926**

Distribution in Iran: Golestan (Ghahari & Colonnelli 2012), Lorestan (Kieseritzky 1926 as *Rhynchites* (*Epirhynchites*) *zaitzevi* Kieseritzky, 1926; Legalov et al, 2010 as *Epirhynchites* (*Tshernyshevinius*) *zaitzevi*, Iran (no locality cited) (Winkler, 1930; Ter-Minassian 1950; Farahbakhsh 1961*;* Balachowsky, 1963; Modarres Awal 1997, 2012; Legalov, 2007).

New material examined: Kuhgiloyeh & Boyerahmad province, Sisakht, 1♂, 1♀, leg. D. Baiocchi, det. E. Colonnelli, 14.05.2013; Kordestan province, Kamiaran, 1♂, leg. D. Baiocchi, det. E. Colonnelli, 23.06.2012 (Colonnelli, personal collection); Mazandaran province, 45km N Firuz-Kuh - Do-Ab, 1♂, 2♀♀, leg. F. Pavel, det. S. Biondi, 14.05.2005 (Biondi, personal collection).

General distribution: Iran.

Host plants in Iran: *Prunus dulcis* (Mill.) D.A. Webb (Rosaceae) (Farahbakhsh 1961; Radjabi, 1991; Modarres Awal, 1997, 2012).

Subgenus *Rhynchites* D.H. Schneider, 1791

***Rhynchites* (*Rhynchites*) *bacchus* (Linnaeus, 1758)**

Distribution in Iran: Alborz (Farahbakhsh, 1961), Lorestan (Legalov et al, 2010), Tehran and other Northern provinces (Modarres Awal, 1997, 2012), Iran (no locality cited) (Erol, 1994).

New material examined: East Azarbaijan province, Kaleibar, 1600/1700 m, 38°52'N 47°0'E, leg. Pad. Mal., det. S. Biondi, 1♂, 26.05.1999; Guilan province, Mt. Talesh - Khalkhal, 1600 m, 37°51'N 48°36'E, 1♀, leg. Pad. Mal., det. S. Biondi, 24.05.1999 (both S. Biondi, personal collection).

General distribution: Albania, Algeria, Armenia, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, China, Croatia, Czech Republic, France, Georgia, Germany, Greece, Hungary, Iran, Israel, Italy, Kazakhstan, Lithuania, Luxembourg, Macedonia, Moldavia, Montenegro, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Turkmenistan, Ukraine, Uzbekistan.

Host plants in Iran: Cultivated Rosaceae of the genera *Malus*, *Pirus*, *Prunus*, and occasionally on *Laurus* (Lauraceae) (Farahbakhsh, 1961; Modarres Awal, 1997, 2012). This leaf-roller is a very important pest of cultivations of several fruits all over the world, since adults and larvae feed within plant tissues such as buds, flowers and soft fruits (Korotyaev, 1984; Ulusoy, Vatansever, & Uygun, 1999).

***Rhynchites* (*Rhynchites*) *lenaeus* Faust, 1891**

Distribution in Iran: West Azarbaijan (Sakenin et al, 2018).

General distribution: Armenia, Azerbaijan, Bulgaria, Georgia, Greece, Israel, Macedonia, Russia (South European Territory), Slovakia, Syria, Turkey (Alonso-Zarazaga et al, 2017), Iran (Sakenin et al, 2018).

Host plants in Iran: *Prunus* (Rosaceae) (Sakenin et al, 2018).

Genus *Tatianaerhynchites* Legalov, 2002

***Tatianaerhynchites aequatus* (Linnaeus, 1767)**

Distribution in Iran: Ardabil, East Azarbaijan, Fars (Borumand, 1998 as *Caenorrhinus aequatus*; Legalov et al, 2010; Modarres Awal, 2012 as *Rhynchites aequatus*), Golestan (Legalov, 2002; Ghahari & Colonelli, 2012), Northern Khorasan (Hoffmann, 1968), Razavi Khorasan, Mazandaran (Legalov et al, 2010), West Azarbaijan (Akbarzadeh Shoukat, 2002, 2004 as *Coenorhinus aequatus*; Legalov et al, 2010), Iran (no locality cited) (Modarres Awal, 1997 as *R*. *aequatus*).

General distribution: Albania, Armenia, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Iran, Israel, Italy, Jordan, Kazakhstan, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Moldavia, Montenegro, the Netherlands, Norway, Poland, Portugal, Romania, European Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Turkey, Turkmenistan, Ukraine.

Host plants in Iran: *Colutea* sp. (Fabaceae) (Hoffmann, 1968), most probably accidentally.

Comments: *Tatianaerhynchites aequatus* is one of the most important pests of apple, *Malus domestica*, orchards at Orumieh (West Azarbaijan) (Akbarzadeh Shoukat, 2002, 2004).

Genus *Temnocerus* Thunberg, 1815

***Temnocerus coeruleus* Fabricius, 1798**

Material examined: Golestan province, Minudasht, Tuska-Chal, 37º12’6’’N 55º32’0’’E, 1 ex, leg. Z. Karimian, det. E. Colonelli, June 2003. New record for Iran.

General distribution: Algeria, Andorra, Austria, Azerbaijan, Belgium, Bosnia Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Moldavia, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine.

***Temnocerus nanus* (Paykull, 1792)**

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

General distribution: Algeria, Andorra, Austria, Belarus, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Mongolia, the Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovenia, Spain, Sweden, Switzerland, Turkey, Turkmenistan, Ukraine (Alonso-Zarazaga et al, 2017), Iran (Samin et al, 2016).

DISCUSSION

The fauna of Iranian Attelabidae is rather diverse with 30 recorded species in 12 genera, of which *Rhynchites* with seven species is the richest (Fig. 2). Modarres Awal (1997) reporded *Pselactus spadix spadix* (Herbst, 1795) (Cossoninae) on *Populus* as *Rhynchites culinaris* (Germar, 1819) by evident mistake of the genus name. Subsequently, the same author (Modarres Awal 2012) recorded again the same *Pselactus* species with the same habitat under its synonym name of *Rhyncolus culinaris* whose authorship was incorrectly attributed to Dejean (1821). *Rhynchites* (*Epirhynchites*) *zaitzevi* (Kieseritzky, 1926) and *Involvulus* (*Teretriorhynchites*) *icosandriae iranensis* Legalov, 2004 are so far the only Attelabidae endemic to Iran.

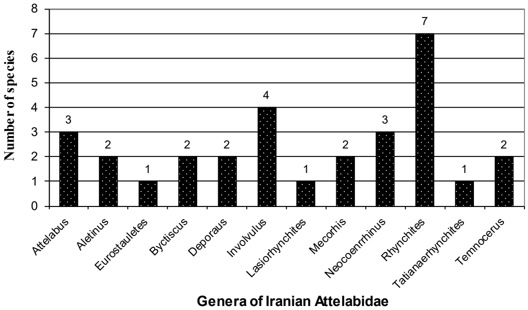

Fig. 2. Species diversity of the Iranian Attelabidae.

Among the countries adjacent to Iran (Fig. 3), apparently only the fauna of Turkey with 39 recorded species has been studied rather well. Since we have not any comprehensive study on Iranian Attelabidae fauna besides the faunistic and literature records quoted in this article, we expect that field work, in such a diverse country like Iran, may result in a substantial increase of the number of species in the future.

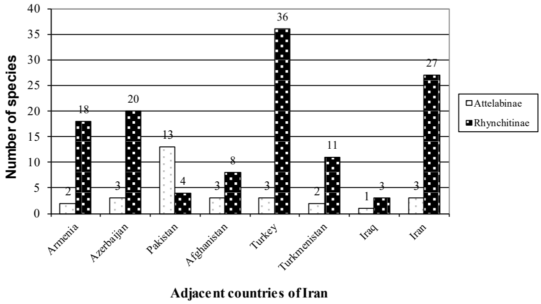

Fig. 3. Comparison of species diversity of Attelabidae in Iran with neighbouring countries.

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Description of a New Species of Australian *Cerdistus* (Asilidae: Asilinae)

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ABSTRACT

A new species of *Cerdistus* was collected from Minnawarra in South Australia. Herein we describe *Cerdistus londti* sp. nov., with the addition of SEM images. Specimens of this species from two localities in South Australia were keyed out and we also include DNA (COI) barcodes for comparison to other species belonging to the *Cerdistus/Neoitamus* complex in Australia.

Key words: New species, *Cerdistus londti*, South Australia.

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INTRODUCTION

The genus *Cerdistus* (Diptera: Asilidae, Asilinae) as it is currently taxonomically delimited, was described in 1849 by Loew and has a worldwide distribution. The genus is very diverse and widespread in Australia. The name *Cerdistus* is adopted as a convenience being used in a liberal sense, not in a confined one as Loew used it, since some Australian species have also been described in the genus *Neoitamus* (White, 1917; Hardy, 1926). The genus *Cerdistus*, in Australia, was first taxonomically treated as a group by G.H. Hardy in 1926 and contained 17 species. Nine years later an additional nine species were recognized (Hardy, 1935). In his Asilid catalogue of the Australasian/Oceanian region Daniels (1989) listed 33 valid species of the genus *Cerdistus* and five in the genus *Neoitamus* as occurring in Australia. Since that time a further five species have been described:*Cerdistus judyjanei* Lavigne (Lavigne, 2010), *C. hudsoni* Lavigne (Lavigne, 2011), *C. weylandi* Lavigne & McAllister (Lavigne & McAllister, 2011), *C. mcarthuri* Lavigne & McAllister (Lavigne & McAllister, 2012) and *C. ascanii* Lavigne & Stevens (Lavigne & Stevens, 2017a).

During a survey of insects in Minnawarra, South Australia in 2006, as part of the long term Minnawarra Biodiversity Project, several specimens of an undescribed species of *Cerdistus* were collected. These specimens were found to be conspecific with two males previously collected in the Adelaide Hills and one on Mt Lofty, also undescribed. Herein we describe the new species as *Cerdistus londti* sp. nov., which increases the number of *Cerdistus* known from Australia to 39 species.

MATERIAL AND METHODS

Collection details

Malaise traps were set up in an area dominated by Stringybark (*Xanthorrhes* sp.) near a swamp at Minnawarra, 2.63 km west of Spring Mount (35°26'11"S 138°30'42"E). Specimens of *Cerdistus londti* sp. nov. were collected in the traps during the period, 21.12.2004 to 05.01.2005. Specimens of the new species originally keyed out in an unpublished key to an undescribed species in the genus *Cerdistus*. A male and female recovered from ethanol in the traps was taken to University of Gazi, Ankara, Turkey and SEM photographs were taken of the specimens.

Three specimens of the new *Cerdistus* from two South Australian locations from Minnawarra and Bugle Ranges (SA) were compared to eight closely related *Cerdistus* species (*mcarthuri*, *hudsoni*, *judyjanei*, *weylandi*, *vittipes, ascanii, margitis*), two *Neoitamus* species (*maculatoides, socius*), and *Neocerdistus acutangulatus.*

All specimens were analysed by the Canadian Centre of DNA Barcoding, Guelph, Canada using the 'barcode region' of the cytochrome *c* oxidase I (COI) gene (Hebert, Ratnasingham, & deWaard, 2003; Ratnasingham & Hebert, 2007), which has shown to be an ideal addition to taxonomic studies (Porco et al, 2012; Stevens & D'Haese, 2016; D'Haese, Stevens, & Weiner, 2017; Lavigne & Stevens, 2017b). Mitochondrial DNA was extracted from a single leg from each specimen and amplified following standard procedures published elsewhere (Hajibabaei, Janzen, Burns, Hallwachs,

& Hebert, 2006). Sequences were aligned using SEQUENCHER (Gene Codes ver. 5.1) sequence editor and PAUP* ver. 4.0b10 (Swofford, 2002) was used to calculate sequence divergence values (uncorrected patristic distance using all 3 codon positions) and generate a Neighbor-Joining phylogram (using p-distance) among the haplotypes. Sequences for all species are archived and available in the BOLD public data portal (http://www.boldsystems.org/index.php/databases) in the MSAR (Australian Robberflies) project, together with images and metadata (Table 1).

RESULTS AND DISCUSSION

Cerdistus londti sp. nov. is a striking species, when observed in the wild, due to its bright orange and complex genitalia. It readily stands out when resting on perching sites.

Diagnosis

Brownish species with grey pleura; scape and pedicel of antennae orange; thorax with long, strong dorsocentral bristles; femora darkened dorsally; genitalia compact, orange.

Description

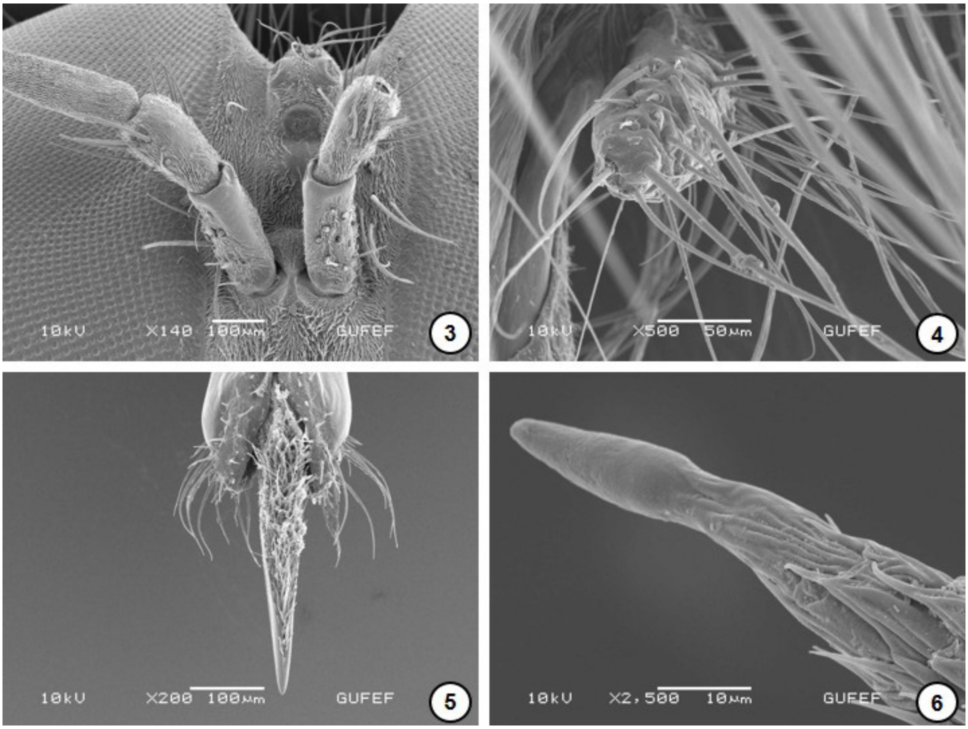
Cerdistus londti sp. nov. (Figs. 1, 2)

Holotype Male: Body length: 12.5 mm. (Range for species: 12 - 13 mm).

Head: Face golden tomentose, flat. Mystax contains 16-20 slender slightly curved white setae, few black setae above. Facial cover ¼. Ocellar tubercle with single short seta beneath each lateral ocellus (Fig. 3). Bristles on occiput black, strongly curved; occiput grey tomentose with dense white slender hairs. Palpus one segmented, shining black, with white abundant setae (Fig. 4). Proboscis shining black, with abundance of microtrichia below (Fig. 5).

Antennae: Basal segment (scape) black (Fig. 3), 2nd segment (pedicel) reddish brown, scape 1/3rd longer than pedicel, 3rd segment linear twice length of pedicel; 4th micro-segment 1/9th length of 3rd segment, style narrow, elongate, 0.83 times length of 3rd segment, tip bare lacking apical spine (Fig. 6). Beard white.

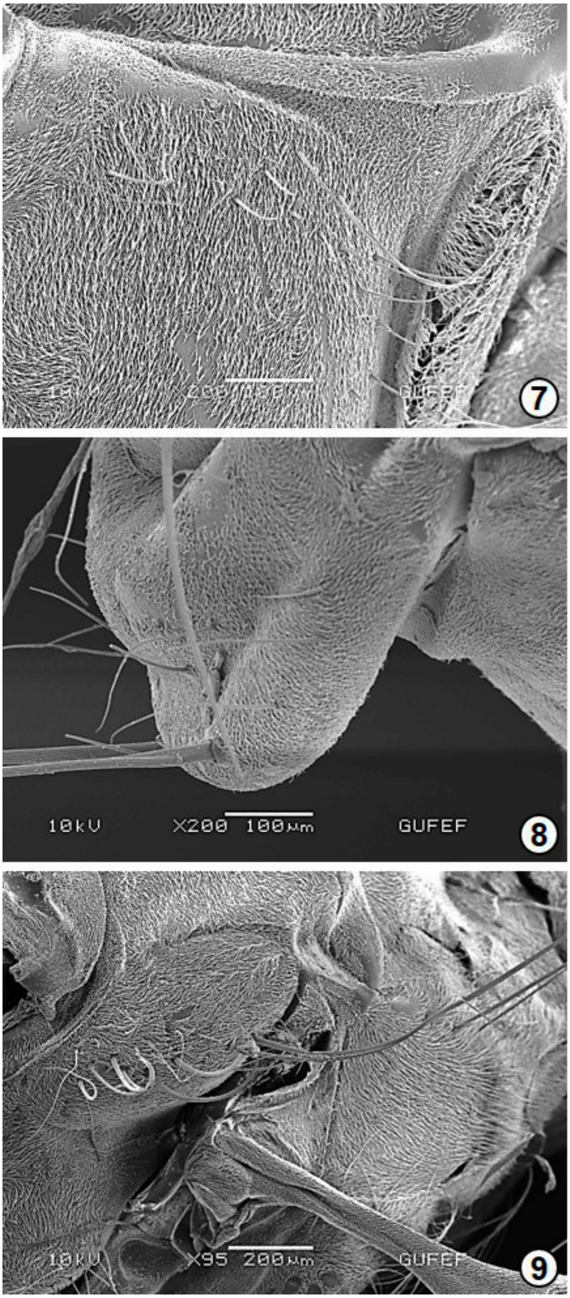
Figs. 1-2. Holotype of *Cerdistus londti* sp. nov. 1. Lateral view of holotype male *C. londti* sp. nov. (SAMA Database No. 29-001066). Photo: G. Weber & R. Lavigne. 2. Dorsal view of holotype male *C. londti* sp. nov. (SAMA Database No. 29-001610). Photo: G. Weber & R. Lavigne.



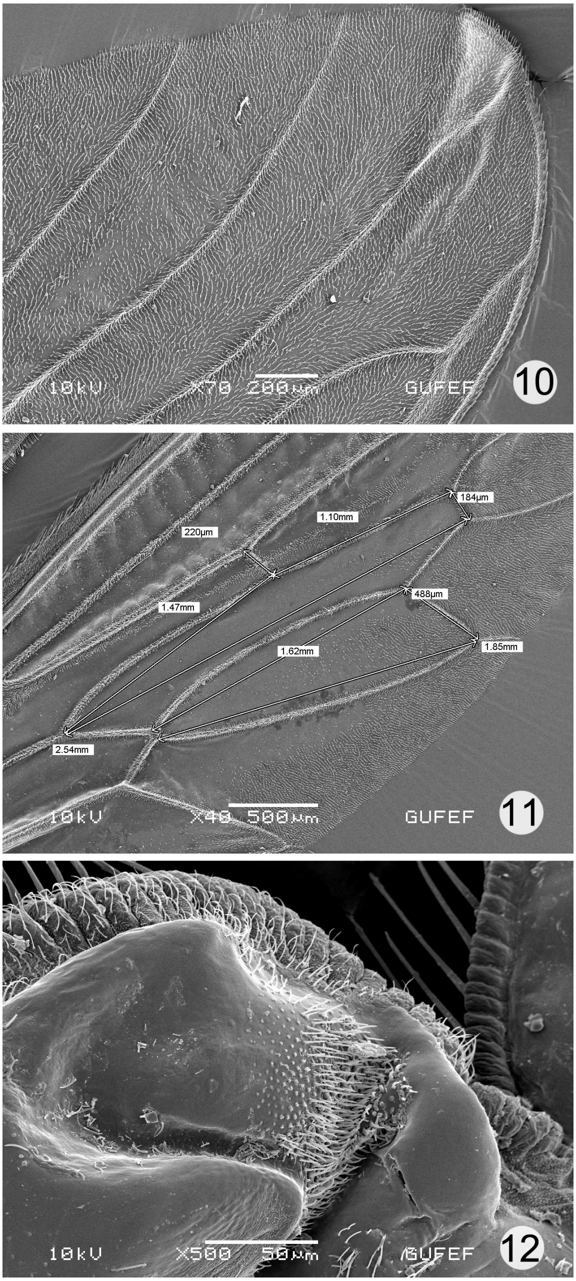
Figs. 3-6. Head of *C. londti* sp. nov. 3. SEM of ocellar tubercle of *C. londti* sp. nov. showing structure and location of setae plus illustrating bare area on inner surface of scape. 4. SEM of one segmented palp showing abundance of apical setae. 5. SEM of underside of proboscis showing abundance of microtrichia and bare tip. 6. SEM of style illustrating bare tip.

Thorax: Greyish-brown tomentose dorsally and grey tomentose laterally with dark brown bare areas dorsally (Fig. 2). Postsutural area of scutum with dorsocentro bristles. Humerus greyish brown tomentose with patch of white setae/hair. Anepisternum grey tomentose with few white hairs on both anterior and dorsal margins (Fig. 7). Anepimeron with white hairs on dorsal margin. Dorsocentral bristles long, black extending anteriorly to just beyond the transverse suture. Scutellar disk, grey tomentose with 2 marginal white bristles (Fig. 8); and scattered medium length white hairs on disk. Mesonotal bristles stout, black: 2 black presutural bristles, 1 supra-alar bristle (1 white, 1 black), 1 white post-alar bristle. Anatergite grey tomentose only without patch of setae. Katatergite (metapleural) bristles 10-12, cream colored, extremely long (Fig. 9). Metanepisternum (hypopleural) bristles stout, 2-4, white.

Wings: Multiple cells filled with microtrichae especially apically (Fig. 10), Discal cell bare, x-vein almost in middle of discal cell (Fig. 11). Sensory pegs/microtrichia at junction of base of wing with thorax (Fig. 12). Halteres light brown.

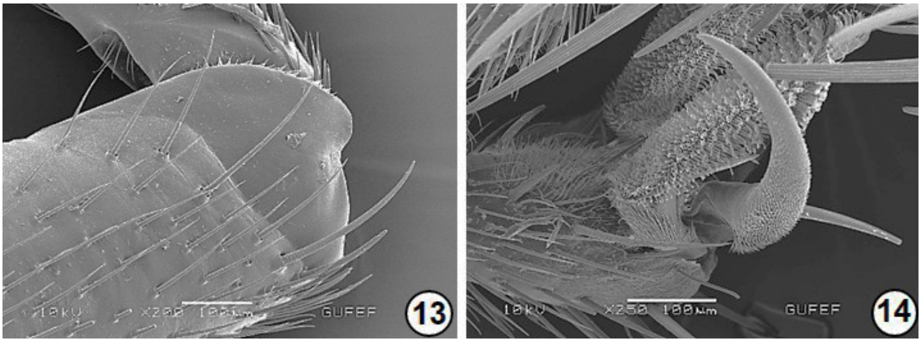


Figs. 7-9. Thorax of *C. londti* sp. nov. 7. SEM of anepisternum showing white hairs on dorsal and lateral margins amongst microtrichia. 8. SEM of scutellum showing two striated marginal setae and scattered hairs. 9. SEM of katatergite with 8 metaplural setae.



Figs. 10-12. Wing of *C. londti* sp. nov. 10. SEM of apex of wing illustrating the dense cover of microtrichia. 11. SEM of position of discal cross-vein on wing and the absence of microtrichia in the discal cell. 12. SEM of patch of sensory pegs/microtrichia at junction of base of wing with thorax.

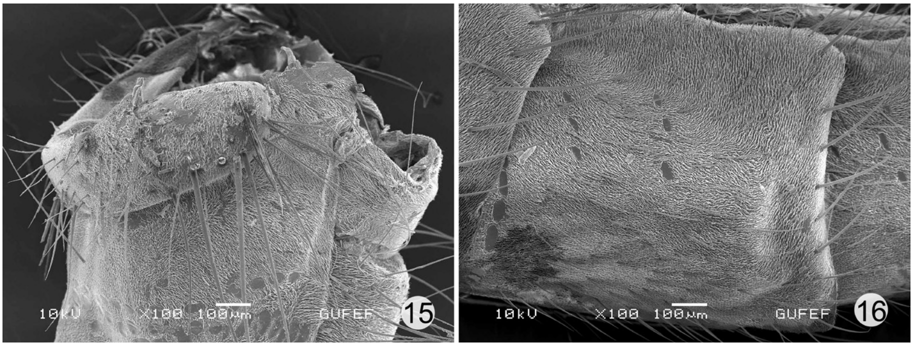
Legs: Legs bare of tomentum, shining; bristles primarily white, except those at apex of tibiae and on tarsi, black. Femora bicolored, dark brown on anterior surface, light brownish orange posteriorly, covered dorsally with dense tiny black setae, apex bare (Fig. 13), tibia orange, darkened at apex, with light brown setae; first tarsal segment bicolored, orange basally. Claws black with brown base, covered with microtrichia (Fig. 14); pulvilli cream coloured; empodia short, brown, 2/3rd length of claws.



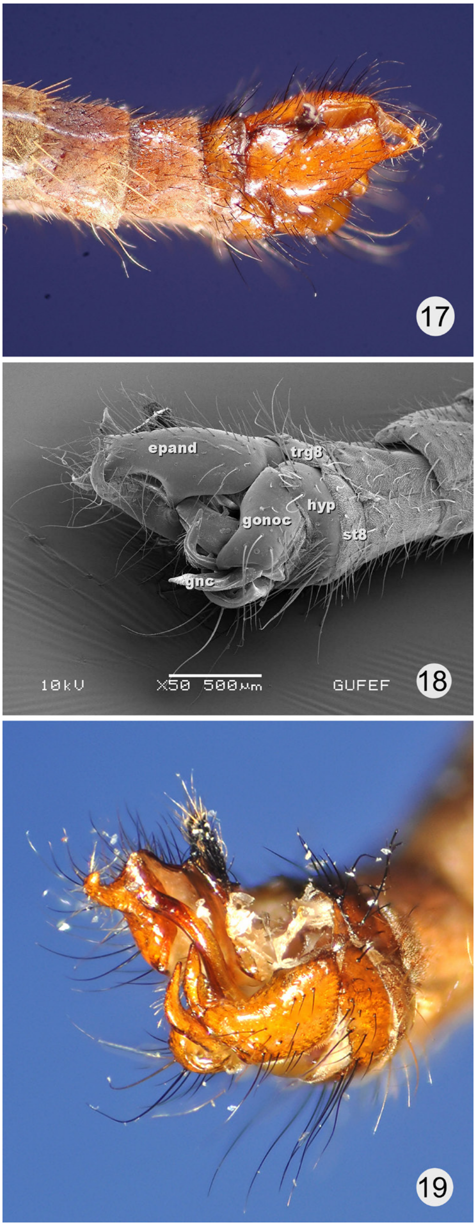
Figs. 13-14. Leg of *C. londti* sp. nov. 13. SEM showing bare area at apex of femur. 14. SEM showing the microtrichia covering the claw.

Abdomen: Tergites yellowish grey pollinose. Two to three exceptionally long white setae dorsolaterally on tergite 1 (Fig. 15). Row of setae on apical margin of tergites 2-5. Sternites yellowish-grey pollinose, with sparsely scattered white hairs; Two parallel diagonal rows of bare spots on sternites 5-6 (Fig. 16).

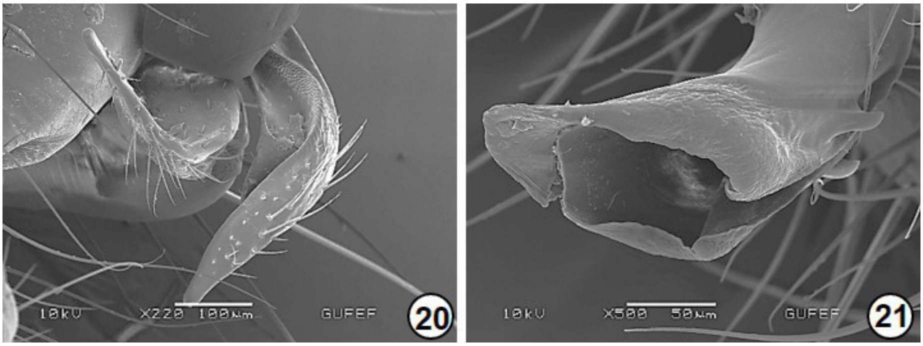
Terminalia (Figs. 2- 4 - paratype SAMA 29-001601): Orange without pubescence. Lower margin of epandria extended forward into inwardly curved digits (Figs. 17-19). Epandrium with double row of long black curved setae on dorsal surface, otherwise bare. Hypandrium rectangular with backward directed black setae (Fig. 18). Gonocoxite ½ length of epandria with patch of long black slender downwardly directed setae. Gonostylus orange, triangular with sharp apex (Fig. 20). Aedeagus elongate, darkened at apex, opening of aedaegus with subapical paired protrusions (Fig. 21). Cerci, black, with short white hair apically.



Figs. 15-16. Abdomen of *C. londti* sp. nov. 15. SEM showing the exceptionally long white setae dorsolaterally on tergite 1. 16. SEM showing the two parallel diagonal rows of bare spots on sternites 5-6.



Figs. 17-19. Male genitalia of *C. londti* sp. nov. 17. Close-up of lateral view of genitalia of *C. londti* sp. nov. showing structure of epandria with crossing tips. Photo: G. Weber & R. Lavigne. 18. SEM of male terminalia of *C. londti* sp. nov. illustrating shape of hypandrium and gonocoxite, epand, epandrium; gonoc, gonocoxite; gnc, gonostylus; hyp, hypandrium; trg8, tergite 8; st8 sternite 8. 19. Partially dissected male terminalia of *C. londti* sp. nov. showing shape of hypandrium and gonocoxite (SAMA Database 29-001560). Photo: A. McArthur & R. Lavigne.

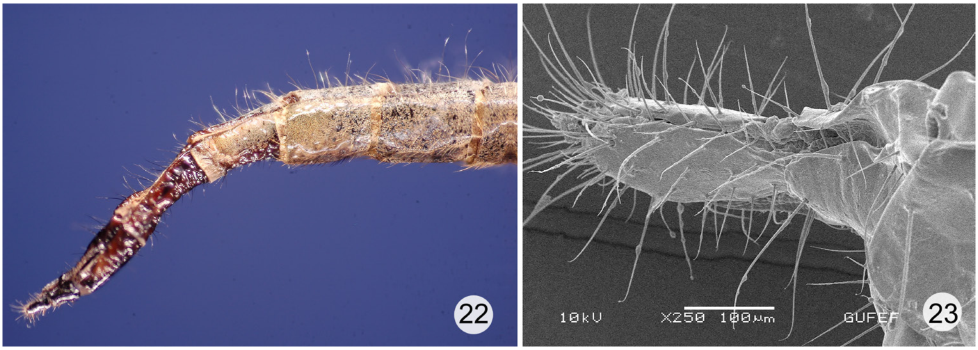


Figs. 20-21. Male genitalia of *C. londti* sp. nov. 20. SEM showing the setae on the outer surface of the gonostylus. 21. SEM of the opening of the aedeagus with paired protrusions subapically.

Material examined: Holotype Male, S AUST: Mt Lofty/ 34°59'S 138°43'E/ 29 Dec 1973-6th Jan 1974/ 10th Aust. Boy Scout Jamboree. 2nd label: Acc. No.: SAMA Database No./ 29-001066.

Paratypes Male, S AUST: Quinyambie Stn./ Coonanna Bore/ 29°50'50"S 140°46'55"E/ 25.10.2008-01.11.2008/at Light / Camp/ Waterhouse Svy. 2nd label: Acc. No.: SAMA Database No. 29-002568. Male, S AUST: Bugle Ranges, 8 km S, Mt Barker/ 35°07'40"S 138°51'40"E/ 02/12/94/ insect net/ Ma/llee scrub R.J. Lavigne 2nd label: Acc. No.: SAMA Database No. 29-000321. Male, S AUST: Bugle Ranges, 8 km S, Mt Barker/ 35°07'40"S 138°51'40"E/ 01/02/02/ aluminum pan trap/ R.J. Lavigne 2nd label: Acc. No.: SAMA Database No. 29-001156. Male, S AUST: 2.63 km W Spring Mount/ Minnawarra/ 35°26'11"S 138°30"42"E/ 21.12.2005-04.01.2006/ malaise trap/ "Stringybark, Xanthorrhes: above swamp/ Museum SEG Svy/ MIN002. 2nd label: Acc. No.: SAMA Database No. 29-001560; female: same data, except SAMA Database No. 29-001561 and SAMA Database No. 29-001564. Three males, same data except SAMA Database No. 29-001562, SAMA Database No. 29-001563 & SAMA Database No. 29-001565. Two males, S AUST: 2.29 km SW Spring Mount/ Minnawarra/ 35°27'01"S 138°31"09'E/ 21.12.2005-04.01.2006/ malaise trap/ "Stringybark, Bursaria spinosa, grasses"/ Museum SEG Svy/ MIN008. 2nd label: Acc. No.: SAMA Database No. 29-001566 & SAMA Database No. 29-001567; Male, S AUST: 1.8 km NW Spring Mount/ Minnawarra/ 35°25'56"S 138°31"26'E/ 21.12.2005-04.01.2006/ malaise trap/ "Stringybark, Xanthorrhes, grasses on stony hilltop"/ Museum SEG Svy/ MIN009. 2nd label: Acc. No.: SAMA Database No. 29-001568. Male (8), S AUST: 1.96km NW Spring Mount, Minnawarra/ 35°25'46"S 138°31'26"E/ 21.12.2005-04.01.2006/ malaise trap/ "Stringybark, low shrubs, grasses""/ Museum SEG Svy/ MIN004. 2nd label: Acc. No.: SAMA Database No. 29-001570. Males (7), same data as 29-001570 except SAMA Database No. 29-001601, SAMA Database No. 29-001603, SAMA Database No. 29-001607, SAMA Database No. 29-001608, SAMA Database No. 29-001610, SAMA Database No. 29-001611, SAMA Database No. 29-001613. Female, S AUST: 1.96km NW Spring Mount, Minnawarra/ 35°25'46"S 138°31'26"E/ 21.12.2005-04.01.2006/ malaise trap/ "Stringybark, low shrubs, grasses""/ Museum SEG Svy/ MIN004. 2nd label: Acc. No.: SAMA Database No. 29-001569. Females (13) same data as 29-001569 except SAMA Database No. 29-001571, SAMA Database No. 29-001572, SAMA Database No. 29-001573, SAMA Database No. 29-001602, SAMA Database No. 29-001604, SAMA Database No. 29-001605, SAMA Database No. 29-001606, SAMA Database No. 29-001609, SAMA Database No. 29-001612, SAMA Database No. 29-001614, SAMA Database No. 29-001615, SAMA Database No. 29-001616, SAMA Database No. 29-001617.

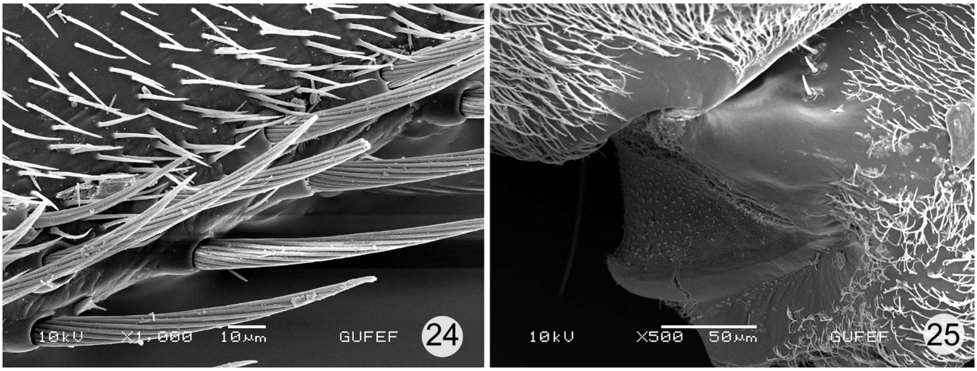
Female: Similar to male, except all post ocular bristles white; abdomen 7 segmented; Ovipositor: shining brown, tip with multiple setae (Fig. 22). Tip of ovipositor with multiple hairs (Fig. 23).



Figs. 22-23. Ovipositor of female *C. londti* sp. nov. 22. Lateral view of female *C. londti* sp. nov. ovipositor (SAMA Database No. 29-001569). Photo: G. Weber & R. Lavigne. 23. SEM of tip of ovipositor of *C. londti* sp. nov. female.

Brief comments on microtrichia

Microtrichia occur on all parts of the body of *Cerdistus londti* and are typically minute scattered curved unsocketed hairlike structures as opposed to macrosetae which are longer, broader, striated, socketed and often confined to veins (Fig. 24). Additionally, modified minute spine-like or amorhous structures are seen at points of articulation, such as at leg junctions (Fig. 25). An unusual feather shaped microtrichia was found only on the female *C. londti* above insertion point of hind wing (Fig. 26). We are unable to ascertain its function.



Figs. 24-25. Microtrichia and macrotrichia. 24. SEM illustrating the difference between microtrichia and macrotrichia. 25. SEM illustrating the modified minute spine-like or amorhous structures seen at points of articulation at coxal junction with thorax.

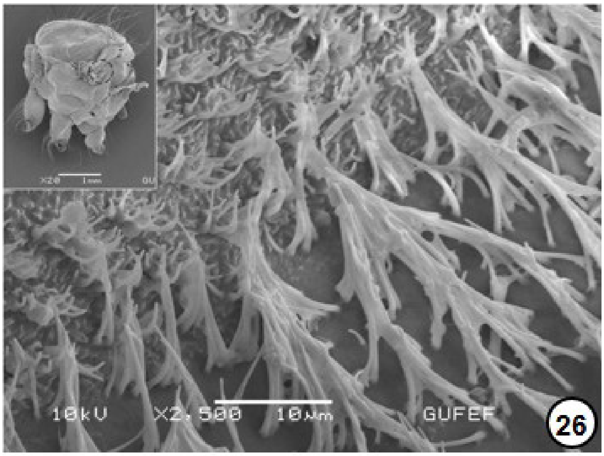


Fig. 26. SEM of patch of unusual feather shaped microtrichia found beneath the post alar wall on female thorax; insert shows location.

Etymology

This species, *Cerdistus londti*, is named in honour of Dr. Jason Londt in recognition of his over forty years of work on Afrotropical Asilidae. He has published many excellent papers describing some 45 new genera and more than 580 new species.

Depository

The holotype and 32 paratypes of *C. londti* sp. nov. are deposited in the South Australia Museum (SAMA) Entomology Collection in Adelaide.

Distribution

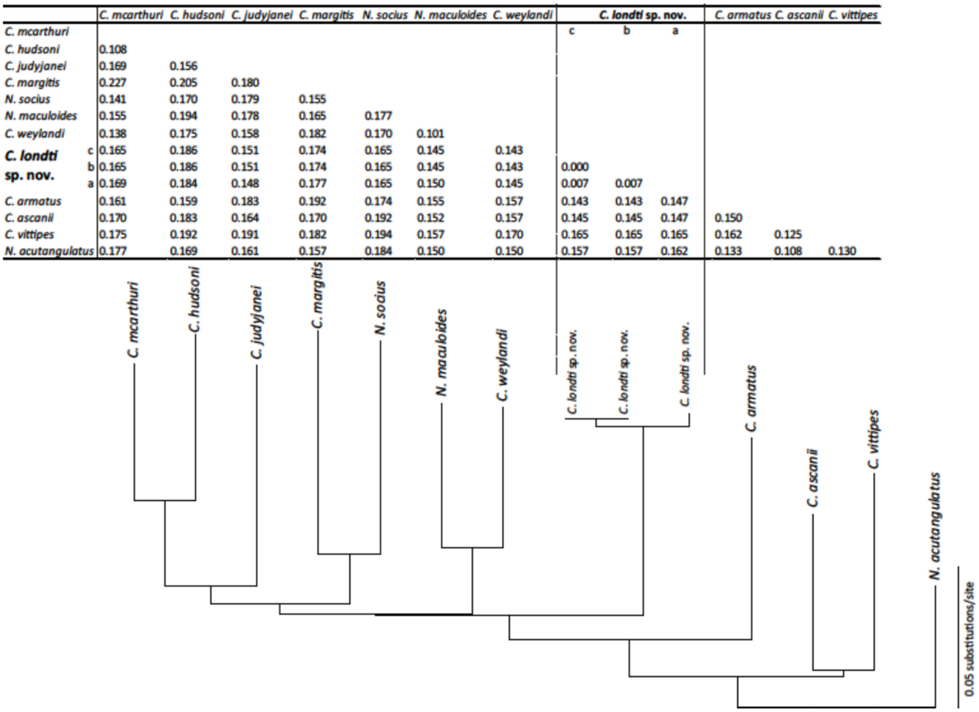
Thus far, *Cerdistus londti* sp. nov. has been collected only in four locations in southern South Australia, three from a limited area near Kangaroo Island: Minnawarra Conservation Park, Mt Lofty Conservation Park, Bugle Ranges and the fourth from Coonanna Bore, 435 miles northeast of Kangaroo Island close to the New South Wales border. The vegetation, Malee scrub, is common to all locations.

Molecular (DNA) barcode

The three specimens of *Cerdistus londti* sp. nov. were compared with other species that belong to the *Cerdistus/Neoitamus* complex in Australia (Table 1). The neighbor-joining tree and minimal sequence divergence (0-0.7%) supports the close intraspecific relationship among the three specimens of *C. londti* sp. nov. (a, b, c) and indicate that they all represent a single species (Fig. 27). Sequence divergence between species reveal divergences between 10.1% (*C. weylandi* and *N. maculoides*) to 19.4% (*C. vittipes* and *N. socius*) (Table 1). Sequence divergence of between 14.3% between *C. londti* sp. nov. and its nearest neighbours *C. weylandi* and adds further support to the species diagnosis of *C. londti* sp. nov. (Fig. 27).

Table 1. Collection data for each Cerdistus/Neoitamus species, and Neocerdistus as outgroup, selected for sequencing. Species relates to those used in Fig. 27. All sequences can be obtained using the 'code' in the BOLD public data portal (http://www.boldsystems.org/index.php/databases).

Species	Code	BOLD sequence code	Collection date	Location	GPS
Neoitamus maculatoides	MS_CER_N101c	MSAC1459-14	23.03.2008	Vivonne Bay, heathlands W of Point Ellen, KI, SA	35.993°S, 137.183°E
Neoitamus socius	MS_CER_29-002880	MSAC1285-14	07.07.1995	Czech Republic, Southern Moravia	49.362°N, 016.406°E
Cerdistus vittipes	MS_CER_29-001079	MSAC1258-14	09.10.1991	W Eyre Pen, Lake Newland CP, SA	33.400°S, 134.867°E
Cerdistus weylandi	MS_CER_29-001839	MSAC1266-14	18.05.2005	Monarto Zoological Park, SA	35.106°S, 139.137°E
Cerdistus mcarthuri	MS_CER_29-001857	MSAC1268-14	01.12.2000	Gluepot Reserve, SA	33.733°S, 139.983°E
Cerdistus judyjanei	MS_CER_29-002573	MSAC1278-14	25.10.2008	Coonanna Bore, Quinyambie Stn., SA	29.847°S, 140.782°E
Cerdistus hudsoni	MS_CER_29-003368	MSAC1286-14	27.11.2007	Perth, Kensington Bushland, WA	31.983°S, 115.883°E
C. ascanii	MS_CER_N048c	MSAC1390-14	27.01.2008	Belair National Park, SA	35.010°S, 138.656°E
Cerdistus margitis	MS_CER_29-001712	MSAC1262-14	22.10.1996	Cheesman Peak, SA	27.333°S, 130.217°E
Cerdistus armatus	MS_CER_29-002500	MSAC1276-14	13.01.1971	Coorong Nat. Pk., SA	36.000°S, 139.500°E
Cerdistus londti sp. nov.	MS_CER_N009a	MSAC1316-14	12.02.1994	Bugle Ranges, SA	35.150°S, 138.883°E
Cerdistus londti sp. nov.	MS_CER_N009b	MSAC1317-14	21.12.2005	Minnawarra, SA	35.429°S, 138.524°E
Cerdistus londti sp. nov.	MS_CER_N009c	MSAC1318-14	21.12.2005	Minnawarra, SA	35.429°S, 138.524°E
Neocerdistus acutangulatus	MS_CER_29-001955	MSAC1270-14	23.03.1993	ILLamurta Spr CP, NT	24.314°S, 132.686°E



	C. mcarthuri	*C. hudsoni*	*C. judyjanei*	*C. margitis*	*N. socius*	*N. maculoides*	*C. weylandi*	**C. londti sp. nov.** c	b	a	*C. armatus*	*C. ascanii*	*C. vittipes*
C. mcarthuri													
C. hudsoni	0.108												
C. judyjanei	0.169	0.156											
C. margitis	0.227	0.205	0.180										
N. socius	0.141	0.170	0.179	0.155									
N. maculoides	0.155	0.194	0.178	0.165	0.177								
C. weylandi	0.138	0.175	0.158	0.182	0.170	0.101							
C. londti sp. nov. c	0.165	0.186	0.151	0.174	0.165	0.145	0.143						
b	0.165	0.186	0.151	0.174	0.165	0.145	0.143	0.000					
a	0.169	0.184	0.148	0.177	0.165	0.150	0.145	0.007	0.007				
C. armatus	0.161	0.159	0.183	0.192	0.174	0.155	0.157	0.143	0.143	0.147			
C. ascanii	0.170	0.183	0.164	0.170	0.192	0.152	0.157	0.145	0.145	0.147	0.150		
C. vittipes	0.175	0.192	0.191	0.182	0.194	0.157	0.170	0.165	0.165	0.165	0.162	0.125	
N. acutangulatus	0.177	0.169	0.161	0.157	0.184	0.150	0.150	0.157	0.157	0.162	0.133	0.108	0.130

Fig. 27. Neighbor-Joining tree and sequence divergence (uncorrected P-distance) for *Cerdistus/ Neoitamus* complex compared to *C. londti* sp. nov.

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

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