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Original article (Orijinal araştırma)

"In different shades of purple": effects of different concentrations of commercial black chokeberry fruit extract [*Aronia melanocarpa* (Michx) Elliott] on fitness components and wing morphology of the fruit fly, *Drosophila melanogaster* Meigen, 1830[1]

"Mor rengin farklı tonlarında": ticari konsantrasyondaki farklı siyah chokeberry meyve özütü konsantrasyonlarının [*Aronia melanocarpa* (Michx) Elliott] meyve sineği *Drosophila melanogaster* Meigen, 1830'in hareket özelliği bileşenleri ve kanat morfolojisi üzerindeki etkileri

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Abstract

It is now widely accepted that bioactive compounds of fruits and vegetables reduce oxidative stress, thus having the beneficial effect of decreasing the risk of many human diseases. The aim of this *in vivo* study was to evaluate the possible protective effects of *Aronia melanocarpa* (Michx) Elliott fruit extract using *Drosophila melanogaster* Meigen, 1830 larvae. Study was done in the year 2016, in Department of Genetics of Populations and Ecogenotoxicology at the Institute for Biological Research, University of Belgrade. Simultaneously with treatments, co-treatments with the same concentrations of black chokeberry fruit extract mixed with a methyl methanesulfonate (MMS) were performed. Fitness traits and morphological characters were monitored. Flies fed on undiluted fruit extract, on MMS + undiluted fruit extract and on MMS, exhibited a prolonged developmental time, lower viability and negatively-impacted wing development. Positive biological effects were observed in flies that developed on substrates with 2 and 25% *A. melanocarpa* extract. Only a mixture of MMS + 25% fruit extract showed positive effects on both fitness components and wing development in comparison with other MMS co-treatments, indicating the ability of this concentration to protect the cells from MMS-induced damage.

Keywords: developmental time, dynamic of eclosion, methyl methanesulfonate, viability, wing shape, wing size

Öz

Son yıllarda, meyve ve sebzelerin içindeki biyoaktif bileşiklerin oksidatif stresi düşürdüğü, böylece birçok insan hastalığının riskini azaltan faydalı bir etkiye sahip olduğu yaygın olarak kabul edilmektedir. Mevcut *in vivo* çalışmanın amacı *Drosophila melanogaster* Meigen, 1830 larvası kullanılarak *Aronia melanocarpa* (Michx) Elliott'nın meyve özütünün muhtemel koruyucu etkilerini değerlendirmektir. Çalışma 2016 yılında, Belgrad Üniversitesi, Biyolojik Araştırma Enstitüsü'ndeki, Popülasyon Genetiği ve Ekogenotoksikoloji Bölümü'nde gerçekleştirilmiştir. Uygulamalar ile aynı anda, metil metansülfonat (MMS) ile karıştırılmış aynı konsantrasyondaki siyah chokeberry meyve özütüyle yan uygulamalar yapılmıştır. Hareket özelliği ve morfolojik karakterler izlenmiştir. Konsantre meyve özütünde, MMS + Konsantre meyve özütünde ve yalnızca MMS'de gelişen sinekler, uzun gelişme süresi, daha düşük yaşama gücü sergilemiş ve kanat gelişimi de olumsuz etkilenmiştir. Olumlu biyolojik etkiler %2 ile 25 arası *A. melanocarpa* özütlü substratlarda gelişen sineklerde gözlenmiştir. Sadece MMS + %25 meyve özütü karışımı, diğer MMS yan uygulamaları ile karşılaştırıldığında hareket bileşenleri ve kanat gelişiminin her ikisinde de tercih edilebilir etkiler göstermiş, bu karışımın MMS tarafından verilen hasara karşı hücreyi koruduğuna işaret etmiştir.

Anahtar sözcükler: gelişme süresi, yumurtadan çıkış dinamiği, metil metansülfonat, yaşama gücü, kanat şekli, kanat boyutu

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Introduction

The modern way of life and development of new technologies have increased the presence of physical and chemical agents in the environment that can cause changes in genetic material and cell damage. Mutations often cause different harmful effects, and their detection and prevention are imperatives of modern fundamental and applied genetic research. In this regard, the detection of substances which may have an adverse or protective effect is one of the most topical challenges of modern science. Many agents of biological and chemical origin exhibit great potential for primary prevention of diseases associated with an increased frequency of mutations (Gasiorowski et al., 1997; Zafra-Stone et al., 2007). Therefore, identification and purposeful use of natural antimutagens which adversely affect spontaneous and induced mutagenesis are of special interest.

It is widely accepted that some of the protective mechanisms in cells are associated with the activity of bioactive components of fruits and vegetables. Bioactive compounds may reduce oxidative stress, and hence have a beneficial effect by reducing the risk of many human diseases (Chrubasik et al., 2010). Darker fruits (e.g., blue and red berries) are known for their high antioxidant content. They are rich in polyphenols with reported beneficial effects on human health (Bermudez-Soto et al., 2007). Most of these polyphenols, which are responsible for the purple, blue, violet and black color of these fruits, are flavonoids from the anthocyanin subclass (Valcheva-Kuzmanova et al., 2004). In comparison with blueberries, cranberries and currants, it has been shown that black chokeberry [*Aronia melanocarpa* (Michx) Elliott] has a significantly higher content of anthocyanins and phenolic compounds (Jakobek et al., 2007; Kokotkiewicz et al., 2010). The biological effects of black chokeberry are mostly due to its high antioxidative activity, which is expected to diminish the formation of reactive oxygen species and oxidative DNA-damaging agents. Many important characteristics of black chokeberry, including its detailed chemical composition, antioxidant features, flavor and nutritional value, and high resistance to pollution and pests, have been thoroughly discussed (Sidhu & Zafar, 2012). *Aronia melanocarpa* contains high concentrations of not only antioxidants (anthocyanins, biophenols, catechins, flavonoids and tannins), but also vitamins (A, B2, B6, B9, C and E) and very rare bioflavonoids (vitamin P). Due to the wide range of multifunctional biological effects, interest in studying its biological activity has grown rapidly over the past decades.

Aronia melanocarpa is used in traditional medicine (Slimestad et al., 2005) and also as a food colorant (Briddle & Timberlake, 1997), a food resource, and an ornamental plant (Kokotkiewicz et al., 2010). Many health-promoting activities of black chokeberry extracts were observed in both *in vitro* and *in vivo* studies (Kokotkiewicz et al., 2010). Recent papers suggest that *A. melanocarpa* and its products may be effective in prevention and treatment of the toxic action of some xenobiotics in humans (Borowska & Brzóska, 2016; Case et al., 2016).

Given the limited knowledge on the effects of anthocyanins and phenolic compounds in *in vivo* systems, we considered study of the effects of *A. melanocarpa* extract to be worthwhile, especially during embryonic development. Furthermore, its protective effect when applied in synergy with toxic/mutagenic/carcinogenic substances is insufficiently known. It has been cautioned that more rigorous studies need to be carried out “before putative therapeutic uses can be confidently recommended for chokeberry products” (Chrubasik et al., 2010).

To our knowledge, different *in vivo* studies of the biological effects of black chokeberry were previously conducted on mammals (Valcheva-Kuzmanova et al., 2005; Valcheva-Kuzmanova & Zhelyazkova-Savova, 2009; Kim et al., 2013; Sharif et al., 2013). Recently, Jo & Imm (2017) reported about effects of black chokeberry extract on lifespan and age-related oxidative stress in *Drosophila melanogaster* Meigen, 1830. The present work represents one of the few experimental researches in which fruit fly was used as a model system to study the biological effects of *A. melanocarpa*.

Drosophila melanogaster is a suitable test organism for examining the protective or genotoxic potential of different compounds. Metabolic activation of the enzymes in fruit flies is similar to that in the mammalian liver (Reiter et al., 2001; Lloyd & Taylor, 2010), allowing at least partial extrapolation of the results obtained. The developmental time, dynamics of embryonic and post-embryonic development and viability represent fitness components important for understanding the possible effects of various environmental factors and chemical substances. The developmental capacity coordinates the expression of morphological, physiological and behavioral traits. Organisms have different investments in development of these features, depending on the environmental conditions. The *Drosophila* wing represents one of the appropriate models to perceive developmental changes reflected on the morphological level in the presence of different nutritional and chemical compounds. The availability and allocation of resources over larval time in adults can be perceived through analysis of the wings, i.e., wing morphology depends on environmental conditions during larval development (McGuigan, 2009). In this connection, we assumed that changes in fitness components and wing size and shape in fruit flies may indicate effects of both natural fruit extracts and mutagenic chemicals. Accordingly, co-treatments with methyl methanesulfonate (MMS) were applied simultaneously with pure black chokeberry treatments. Methyl methanesulfonate is a strong mutagen classified as a carcinogen, teratogen, and an agent which may cause developmental toxicity (Anonymous, 1987). It is also an apoptosis inducer, as well as an alkylating agent. In *D. melanogaster*, somatic and sex-linked recessive lethal mutations were induced after exposure of larvae or adults to MMS in their food (Mitchell et al., 1981).

Material and Methods

This study was conducted in 2016 at the Department of Genetics of Populations and Ecogenotoxicology, Institute for Biological Research, University of Belgrade.

Aronia melanocarpa extract

A commercial fruit extract of *A. melanocarpa* made by the Armedina Company, Belgrade, Surčin, Boljevci, Serbia, from fruits that originated from the Siberian region (Russian Federation) was used in the work. The extract was prepared without preservatives, alcohols, gluten or heat treatment. The *A. melanocarpa* extract was free of proteins and fats and contained 13.6% carbohydrates and a minimum of 0.6% vitamin P. The fruit extract was added to a standard cornmeal substrate instead of water in an amount needed to obtain the required concentration. The undiluted fruit extract was taken as a 100% solution.

Drosophila melanogaster strain

Wild type *D. melanogaster* stock, Canton S, obtained from the Bloomington Drosophila Stock Center, Indiana University, Bloomington, IN, USA, was used in the experiment. This strain was maintained under the optimal laboratory conditions for *D. melanogaster* (25°C, 60% RH, 300 lux 12:12 h L:D photoperiod).

Chemicals

Methyl methanesulfonate (MMS, CAS no. 66-27-3, Sigma-Aldrich, St Louis, MI, USA) at half of the half lethal dose (LD_{50}), 1.5 mM of MMS, was used in the experimental procedure.

Experimental procedure

Standard cornmeal substrate (ST) for *Drosophila* was used for strain maintenance and egg production. The substrate was composed of 9% sugar, 10% cornmeal, 2% agar and 2% yeast. As a mold-inhibiting complement, nipagin dissolved in 96% alcohol was also added to the substrate.

Eggs (60 per vial) were randomly collected from 30 females, 3-8 d old, 8 h after they had been laid in Petri dishes. Egg-to-adult development was completed under optimal laboratory conditions, in 60 ml vials.

Three experimental groups were formed (Figure 1). The first experimental group included negative (ST) and positive (1.5 mM concentration of methyl methanesulfonate, MMS, in standard medium) control subgroups. The second experimental (treatment) group consisted of the following three subgroups: flies fed on undiluted fresh fruit extract, flies fed on 2% fruit extract and flies fed on 25% fruit extract. Experimental subgroups consisting of flies developed on undiluted fresh fruit extract, 2% fruit extract and 25% fruit extract, each supplied with 1.5 mM MMS, constituted the third experimental (co-treatment) group. Each control, treatment and co-treatment subgroup was replicated four times (Figure 1A).

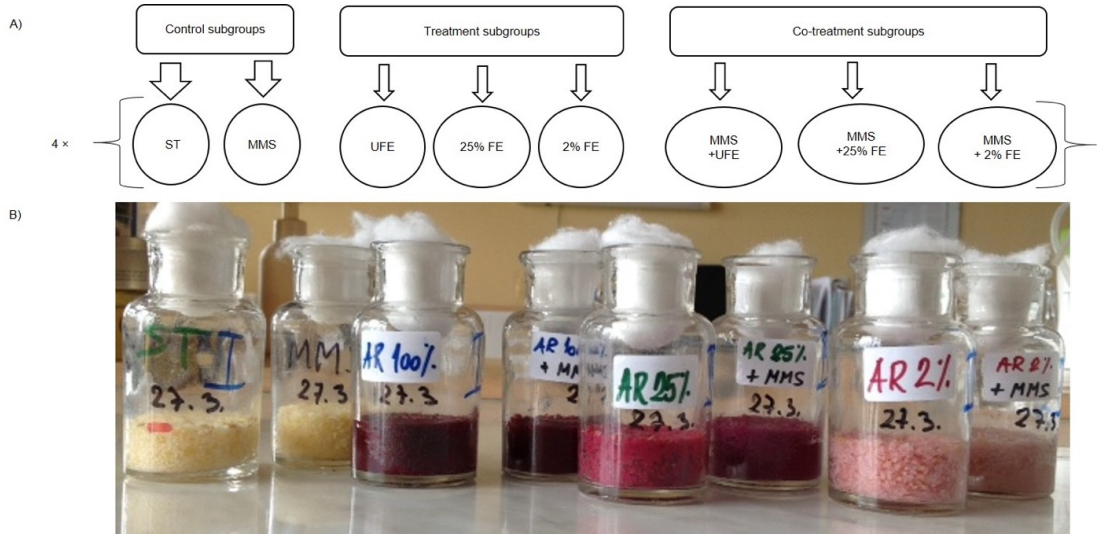

Figure 1. A) Scheme of the experimental design (Abbreviations: ST - standard cornmeal substrate - negative control subgroup; MMS - methyl methanesulfonate - positive control subgroup; UFE - undiluted fruit extract; 25% FE - 25% fruit extract; 2% FE - 2% fruit extract; MMS + UFE - MMS + undiluted fruit extract; MMS+25% FE - MMS + 25% fruit extract; MMS + 2% FE - MMS + 2% fruit extract); B) Bottles with *Drosophila melanogaster* flies developed in the aforementioned food regimes.

Fitness components

In all experimental groups (control, treatment and co-treatments subgroups), flies were scored for developmental time (D_t), hatching dynamics (Δn_t) and viability (V_i). Developmental time was measured in days, once all the adults have emerged, using the formula: $D_t = (\sum n_d \times d) / \sum n_d$, where n_d is the number of flies emerging d days after the eggs were laid. Dynamics of hatching was scored as the number of eclosed individuals at the same time of each day. Viability was calculated as the ratio of emerged adults to the number of laid eggs, according to the formula $V_i = n/N$, where N is the total number of eggs, while n is the number of adults emerging from the total number of eggs.

Statistical analysis

The Kolmogorov-Smirnov test was used to test the normality of the data for both developmental time and viability. The values of developmental time and viability were analyzed by one-way ANOVA. The Dunnett's post-hoc procedure was used to test the statistical significance of treatments and co-treatments in comparison with the negative control group.

Analysis of wing size and shape

Right wings of both sexes were used in an analysis of wing size and shape in the three experimental groups. All wings were individually photographed. Digital images were obtained on a Leica DM RB photomicroscope (Leica, Wetzlar, Germany) connected with a DFC320 CCD camera (Leica). A measurement scale of 0.5 mm was added to each wing photo in Adobe Photoshop CS5.1 (Adobe Inc, San

Jose, California, USA). Measurement units were 0.1 mm. Wings were marked with 11 reference marks using tpsDig2w32, a computer program for digitizing reference marks and outlines for geometric morphometric analyses (see: life.bio.sunysb.edu/morph/soft-dataacq.html) (Figure 2).

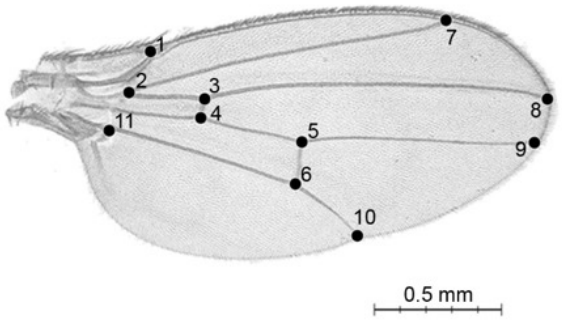

Figure 2. *Drosophila melanogaster* wing with 11 reference marks and measurement scale.

Wing size and shape were analyzed using the geometric morphometry approach in MorphoJ software (www.flywings.org.uk/MorphoJ_page.htm). Principal component analysis (PCA) was used to determine the total variability of wing size and shape in the whole sample, based on Procrustes coordinates. Regression of wing shape on centroid size was performed in order to determine whether there is a dependence between size and shape. Differences of wing centroid size as a function of treatments and co-treatments in comparison with the negative control group, as well as between the sexes, were tested using one-way ANOVA. Moreover, MANOVA was performed in order to test the variability of wing shape as a function of treatments and co-treatments in comparison with the negative control group, as well as between the sexes.

STATISTICA® version 5.0 (StatSoft, Inc., Tulsa, OK USA; www.statsoft.com) software was used for all statistical calculations.

Results

The Kolmogorov-Smirnov test confirmed that normality of variances existed in all cases, so it was not necessary to perform data transformation (Zar, 1999).

Developmental time

Mean developmental time in each experimental group is given in Table 1. The shortest developmental time (13.51 d) was observed in the negative control group (ST) and the longest (18.88 d) in the co-treatment that involved MMS with undiluted fruit extract.

One-way ANOVA indicated that different concentrations of *A. melanocarpa* in treatments and co-treatments significantly affected developmental time ($F = 72.2$; $p < 0.001$). Dunnett's post-hoc test showed significantly longer developmental time in all experimental groups in comparison with the negative control group ($p < 0.001$), except in treatments with 2 and 25% fruit extract where that flies had significantly shorter development in comparison with the other experimental groups (LSD post-hoc test, $p < 0.001$).

Table 1. Developmental time (d, mean ± SE) of *Drosophila melanogaster* in control, treatment and co-treatment subgroups

Groups	Subgroups	Developmental time
Control	negative control (ST)	13.51 ± 0.07
	positive control (MMS)	17.80 ± 0.09[a]
Treatment	2% fruit extract	13.78 ± 0.06[a,b]
	25% fruit extract	14.32 ± 0.04[a,b]
	undiluted fruit extract	18.87 ± 0.14[a]
Co-treatment	MMS + 2% fruit extract	17.50 ± 0.09[a]
	MMS + 25% fruit extract	15.06 ± 0.05[a]
	MMS + undiluted fruit extract	18.88 ± 0.14[a]

[a] $p < 0.001$, significantly longer developmental time in all experimental groups in comparison with the negative control group;
[b] $p < 0.001$, significantly shorter development in comparison with the other experimental groups.

Dynamics of hatching adults

For developmental time and dynamics of hatching, two groups were evident. Treatments with 2 and 25% *A. melanocarpa* fruit extract and MMS + 25% fruit extract co-treatment were in the first group, together with the negative control group. The second group consisted of the remaining co-treatments with MMS, treatment with the undiluted fruit extract and the positive control group.

Hatching of adults of the negative control group and from the substrate with 2% fruit extract started on day 12, while it started on days 13 and 14 from substrates with 25% fruit extract and MMS + 25% fruit extract, respectively (Figure 3). The peak of eclosion in the experimental groups with 2% fruit extract, 25% fruit extract and MMS + 25% fruit extract was observed 1 and 2 d later (51.7% hatched individuals from the substrate with 2% fruit extract on day 14, 64.0% hatched individuals from the substrate with 25% fruit extract on day 14, and 72.7% hatched individuals from the substrate with MMS + 25% extract on day 15) in comparison with the negative control group (46.5% hatched individuals on day 13). Adults from these groups finished eclosion between day 16 (those that developed on substrates with 2% and 25% fruit extract) and day 17 (in the negative control group and in flies that developed on the substrate supplied with MMS + 25% fruit extract).

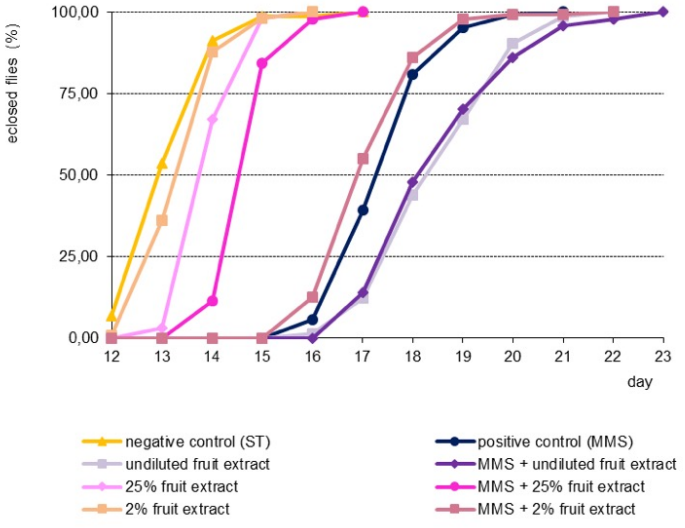

Figure 3. Dynamics of hatching of *Drosophila melanogaster* adults in all experimental subgroups.

Eclosion of adults from undiluted *A. melanocarpa* fruit extract, MMS, and co-treatments with MMS started from day 16 (MMS + 2% fruit extract) and day 17 (MMS + undiluted fruit extract). The highest number of eclosed adults (42.7%) was detected on day 17 in the MMS + 2% extract co-treatment and on day 18 on substrates with undiluted *A. melanocarpa* fruit extract (31.7%), MMS (41.6%), and their co-treatment (34.0%). Hatching in these experimental groups was finished on day 21 (from the substrate with MMS), on day 22 (from the substrate with undiluted fruit extract and that with MMS + 2% fruit extract), and on day 23 (from the substrate with MMS + undiluted fruit extract).

Viability

The ratio of eclosed adults and eggs laid was used to indicate viability, as it further contributes to the overall picture of complete development. Figure 4 shows the number of eclosed *D. melanogaster* adults in the control conditions and during exposure to the treatments and co-treatments. The lowest viability was found in treatment and co-treatment with the undiluted fruit extract, while individuals that developed on the substrate with 25% fruit extract showed the highest viability. From the highest to the lowest, viability in the experimental subgroups were as follows: 25% fruit extract > standard substrate, negative control subgroup > 2% fruit extract > MMS + 25% fruit extract > MMS + 2% fruit extract > MMS, positive control subgroup > MMS + undiluted fruit extract > undiluted fruit extract. One-way ANOVA showed significant differences in viability among treatments and co-treatments ($F = 8.83$; $p < 0.001$). Dunnett's post-hoc test indicated that viability was significantly lower in the case of treatment and co-treatment with undiluted fruit extract in comparison with the negative control group ($p < 0.05$).

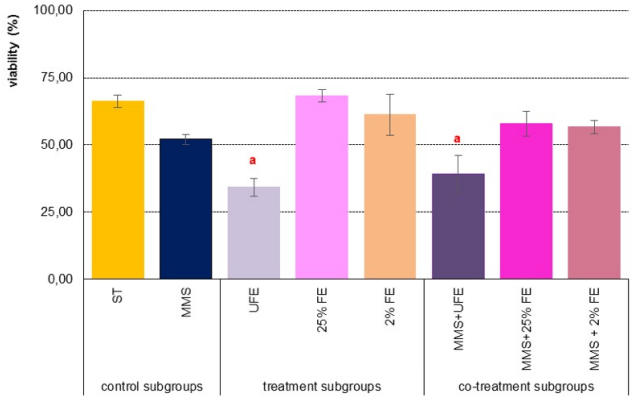

Figure 4. Mean viability ± SE (%) of *Drosophila melanogaster* in control treatment and co-treatment subgroups (Abbreviations: ST - standard cornmeal substrate - negative control subgroup; MMS - methyl methanesulfonate - positive control subgroup; UFE - undiluted fruit extract; 25% FE - 25% fruit extract; 2% FE - 2% fruit extract; MMS + UFE - MMS + undiluted fruit extract; MMS + 25% FE - MMS + 25% fruit extract; MMS + 2% FE - MMS + 2% fruit extract; a - significantly lower viability in comparison with the negative control subgroup (Dunnett's post-hoc test $p < 0.05$).

Analysis of wing size and shape

Table 2 shows mean values and the standard error of centroid size (CS). PCA showed separation of all of the aforementioned groups in regard to wing shape and size, as well as sexual dimorphism in those traits. The first principal component (PC1) described 23.1% of the variability in the whole sample, and PC2 and PC3, 12.7 and 12.4%, respectively. Most of the variability of wing size and shape in females from all experimental groups is described by the first four principal components, which constituted 55.1% of the total variability (PC1 = 19.1%, PC2 = 13.4%, PC3 = 12.6%, and PC4 = 10.1%). For males, most of the variability in wing size and shape was defined by the first three principal components, which comprised 46.1% of total variability (PC1 = 21.6%, PC2 = 13.0%, and PC3 = 11.6%). The regression curve showed that the allometry is 10.4% of the size of the whole sample ($p < 0.0001$).

Table 2. Wing centroid size (mean ± SE, in mm) in *Drosophila melanogaster* females and males from control, treatment, and co-treatment subgroups

Groups	Subgroups	Females	Males
Control	negative control (ST)	2.30 ± 0.08	2.07 ± 0.08
	positive control (MMS)	2.30 ± 0.11	2.06 ± 0.08
Treatment	2% fruit extract	2.39 ± 0.11[a]	2.12 ± 0.08[a]
	25% fruit extract	2.32 ± 0.11	2.09 ± 0.10
	undiluted fruit extract	2.17 ± 0.11[b]	2.00 ± 0.10[b]
Co-treatment	MMS + 2% fruit extract	2.34 ± 0.12	2.09 ± 0.09
	MMS + 25% fruit extract	2.33 ± 0.09	2.05 ± 0.08
	MMS + undiluted fruit extract	2.16 ± 0.10[b]	1.97 ± 0.13[b]

[a] significantly larger wings in comparison with the negative control subgroup;
[b] significantly smaller wings than flies from the negative control subgroup.

ANOVA (Table 3) and MANOVA (Table 4) confirmed sexual dimorphism for both centroid size and wing shape. Females possessed significantly larger wings than males in all experimental groups.

Table 3. Sexual dimorphism in centroid size, tested by ANOVA, in all experimental subgroups

Groups	Subgroups	df	Error df	MS	Error MS	F	
Control	negative control (ST)	1	90	11.876	0.0069	172.10	***
	positive control (MMS)	1	92	12.877	0.0098	131.66	***
Treatment	2% fruit extract	1	99	18.426	0.0089	207.60	***
	25% fruit extract	1	124	17.679	0.0112	157.94	***
	Undiluted fruit extract	1	52	0.3565	0.0110	32.47	***
Co-treatment	MMS + 2% fruit extract	1	79	12.595	0.0122	103.40	***
	MMS + 25% fruit extract	1	73	14.987	0.0081	184.16	***
	MMS + Undiluted fruit extract	1	57	0.5694	0.0131	43.32	***

*** $p<0.001$

Table 4. Sexual dimorphism in wing shape, tested by MANOVA, in all experimental subgroups

Groups	Subgroups	df	Error df	Wilk's	F	
Control	negative control (ST)	18	73	0.2214	22.41	***
	positive control (MMS)	18	75	0.2332	13.70	***
Treatment	2% fruit extract	18	82	0.1542	24.99	***
	25% fruit extract	18	107	0.2214	20.91	***
	Undiluted fruit extract	18	35	0.2589	5.56	***
Co-treatment	MMS + 2% fruit extract	18	62	0.2158	12.52	***
	MMS + 25% fruit extract	18	56	0.2407	9.82	***
	MMS + Undiluted fruit extract	18	40	0.1175	16.69	***

*** $p<0.001$

The ANOVA showed significant differences in centroid size between the control groups and some of the treated groups (Table 5). Females and males treated with 2% extract had significantly larger wings compared with females and males from the negative control group. Flies that developed on undiluted fruit extract and MMS + undiluted fruit extract possessed smaller wings than flies from the negative control subgroup (Table 5).

Table 5. One-way ANOVA of wing centroid size in females and males subjected to treatments and co-treatments compared with the negative control subgroup

Groups	Subgroups	Sex	df	Error df	MS	Error MS	F	
Control	MMS - positive control	♀	1	105	0.0000	0.0095	0.00	
		♂	1	77	0.0036	0.0068	0.53	
Treatment	2% fruit extract	♀	1	95	0.1839	0.0093	19.84	***
		♂	1	94	0.0467	0.0066	7.10	**
	25% fruit extract	♀	1	111	0.0127	0.0097	1.31	
		♂	1	103	0.0036	0.0091	0.40	
	Undiluted fruit extract	♀	1	70	0.2997	0.0085	35.24	***
		♂	1	72	0.0949	0.0083	11.44	**
Co-treatment	MMS + 2% fruit extract	♀	1	89	0.0326	0.0108	3.01	
		♂	1	80	0.0049	0.0077	0.63	
	MMS + 25% fruit extract	♀	1	89	0.0217	0.0078	2.77	
		♂	1	74	0.0139	0.0070	1.99	
	MMS + Undiluted fruit extract	♀	1	72	0.3223	0.0078	41.55	***
		♂	1	75	0.2226	0.0108	20.57	***

**p<0.01; ***p<0.001; ♀ - female, ♂ - male

The MANOVA showed significant differences of wing shape without allometry effects between the control group and treatments with 2% fruit extract and undiluted fruit extract, as well as between the control group and co-treatment with MMS + undiluted fruit extract in both sexes (Table 6). It was also noticed that only females in treatment with MMS and in co-treatment with MMS + 2% fruit extract had a significantly different wing shape without allometry effects in comparison with the negative control group. Differences of wing shape for females and males are shown in Figure 5.

Table 6. One-way MANOVA of wing shape without allometry effects in females and males subjected to treatments and co-treatments compared with the negative control subgroup

Groups	Subgroups		df	Error df	Wilk's	F	
Control	positive control (MMS)	♀	18	77	0.617	2.66	**
		♂	18	75	0.759	1.33	
Treatment	2% fruit extract	♀	18	92	0.533	4.49	***
		♂	18	85	0.625	2.83	***
	25% fruit extract	♀	18	50	0.841	0.53	
		♂	18	54	0.852	0.52	
	Undiluted fruit extract	♀	18	71	0.475	4.35	***
		♂	18	61	0.519	3.14	***
Co-treatment	MMS + 2% fruit extract	♀	18	70	0.627	2.31	**
		♂	18	56	0.658	1.62	
	MMS + 25% fruit extract	♀	18	54	0.869	0.45	
		♂	18	52	0.823	0.62	
	MMS + Undiluted fruit extract	♀	18	87	0.661	2.47	**
		♂	18	59	0.552	2.66	**

**p<0.01; ***p<0.001; ♀ - female, ♂ - male

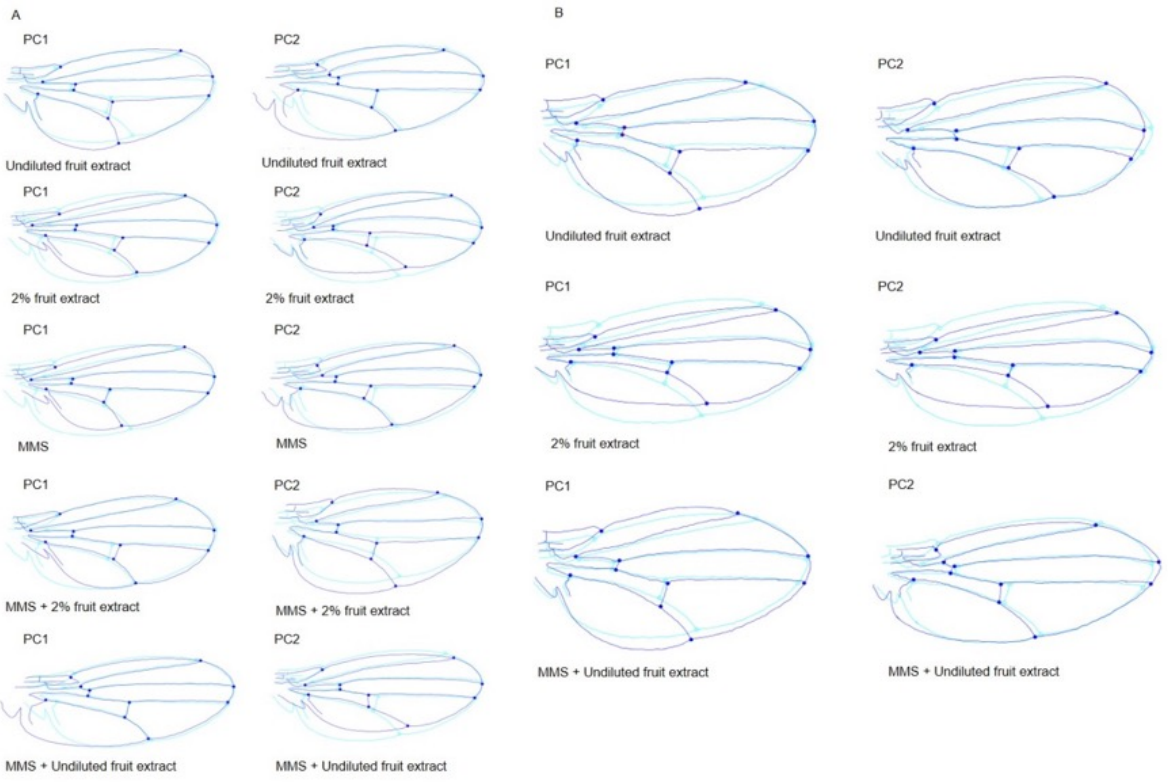

Figure 5. Differences in wing shape in females (A) and in males (B) from the positive control subgroup, treatment subgroups and co-treatment subgroups in relation to the negative control subgroup.

Discussion

It is already known that bioactive substances, including components from black chokeberry, may reduce the induction of mutagenesis and prevent many degenerative diseases, cancer among them. However, some of those components can be toxic or even increase mortality (Bjelakovic et al., 2008). Given that a large number of bioactive plant products are used as supplements in human and animal diets, it is crucial to determine both their protective and their harmful concentrations (doses). In the study presented here, different concentrations of *A. melanocarpa* fruit extract were added to the food of *D. melanogaster* larvae, and its biological effects were monitored during development (fitness traits) and in adults of the first generation of offspring (morphological traits).

During insect development, larval pattern building and metamorphosis are considered as two different developmental processes that contribute to the formation of adults (Nascimento et al., 2002). It is known that the developmental time of each preimaginal stage, including the embryonic period (phase), regulates the time needed for body development, fitting the developmental time into the environmental conditions. Accordingly, the developmental time represents an adaptive trait and is thereby a crucial selective component in the existence of insects living in environments that are changeable in regard to the source and quality of the food. Whereas, viability, which reflects successfully completed development from eggs to adults, can be also considered as an important factor in assessing the effect of different environmental conditions.

The obtained data indicate that a significantly shorter development and higher viability were observed only in flies that developed on substrates containing 2 and 25% *A. melanocarpa* fruit extract in comparison to the other treatments and co-treatments. However, flies that developed on undiluted fruit extract, on MMS

+ undiluted fruit extract, and on MMS alone exhibited a prolonged developmental time and had lower viability. Only a mixture of MMS + 25% extract showed positive effects on fitness components in comparison with other MMS co-treatments. This may indicate a possible chemopreventive potential of black chokeberry when used as a fruit extract diluted to quarter-strength. Previously published studies confirmed the wide range of positive biological effects of *A. melanocarpa*, including antioxidant, antibacterial, antiviral, antimutagenic, anticancerogenic, antidiabetic and antiinflammatory effects, as well as its cardioprotective, hepatoprotective and gastroprotective properties, under both *in vivo* and *in vitro* conditions (Gasiorowski et al., 1997; Simeonov et al., 2002; Surh, 2003; Matsumoto et al., 2004; Valcheva-Kuzmanova et al., 2004; Ohgami et al., 2005; Puupponen-Pimiä et al., 2005; Valcheva-Kuzmanova et al., 2007; Zafra-Stone et al., 2007; Nikolova et al., 2012).

Considering these results, black chokeberry fruit extract could be expected to have a protective effect against MMS, i.e., it can fix disturbances induced during development. However, the present study showed that the influence of black chokeberry fruit extract on fitness components was not beneficial in all cases and was even detrimental in higher concentrations.

Wing size and shape in *Drosophila* represent a convenient model for investigation, for both measurements and analysis, of the developmental control of phenotypic variation (Houle et al., 2010). The polygenic determination of wing shape, which is independent from that of wing size, is based on the action of genes generally involved in development and/or metabolic functions, especially those related to the regulation of different cellular processes, such as motility, adhesion, communication, and signal transduction (Carreira et al., 2011). The final size and shape of the *Drosophila* wing are the result of tight coordination of cell proliferation, apoptosis, cell allocation and mitotic orientation (de Celis, 2003; Palsson & Gibson, 2004; Baena-López et al., 2005; Dworkin & Gibson, 2006; Trotta et al., 2011). In that sense, the patterns of wing size and shape variation were useful in identifying possible morphological responses to different concentrations of *A. melanocarpa* extract, as well as to co-treatments with MMS.

In the present study, females had significantly larger wings than males in all experimental groups, as expected, since sexual dimorphism in body size is well known in *D. melanogaster* (Testa et al., 2013; Takahashi & Blanckenhorn, 2015). However, females and males treated with undiluted fruit extract and with MMS + undiluted fruit extract possessed significantly smaller wings than females and males from the negative control subgroup. The used of 1.5 mM MMS in the experimental procedure, as half of the half lethal dose (LD50), had no significant effect on wing centroid size comparing to negative control subgroup. Significant differences in wing centroid size obtained in treatment undiluted fruit extract and MMS + undiluted fruit extract can be explained as the effect of fruit extract. Flies feeding on these substrates exhibited a prolonged developmental time and had lower viability and therefore, possibly, had significantly smaller wing centroid size comparing to negative control subgroup. Also, the high amount of sugar in undiluted fruit extract can cause smaller wings.

Females and males treated with 2% fruit extract had significantly larger wings compared with females and males from the negative control subgroup, and these differences can be influenced by the sugar linked to the anthocyanidin. The biological activity of *A. melanocarpa* extracts have been reported to be influenced by the sugar units linked to the anthocyanidin (Bräunlich et al., 2013).

Flies in the co-treatment MMS + 2% fruit extract had slightly smaller wings and prolonged developmental time, but there were no differences compared to negative control group. The co-treatments effect was mostly reflected through the components of the fitness. This suggests the possibility that high concentrations of *A. melanocarpa* fruit extract have a negative impact on wing size. In the case of wing shape, significant differences were observed between some of the treatments and co-treatments in comparison with the negative control subgroup, also confirming their impact on wing development. Carreira et al. (2011) shows that more than 63% of induced mutations affected wing shape in one or both sexes,

although only 33% showed significant differences in both males and females. The reason for the absence of differences in wing shape between males from the positive subgroup comparing to negative control subgroup can be the results of sex-linked recessive changes that are expressed only in homozygous conditions. Hence, differences in wing shape between females from the MMS subgroup compared to the negative control subgroup were observed. Many genes contributing to the wing shape in a sexually dimorphic manner because of the complexity of its genetic architecture (Carreira et al., 2011). Different levels of carbohydrates and proteins in substrates on which *Drosophila* is feeding may change the duration and stability of development and, therefore, the size, shape and symmetry of certain adult traits (Shingleton, 2010; Trajković et al., 2013).

Aronia melanocarpa represents one of the richest plant sources of procyanidins and anthocyanins, which are potent antioxidants that destroy free radicals (Santrucek & Krepdka, 1988) and as a result have a wide range of potential medical, therapeutic and nutritive effects (Lala et al., 2006). It was found that anthocyanins isolated from black chokeberry significantly inhibit the mutagenic activity of benzo (a) pyrene and 2-aminofluorene (Gasiorowski et al., 1997). In addition, in the presence of black chokeberry extract, the histopathological changes caused by N-nitrosamines in the rat liver did not occur (Atanasova-Goranova et al., 1997). Beneficial effects of *A. melanocarpa* on behavior were also observed in animal models, such effects being expressed in increased locomotion, and reduction of anxiety-like and depression-like behavior (Tomić et al., 2016). However, the results of our research strongly indicate the need to define the range of concentrations which can provide a proper balance and be safely used for human disease prevention and therapeutic purposes. Otherwise, without accurate determination, either of both extremes, oxidative and antioxidative stress, might arise and could be deleterious (Poljsak et al., 2013). In conclusion, to judge from the results of analyzing both fitness and morphological traits in the present study, it would appear that *A. melanocarpa* extract in a concentration of 25%, administered together with MMS, decreases the deleterious effects of MMS during development.

Further studies using different genotoxic tests are needed in order to analyze mechanisms governing the action of black chokeberry fruit extract and its components. The similarity of metabolic pathways in *Drosophila* and mammals (Reiter et al., 2001; Leopold & Perrimon, 2007; Lloyd & Taylor, 2010; Owusu-Ansah & Perrimon, 2014) would make the results of these tests widely applicable.

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Original article (Orijinal araştırma)

Pest status, prevalence and molecular identification of *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 (Coleoptera: Curculionidae) in saffron[1]

Safran alanlarında, *Hoplopteridius lutosus anatolicus* Osella & Lodos,1979 (Coleoptera: Curculionidae)'un zarar durumu, yaygınlığı ve moleküler teşhisi

Cenk YÜCEL[2*] **Didem CORAL ŞAHİN[2]**

Abstract

Saffron (*Crocus sativus* L.) is an endangered plant species that has been cultivated in a small field in Safranbolu District of Karabük Province, Turkey. Although the existence of *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 (Coleoptera: Curculionidae) was known for Turkey, the present study determined its pest status and, prevalence with respect to its endangered host plant saffron in the Safranbolu District of Karabük between 2015 and 2016. *Hoplopteridius lutosus anatolicus* larvae were found to harm saffron corms starting from the second half of May till the end of June with the harmful effects continuing even after harvest during storage. While the pest is not normally found in the newly established saffron fields, *H. lutosus anatolicus* establishes in these fields with a prevalence rate of 4-35% in subsequent years. In addition, CO1 sequences for the molecular identification were performed for quick and reliable identification in future studies and these novel sequences for the pest were uploaded to BOLD and GenBank databases.

Keywords: CO1, corm damage, *Crocus sativus*, molecular taxonomy, Molytinae, Safranbolu

Öz

Safranbolu ilçesinde küçük bir alanda kültürü yapılan safranın (*Crocus sativus* L.) nesli tehlike altındadır. Türkiye'de daha önce varlığı bilinen, ancak yaygınlığı ve konukçu bitkisi olan safrandaki durumu bilinmeyen Safran hortumlu böceği, *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 (Coleoptera: Curculionidae)'nin Karabük ili Safranbolu ilçesindeki safran alanlarındaki yaygınlığı ve yoğunluğunun belirlenmesi amacıyla 2015-2016 yıllarında yapılmıştır. Çalışma sonucunda Safranbolu ilçesi safran alanlarında *H. lutosus anatolicus*'un safran soğanlarında larva zararının mayıs ayının ikinci yarısından haziran sonuna kadar olduğu, hasat sonrasında zararın depoda devam ettiği belirlenmiştir. Sürvey çalışmalarında yeni tesis edilen tarlalarda zararlı görülmezken ilerleyen yıllarda zararlının tarlaya yerleştiği ve bulaşıklık oranını artarak %4-35 olduğu saptanmıştır. Ayrıca ilerleyen dönemde yapılacak çalışmalarda zararlının hızlı ve güvenilir teşhisinin yapılabilmesi için CO1 gen dizilemesi yapılmıştır ve BOLD ve Genbank veri tabanına bu zararlı için yapılan ilk yükleme niteliğindedir.

Anahtar sözcükler: CO1, bitki soğanı zararı, *Crocus sativus*, moleküler taksonomi, Molytinae, Safranbolu

[1] Part of this work was presented as a oral presentation at the XIII. Congress of Ecology and Environment with International Participation (12-15 September 2017) Edirne, Turkey.

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Introduction

Of the 85 colchicums (*Crocus* spp.) found around the world, Turkey has 72 taxa consisting of 36 species and 36 subspecies, with 40 endemic taxa (19 species and 21 subspecies). Of these, only saffron is cultivated commercially (Kravkaz et al., 2006). Saffron (*Crocus sativus* L., Iridaceae) has been cultivated for a long time for cosmetic, culinary, dyeing and medicinal purposes. It is cultivated in very limited areas of Safranbolu District in Karabük Province and the Harran Plains in Şanlıurfa Province in Turkey. Saffron cultivation is a labor-intensive work based on patience with very delicate and attentive care for 3-4 years given to the saffron corms planted during August and September. Saffron is one of the most expensive spices in the world because about 100-140 thousand saffron flowers are needed to produce 1 kg of dry saffron and its cultivation is as difficult. China, India and Iran are the major saffron producers in the world.

There have been relatively few studies on saffron pests and diseases. It is known that *Rhizoglyphus robini* Claparede, 1869 and *Thrips tabaci* Lindeman, 1889 are economically damaging pests, as well as other mite, thrips and nematode species (Kafi et al., 2006; Rahimi et al., 2008). Satyagopal et al. (2014), reported that pest control is needed for *Anaphothrips obscurus* (Müller, 1776), *Thrips flavus* Schrank, 1776, *Microcephalothrips abdominalis* (Crawford, 1910), *Rhizoglyphus echinopus (*Fumouze & Robin, 1868) and *R. robini* since they damage the above ground parts of saffron. Also, Chandel et al. (1996) reported that *Mylabris macilenta* Marseul, 1873 feeds on saffron flowers and causes production losses in the saffron fields infected with the pest in India.

By decreasing the yield and sometimes totally destroying the whole crop, pests only add to the already overwhelming labor-intensive process of saffron cultivation, which threatens the viability of saffron farming. Therefore, in order to prevent economic losses, it is important to determine saffron pests and their relationship with the host plant for different periods during the cultivation process.

An accurate and fast taxonomic identification of pests is crucial for proper agricultural management. However, morphological identification methods are time-consuming, require high taxonomical expertise and usually only provides adult stage identification. To resolve this problem, molecular methods offer a reliable and easy DNA-based identification tool called DNA Barcoding. The DNA Barcoding identifies target species using short DNA sequences as barcodes (Hebert et al., 2003), in particular, a 658-bp fragment of the mitochondrial cytochrome *c* oxidase (CO1) gene. Since DNA Barcoding is an emerging tool, databases should be constructed on the basis of specimens identified by specialists to make identification comprehensive and reliable (Jalali, 2015).

The Molytinae (Curculionidae) genus *Hoplopteridius* Daniel, 1908 (Coleoptera: Curculionidae) has five species distributed only in the Palearctic Region and the species *Hoplopteridius lutosus* (Frivaldszky, 1835) represents four subspecies known from Bulgaria, Italy, Romania and Turkey (Alonso-Zarazaga et al., 2017). One of these subspecies, *Hoplopteridius lutosus anatolicus* Osella & Lodos, 1979 was described by Osella & Lodos (1979) from Safranbolu indicating it is a potential pest of saffron *Crocus vernus* (L.) Hill. In the present study, the status and prevalence of *H. lutosus anatolicus* was determined with data collected in 2015 and 2016 in Safranbolu District, Karabük Province, Turkey.

Material and Methods

Survey and sampling

Studies were conducted in the saffron fields of Safranbolu District, Karabük Province during 2015 and 2016 in order to determine the pest status and prevalence of *H. lutosus anatolicus* (Table 1). Sampling 0.1 ha, a total of 100 saffron corms were collected randomly in 10 different spots along 10 different rows in each field. The presence of the pest and health state of the saffron corms were then visually determined. The number of subsamples was increased according to the field size (Jarvis & Guthrie, 1987).

Table 1. Duration of the surveys and location of fields surveyed

Village	Field 1	Field 2
Merkez	0.1 ha, 5-6 years 41°14'34" N, 32°40'36" E, 425 m	-
Yazıköy	0.2 ha, 2-3 years 41°14'26" N, 32°44'23" E, 478 m	0.3 ha, 0-1 year 41°14'20" N, 32°44'43" E, 490 m
Çercen	0.1 ha, 2-3 years 41°11'26" N, 32°48'18" E, 609 m	0.2 ha, 3-4 years 41°11'43" N, 32°48'31" E, 645 m

Laboratory studies

Fifty damaged corms were randomly collected from each field and kept in a climate chamber at 24±1ºC, 65±5% RH in the dark to allow any insect larvae to mature. Species identification was confirmed based on adult insects from saffron plants obtained from these corms. The collected specimens were preserved in absolute ethanol. The specimens were identified morphologically and from genitalia preparations.

Molecular studies

DNA was extracted from two different populations. Abdomen and three pairs legs were removed, and DNA extracted using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) with slight modifications as described by Magoga et al. (2016). Extracted DNA was used as template for a 658-bp fragment of the mitochondrial CO1 gene amplified by PCR using universal primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer et al., 1994). PCR reactions were performed in a 25µl final volume reaction mix and PCR thermal profile as in Montagna et al. (2017). Successful amplifications were determined by gel electrophoresis and sequenced bidirectionally by ABI Technology (Applied Biosystems, Foster City, CA, USA). The electropherograms obtained were manually edited, checked for double peaks and frameshifts by using Geneious Pro 5.5 (Biomatters Ltd., Auckland, New Zealand), and primers were removed. Each sequence was translated to protein in the EMBOSS transeq tool (www.ebi.ac.uk/Tools/emboss/transeq) to be sure that they complied with an open reading frame. Sequences were aligned at codon level using MUSCLE (Edgar, 2004) in MEGA (Tamura et al., 2013). Finally, consensus sequences were uploaded to the BOLD and GenBank (accession number ADM7695) databases.

Results and Discussion

The study was conducted in five different fields in Safranbolu District, which is probably the complete range of this endangered host in saffron fields of Turkey. In order to determine the prevalence of the pest, two different surveys were conducted on 15 October 2015 after the plantation of corms and on 1 June 2016 just before harvesting. According to the survey, all saffron fields, except the newly planted ones, were found to be infested with the pest. The corms were damaged due to the feeding of the larvae on corms (Figure 1A). It was found that the pest matured into adults and went into hibernation state under the soil. Damage to corms was detected in all fields except the newly established field in Yazıköy. It was determined that corm damage was only caused by the larvae.

Prevalence of the pest and damage to corms were examined in the fields again in June 2016 just before saffron harvest. Pest damage to the corms had increased in four of the five fields, and the already damaged corms had putrefied. No damage to corms was observed in the newly established saffron field located in Yazıköy (Figure 2).

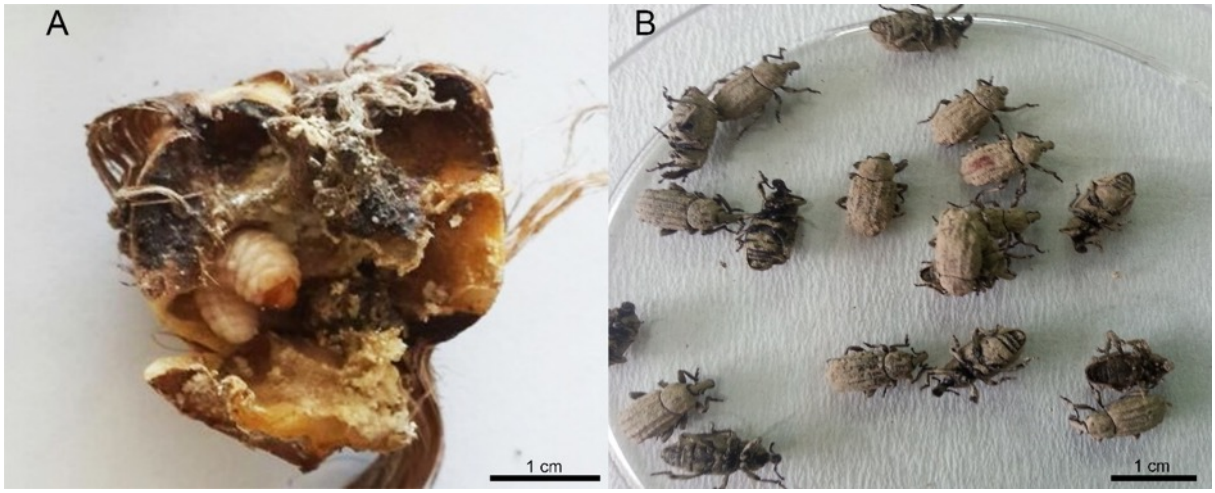

Figure 1. A) Corm damage by larvae of *Hoplopteridius lutosus anatolicus*; B) adults reared from larval stage in laboratory.

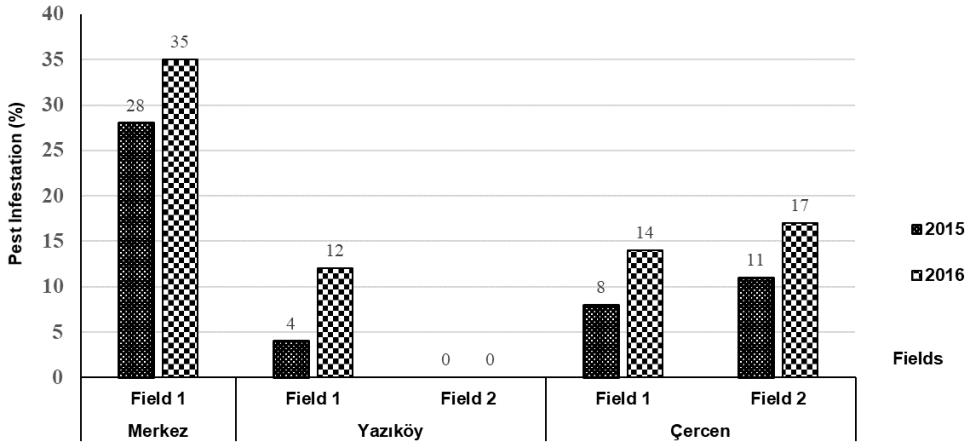

Figure 2. Prevalence of *Hoplopteridius lutosus anatolicus* on saffron corms in 2015 and 2016.

Prevalence rates of 4-28% and 11-35% were found in corms during the first and second samplings, respectively. It was also found that the prevalence rate rises with the age of the plantation (1-4 years). While the pest was not found in the newly planted field, damage to saffron corms was evident in the second year, and it increased in the third and fourth years. All previous studies (except Yücel et al., 2017) were for identification purposes only without any investigation of damage to saffron corms, economic loss, and host-pest interactions. Yücel et al. (2017) reported that the pest damaged saffron corms, which is consistent with our findings.

It is quite difficult to collect the pest since it goes into hibernation after maturing into an adult under the soil away from the corms. For this reason, the infected corms were collected before harvest in June in order to allow the larvae to mature to adults in the laboratory (Figure 1).

The larvae in culture matured into adults within an 87-d period between 3 June 2016 and 29 August 2016. Only 4-26% of larvae matured into adults because of the difficulties maintaining the infested corms in the laboratory (Figure 1B, Table 2). Although the adults were transferred onto sprouting saffron corms, no feeding or mating behavior was observed.

Table 2. Rate (%) of maturation of larvae to adults for 50 infested corms under laboratory conditions

Village	Field 1	Field 2
Merkez	26	-
Yazıköy	8	0
Çercen	4	14

From the present study, species *H. lutosus anatolicus* is reported for the second time in Turkey and for the first time in an agricultural production area. Habitus and genitalia images of the pest are given in Figure 3. Antennal and genitalia features are the morphological characteristics that are used for the identifications (Osella & Lodos, 1979). Osella & Lodos (1979) detected the pest in *Crocus vernus* (L.) (spring crocus) in Safranbolu (Karabük), Ballıdağ (Kastamonu) and Abant (Bolu). Also, Lodos et al. (2003) reported *H. lutosus anatolicus* in *Crocus* sp. in Safranbolu and *Hoplopteridius chaudoirii* (Hochhuth, 1847) in Erciyes Mountain (Kayseri).

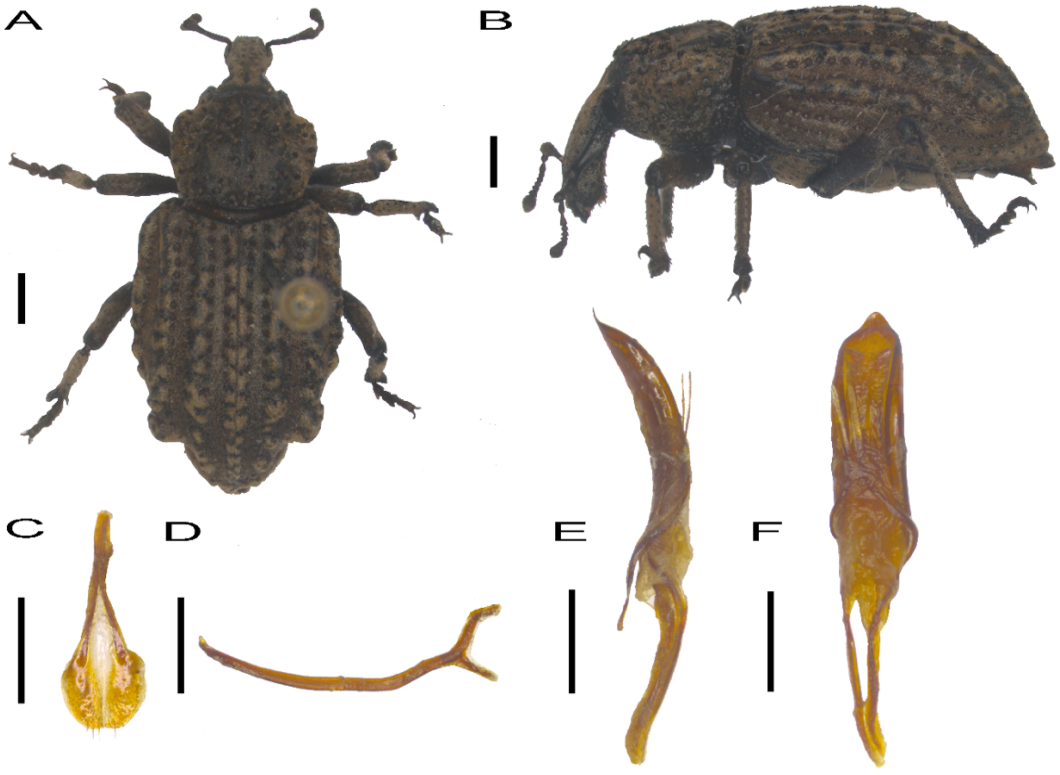

Figure 3. Images of *Hoplopteridius lutosus anatolicus*: A and B) habitus; C) spiculum ventrale (female genitalia); D) male genitalia as spiculum gastrale; E and F) aedeagus (Scale bars = 1 mm).

In Spain, *Ceutorhynchus pulvinatus* Gyllenhal, 1837 (Coleoptera: Curculionidae) was observed in high numbers and other Coleoptera species have been found in saffron fields (Cirujeda et al., 2016). However, no information is given about the level of damage to the saffron. *Mylabris macilenta* has also been found in saffron, but the report provided no information on damage (Chandel et al., 1996).

Only a limited number of studies has been conducted on *Hoplopteridius* and these studies were on detection and distribution (Osella & Lodos, 1979; Lodos et al., 2003). There are no reports about damage to saffron and prevalence of the pest in production areas. In the present study, considerable loss in saffron was confirmed by field observations and producer feedback. It was determined that the pest reaches a high density especially in aging saffron fields in which the producers have not applied adequate pest management. Saffron cropping provides two types of produce. The first is the production of saffron spices from the flowers, and the second is saffron corms sold as planting material. The insect damage is directly to the saffron corms. As evident in Figure 1, the corms are subject to severe damage. In addition, fungal infections occur in the damaged corms. As a result, these corms are no longer able to produce shoots and consequently no flowers. As a result, the usual 15 t/ha corm yield is reduced to 1-1.5 t/ha. Due to the high economic losses, most of the saffron producers have shifted to producing other crops.

In molecular studies, two CO1 sequences (639 and 658 bp), with a base composition of A = 29.4%; C = 19.6%; G = 16.1%; T = 34.9% were successfully obtained after performing quality control analysis. This study provided CO1 sequences of *H. lutosus anatolicus* for BOLD and GenBank databases for the first time. This is a new record for the genus *Hoplopteridius* as well. Due to the absence of sequences in these databases, it was not possible to confirm the morphological identification using molecular analysis. The Blast analysis showed at least 17% nucleotide distance from *Plinthus* Germar, 1817 which has been proposed as one of the closest genera to *Hoplopteridius* (Davidian, 2008). In BIN analysis, it was also determined that the distance to nearest neighbor species (*Plinthus pseudostarcki* Meregalli, 1985) is 17.4%. In addition, certain morphological identification specialists support the accuracy of the sequences. Further studies could be undertaken to increase the number of sequences of the genus in databases.

In conclusion, the present study determined *H. lutosus anatolicus* as a damaging pest for saffron and reported the species for the second time in Turkey after 36 years. The primary damage is caused by pest larvae feeding inside the saffron corms and consequently decreasing the chance of shooting. This, therefore, decreases the flower yield. Although the pest was not detected in a newly established fields, it is established by the second year with moderate prevalence, with damage to corms increasing in the third and fourth years. Saffron corms are stored after harvesting and sold in August as foundation stock. Feeding of the pest on corms permit fungal infections, that cause secondary damage and result in total rotting of the corms.

The present study has determined the status and prevalence of *H. lutosus anatolicus*, which is an important step in preserving and increasing the saffron production of Turkey, which is important both economically and culturally. Moreover, CO1 sequences were submitted to BOLD and GenBank databases for the first time for further DNA Barcoding studies and molecular identification, and are the first sequences submitted for this genus.

Acknowledgments

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Original article (Orijinal araştırma)

A new invasive species in Turkey: *Dacus ciliatus* Loew, 1862 (Diptera: Tephritidae)

Türkiye'de yeni bir istilacı tür: *Dacus ciliatus* Loew, 1862 (Diptera: Tephritidae)

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Abstract

In 2018, a problem that emerged in cucumber cultivation areas of southeastern Anatolia Region (Diyarbakır, Mardin, Siirt and Şırnak) was investigated. *Dacus ciliatus* Loew, 1862 (Diptera: Tephritidae) was found in the region causing damage to melons and watermelons. *Dacus ciliatus* is a polyphagous species, included on in the European quarantine list by the European and Mediterranean Plant Protection Organization, that causes economic losses in plants, especially in the Cucurbitaceae. This report of *D. ciliatus* is the first for agriculture and the fauna Tephritidae in Turkey.

Keywords: Cucurbitaceae, Lesser pumpkin fly, Tephritidae, Turkey

Öz

2018 yılında Güneydoğu Anadolu Bölgesi (Diyarbakır, Mardin, Siirt ve Şırnak) hıyar ekiliş alanlarında ortaya çıkan bir sorun üzerine ele alınmış olup, bölgede başta hıyar olmak üzere kavun ve karpuzlarda zarar yapan türün *Dacus ciliatus* Loew, 1862 (Diptera: Tephritidae) olduğu tespit edilmiştir. *Dacus ciliatus* Avrupa ve Akdeniz Bitki Koruma Organizasyonu tarafından A1 karantina listesinde bulunan ve özellikle Cucurbitaceae familyasındaki bitkilerde ekonomik önemde kayıplara neden olan polifag bir türdür. *D. ciliatus*'un bu raporu Türkiye tarım alanları ve Tephritidae faunası için ilk kayıttır.

Anahtar sözcükler: Cucurbitaceae, Küçük kabaksineği, Tephritidae, Türkiye

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Introduction

Tephritidae (Diptera) is the most economically important family in the world. The worldwide fauna of Tephritidae comprises nearly 4800 species in about 500 genera (Freidberg, 2006; Norrbom, 2010; Pape et al., 2011). According to Carroll et al. (2002), 190 species of fruit flies have economic impacts around the world. To date, 162 species of fruit flies have been recorded from Turkey (Yaran & Kütük, 2016; Yaran et al., 2018). Most species are phytophagous and many pest species have been studied extensively due to the damage they cause to plants of economic interest (Aluja & Norrbom, 1999; Aluja & Mangan, 2008). The family contains numerous fruit-infesting species of economic importance, such as *Ceratitis capitata* (Wiedemann, 1824), *Bactrocera oleae* (Rossi, 1790), *Bactrocera dorsalis* (Hendel, 1912), *Anastrepha fraterculus* (Wiedemann, 1830), *Toxotrypana curvicauda* Gerstaecker, 1860, *Rhagoletis cerasi* (Linnaeus, 1758) and *Rhagoletis pomonella* (Walsh, 1867), and many other noxious pests. A couple of species are profitable and efficaciously used in biological control of some weeds (White & Elson-Harris, 1994).

Fruit flies are one of the most important quarantine pests worldwide. The lesser pumpkin fly, *Dacus ciliatus* Loew, 1862 (Diptera: Tephritidae) is a serious invasive pest of cucurbits throughout Africa, being found from Egypt to South Africa (Hancock, 1989). This fly is also a pest in many parts of Asia. *Dacus ciliatus* has been recorded in Israel (Norrbom et al., 1999) and Oman (Azam et al., 2004). This fly is classified as an A1 quarantine pest by the European and Mediterranean Plant Protection Organization (2018). *Dacus ciliatus* is a brown-orange colored species with yellow spots at the anterior shoulders of the thorax and posteriorly near the abdomen. The wings have an expanded anterior dark-brown band. Fully-grown maggots are about 10 mm long (Azab et al., 1971).

Dacus ciliatus is a polyphagous fly, that damages crops in the Cucurbitaceae (Vayssières et al., 2002). Larvae of *D. ciliatus* develop in the fruits. Infected fruit commonly has oviposition pits around which necrosis can develop. In serious infestations the fruit may fall leaving just the skin (El-Nahal et al., 1971). According to Bhatia & Mahto (1968) and Viraktamath et al. (2003), this species mostly occurs in high population density in cultivated melons and to a lesser degree in wild cucurbits, and causes serious damage to crops India.

This study was conducted in southeastern Anatolia to determine the presence of *Dacus ciliatus*. In this paper, adult and wing figures material are described, and distribution and host plants of *D. ciliatus* are given.

Material and Methods

Fruit infested with larvae were collected from *C. sativus* crops and adults caught by sweep net in Diyarbakır, Mardin, Siirt and Şırnak Provinces of Turkey in 2018 (Figure 1). Samples with larvae were placed in paper bags, examined in the laboratory and placed in the plastic boxes for the emergence of adult flies. The adult flies from traps and containers were placed in 70% ethyl alcohol for identification. Specimens were lodged in the museum collection of the Biological Control Laboratory in Plant Protection Department of Agriculture Faculty, Çukurova University, Adana, Turkey. Photos were taken with a Nikon SMZ 745 T microscope. Species identification was made using the keys and descriptions of Drew et al. (1998) and White (2006).

Figure 1. Locations sampled for *Dacus ciliatus* (satellite image from Anonymous, 2018).

Result and Discussion

***Dacus ciliatus* Loew, 1862** (Figures 2-4)

Material examined. Diyarbakır (37º55'33" N, 40º12'38" E, 701 m), 08.IX.2018, 3♀♀; Mardin, Midyat, (37º25'22" N, 41º22'18" E, 988 m) 08.IX.2018, 5♀♀; Mardin, Ömerli (37º24'30" N, 40º57'16" E, 1.172 m), 08.IX.2018, 3♀♀; Siirt (37º56'03" N, 41º56'32" E, 917 m), 09.IX.2018, 4♀♀; Şırnak, Cizre (37º20'35" N, 42º11'01" E, 460 m), 06.IX.2018, 2♀♀.

Host. Cucurbits, including cucumber, gourd, melon, pumpkin, water melon and others (CABI/EPPO, 2018).

Distribution. Africa, Indian Ocean islands, East Asia and West Asia (including Iran, Israel, Jordan and Oman) (Hancock, 1989; White & Elson-Harris, 1994; Norrbom et al., 1999; Maklakov et al., 2001; Azam et al., 2004; Vayssières et al., 2008).

Remarks. In Turkey this pest was first observed in September 2018 in cucumber in Şırnak. *Dacus ciliatus* is an important pest of Cucurbitaceae and it is an EPPO A1 quarantine pest causing fruit damage and yield losses. Damage due to infestations of *D. ciliatus* was detected in the southeastern Anatolia (including Diyarbakır, Mardin, Siirt and Şırnak).

Dacus ciliatus is smaller than *Bactrocera cucurbitae* (Coquillett, 1849), scutum preponderantly red-brown. Costal band in the wing is thin, before wing apex; enlarged a small spot at apex. Abdomen has two black spots especially in females (White & Elson-Harris, 1994). *Dacus ciliatus* was first reported in India in 1914 (Kandakoor et al., 2013). This species is significant and widespread pest of Cucurbitaceae in Africa and Asia (CABI/EPPO, 2018). Adults flying and shipment of fruit are chief mechanisms of spread. Establishment within a country can have negative economic impacts for exports. *Dacus ciliatus* is considered a phytosanitary hazard in countries with suitable climates and host crops. Future studies on the geographical distribution and impact of this pest in cucurbits in Turkey, as well as in other commercial and native fruit species, should be considered a priority. In addition, natural enemies of *D. ciliatus* should be determined as these could help reduce the rate of spread of this species.

In this paper, we report the occurrence of *D. ciliatus* on *Cucumis sativus* L. (Cucurbitaceae), an important invasive agricultural pest, in Turkey.

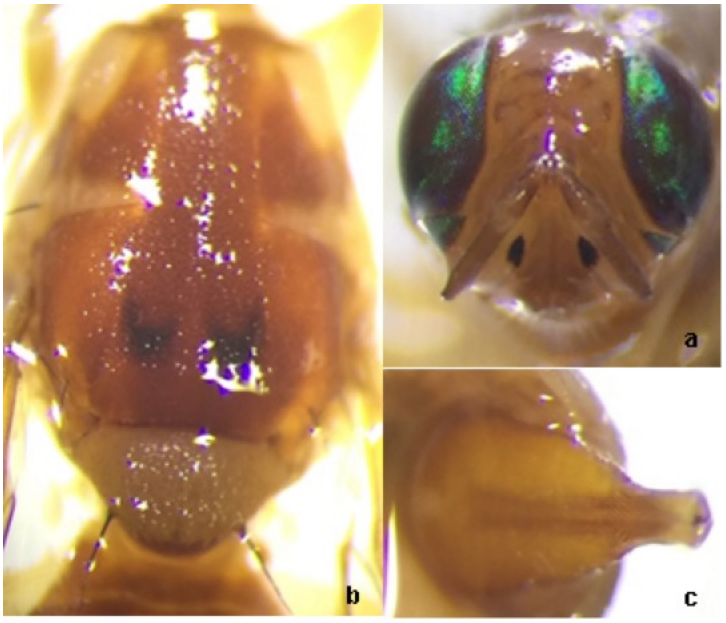

Figure 2. *Dacus ciliatus*: a) female head, b) female thorax and c) ovipositor (Photo: A. F. Çalışkan Keçe).

Figure 3. *Dacus ciliatus*: a) wing and b) adult (Photo: A. F. Çalışkan Keçe).

Figure 4. *Dacus ciliatus*: a) larvae and b, c) damage on cucumber (Photo: A. F. Çalışkan Keçe).

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Original article (Orijinal araştırma)

A new *Leptusa* Kraatz, 1856 (Coleoptera: Staphylinidae: Aleocharinae) species from Turkey[1]

Türkiye'den yeni bir *Leptusa* Kraatz, 1856 (Coleoptera: Staphylinidae: Aleocharinae) türü

Osman SERT[2] **Yavuz TURAN[2*]** **Mahmut KABALAK[2]**

Abstract

In this study, a new Aleocharinae Fleming, 1821 (Coleoptera: Staphylinidae) species, *Leptusa* (*Roubaliusa*) *giresunensis* sp. n. (Coleoptera: Staphylinidae: Aleocharinae), is described from Giresun Province in Turkey. The study was conducted between March 2013 and March 2016 in the Eastern Black Sea Region and new species was collected in 2014-2015. Photographs of the habitus are given and antenna, aedeagus, spermatheca, and both male and female sternite VII-VIII and tergite VIII are illustrated for the new species. Furthermore, species list of the *Leptusa* for Turkey, identification key and maps of the new species and congeners are given. The habitus, aedeagus and spermatheca are distinguished from similar species *Leptusa trapezuntis* Pace, 1989 and *Leptusa flagrifera* Assing, 2009 (Coleoptera: Staphylinidae: Aleocharinae).

Keywords: Aleocharinae, *Leptusa*, new species, Staphylinidae, Turkey

Öz

Bu çalışmada, yeni bir Aleocharinae Fleming, 1821 (Coleoptera: Staphylinidae) türü, *Leptusa* (*Roubaliusa*) *giresunensis* sp. n. (Coleoptera: Staphylinidae: Aleocharinae) Türkiye (Giresun)'den tanımlanmıştır. Çalışma 2013 Mart ve 2016 Mart tarihleri arasında Doğu Karadeniz Bölgesi'nde yürütülmüş ve yeni tür 2014-2015 yılları arasında toplanmıştır. Yeni türün vücut fotoğrafı verilmiş ve anten, aedeagus, spermateka, erkek ve dişi bireye ait 7. ve 8. sternit ve 8. tergitleri çizilmiştir. Ayıca *Leptusa* türlerinin Türkiye listesi, teşhis anahtarı ve yeni tür ile yakın türlerin haritaları verilmiştir. Benzer olan *Leptusa trapezuntis* Pace, 1989 ve *Leptusa flagrifera* Assing, 2009 (Coleoptera: Staphylinidae: Aleocharinae) türlerinden dış morfolojisi, aedeagus ve spermateka yapıları bakımından farklılıkları verilmiştir.

Anahtar sözcükler: Aleocharinae, *Leptusa*, yeni tür, Staphylinidae, Türkiye

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Introduction

The genus *Leptusa* is represented by numerous species in the Palearctic and other zoogeographic regions (Assing, 2004a). According to Schülke & Smetana (2015) *Leptusa* is represented by 409 species and 74 subspecies belonging to 71 subgenera in the Palearctic region. Besides nine species are given as *incertae sedis*.

One of the most diverse endemic species in Turkish is *Leptusa* Kraatz, 1856 in the Staphylinidae. The vast majority of the *Leptusa* species in Turkey have been recorded in the north and east (Assing, 2007). Studies on *Leptusa* in Turkey have been faunistic, new species/records and zoogeographical, particularly those of Volker Assing (Assing, 2002, 2003a, 2004b, 2007, 2009a, b, 2017). Also, the monograph on *Leptusa* by Pace (1989) is highly relevant.

Leptusa in countries neighboring Turkey include 27 species in five subgenera from the Caucasus region (excluding for Turkey) (Assing, 2017). Fourteen species are known from the Russian areas of Greater Caucasus, 17 species from Georgia, five species from Armenia and two from Azerbaijan (Assing, 2017). In addition, eight species are known from Iran, 11 species from Bulgaria and 24 species from Greece (Schülke & Smetana, 2015).

The genus includes 26 species and two doubtful subspecies in seven subgenera (Anlaş, 2009; Assing, 2009a, b, 2011, 2013; Schülke & Smetana, 2015). With the new species, the number of *Leptusa* species in Turkey has been raised to 27.

In Anatolia, 20 species in this genus are endemic. In *Leptusa,* the phenomenon of endemism is very widespread, as its habitats are mainly linked to mountainous regions and also lots of the *Leptusa* species are micropterous and confined to montane, subalpine and alpine habitats (Pace, 1989; Assing, 2017).

In *Leptusa*, a positive identification of species and subgenera generally relies on the male sexual characters (Assing, 2002). In the more recent taxonomic literature on *Leptusa*, the shape and internal structures of the median lobe of the aedeagus has been given particular emphasis, the male secondary sexual characters have been largely ignored. However, it has been shown that features of the male tergites VII-VIII and sternites VII-VIII may also be of considerable taxonomic significance (Assing, 2007).

The aim of the present study was to contribute to the knowledge of the *Leptusa* fauna of Turkey by describing a new species and explain how it differs from related congeners.

Material and Method

This report is based on material collected during field studies in Giresun Province in the Eastern Black Sea Region of Turkey between March 2013 and March 2016. All samples are collected by sifting leaf debris (*Rhododendron* sp.). Insects were killed with ethyl acetate and preserved in 10% acetic acid and 96% alcohol. The coordinates of the specimens were recorded by GPS. In the laboratory, genitalia were extracted using pins and put into KOH for cleaning the adipose tissue. Photographs of the habitus was taken using a Leica MZ 16A stereoscopic microscope, male and female sternite VII-VIII and tergite VIII, aedeagus, and female spermatheca were drawn. Maps were created using ArcMap 10.2.2 (Figures 1&2). The structure of the habitus, aedeagus and spermatheca of *Leptusa giresunensis* sp. n. was compared with *Leptusa trapezuntis* Pace, 1989 (Ordu-Gürgentepe, Akkuş; Giresun and Gümüşhane) and *Leptusa flagrifera* Assing, 2009 (Fuman County, Gilan Province, Iran) (Coleoptera: Staphylinidae: Aleocharinae). The material was deposited in cSrt (private collection of the senior author) in Hacettepe University. From anterior margin of labrum to posterior margin of tergite VIII represents the whole-body length (Figure 3a). The length of the median lobe of the aedeagus was measured from the apex of the ventral process to the base of the capsule. Also, a species list of the *Leptusa* for Turkey is given in Table 1.

Figure 1. Localities of *Leptusa* species collections.

Figure 2. Distribution of the new species.

Table 1. Species list of the *Leptusa* for Turkey

Subgenus	Species	Distribution in Turkey	References
Dendroleptusa Pace,1983	*samia* Assing, 2004	Manisa	Anlaş, 2009; Assing, 2007
Dysleptusa Pace, 1982	*fuliginosa* Aube, 1850	Artvin, Bolu, Düzce, Kastamonu, Rize, Sinop	Anlaş, 2009; Assing, 2003a, 2007, 2009a, 2011, 2013; Pace, 1989
	improvisa Assing, 2009	Antalya	Assing, 2009a
Leptusa Kraatz, 1856	*pulchella* (Mannerheim, 1830)	Artvin, Rize, Trabzon	Anlaş, 2009; Assing, 2003a, 2007
Neopisalia Scheerpeltz 1966= *Stenoleptusa* Scheerpeltz, 1966	*cimmeria* Pace, 1996	Rize	Anlaş, 2009; Assing, 2002, 2003a, 2007; Pace, 1996
	confinis Pace, 1982= *paphlagonica* Pace, 1982= *othmaniorum* Pace, 1983	Balıkesir, Bartın, Bursa, İstanbul, Kastamonu, Kocaeli, Sakarya, Samsun, Sinop, Zonguldak	Anlaş, 2009; Assing, 2002, 2007, 2009a,b, 2011, 2013; Pace, 1982,1989
	crinita Assing, 2007	Rize	Anlaş, 2009; Assing, 2007, 2009a
	diecki Pace, 1983= gurgentepensis Pace, 1989	Giresun, Gümüşhane, Ordu, Samsun, Trabzon	Anlaş, 2009; Assing, 2002, 2003a, 2007, 2009a,b; Pace, 1983a, 1989
	janczyki Pace, 1983	According to Anlaş, 2009 Rize or/and Artvin	Anlaş, 2009; Assing, 2009b; Pace, 1983a, 1989
	korgei Scheerpeltz, 1970	Rize	Anlaş, 2009; Assing, 2002, 2003b; Scheerpeltz, 1970
	longilobata Assing, 2007	Gümüşhane, Trabzon	Anlaş, 2009; Assing, 2007
	nurdaghensis Assing, 2003	Hatay	Anlaş, 2009; Assing, 2003b, 2004a
	rizensis Pace, 1996	Rize	Anlaş, 2009; Assing, 2007; Pace, 1996
	sica Assing, 2003	Rize	Anlaş, 2009; Assing, 2003a, 2007, 2009a
	soganlica Assing, 2007	Trabzon	Anlaş, 2009; Assing, 2007
	spoliata Assing, 2002	Giresun, Ordu	Anlaş, 2009; Assing, 2002, 2004b, 2007
	venusta (Hochhuth, 1849)	Artvin, Kars, Rize, Trabzon	Anlaş, 2009; Assing, 2002, 2003a, 2007; Pace, 1989
Roubaliusa Scheerpeltz, 1966	*trapezuntis* Pace, 1989	Giresun, Gümüşhane, Ordu	Anlaş, 2009; Assing, 2003a, 2007, 2009a; Pace, 1989
	giresunensis sp. n.	Giresun	Giresun (Present paper)

Table 1 (continued).

Subgenus	Species	Distribution in Turkey	References
Stictopisalia Scheerpeltz, 1966	*amisensis* Pace, 1982	Samsun	Assing, 2009b; Pace, 1982, 1989
	asiatica Bernhauer, 1909= *abantensis* Fagel, 1968 = *flagellulifera* Assing, 2009	Adana-Gaziantep (Eastern Osmaniye), Bitlis (Eastern Van Lake), Bolu, Düzce, Kastamonu, Karabük, Kocaeli, Ordu, Sakarya (Gök Tepe), Zonguldak	Anlaş, 2009; Assing, 2003a, 2007, 2009b, 2013, 2014; Bernhauer, 1909; Fagel, 1968; Pace, 1989
	artviniensis Pace, 1982= batumiensis Pace, 1983	Artvin, Rize, Trabzon	Anlaş, 2009; Assing, 2002, 2003b; Pace, 1982, 1983b, 1989
	fibula Assing, 2003	Ordu, Giresun, Gümüşhane, Trabzon	Anlaş, 2009; Assing, 2003a, 2007, 2009a
	ionopolitana Pace, 1982	Kastamonu	Anlaş, 2009; Assing, 2009b, 2014; Pace, 1982, 1989
	merkli Bernhauer, 1900= anatolica Fauve, 1900	Bolu, Bursa, İstanbul, Kocaeli, Sakarya, Yalova	Anlaş, 2009; Assing, 2002, 2003b, 2009b, 2011, 2013, 2014; Bernhauer, 1900
incertae sedis	*taurica* Assing, 2004	Kahramanmaraş	Anlaş, 2009; Assing, 2004a, 2009a,b
	marasica Assing, 2006	Kahramanmaraş	Anlaş, 2009; Assing, 2006

Results and Discussion

Leptusa (*Roubaliusa*) *giresunensis* sp. n.

Type Locality

Holotype: Giresun: Tirebolu, Tirebolu-Doğankent, 29 m, 40°54'30" N, 38°50'55" E, 16.IV.2014, leg: Y. Turan, M. Kabalak, O. Sert, 2015, det. Y. Turan, 1♂ (cSrt).

Paratypes: same data as holotype 1♂, 1♀; Giresun: Dereli, Yavuzkemal, Konuklu village, 1,178 m, 40°38'29" N, 38°18'10" E, 15.IV.2014 and 28.IV.2015, leg: Y. Turan, M. Kabalak, O. Sert, 2014, 2015, det. Y. Turan, 2♂♂, 5♀♀ (cSrt).

Description (Holotype)

Body length 2.91 mm (for all specimens: 2.89-3.61 mm.), body reddish, head slightly darker at least medially, VI and anterior half of III-V and VII abdominal segments blackish, II-XI antenna segments reddish-brown and I darker. Head feebly transverse; punctation barely visible, dense, shallow microsculpture; in dorsal view eyes shorter than postocular region (Figure 3a). Antennomere IV oblong V weakly oblong, VI quadrate VII-X transverse (Figure 3b). Pronotum roughly 1.35 times as wide as long and 1.3 times as wide as head; maximal width in anterior half; punctation dense and fine, barely recognizable distinct microreticulation; elytra without sexual dimorphism, slightly wider than pronotum, at suture about as long as pronotum; humeral angles moderately marked; punctation rather dense; barely visible in the pronounced microsculpture; hind wings reduced. Abdomen segments V-VI widest, barely wider than elytra; all segments microsculpture distinct. Sternite VII-VIII and tergite VIII with sexual dimorphism.

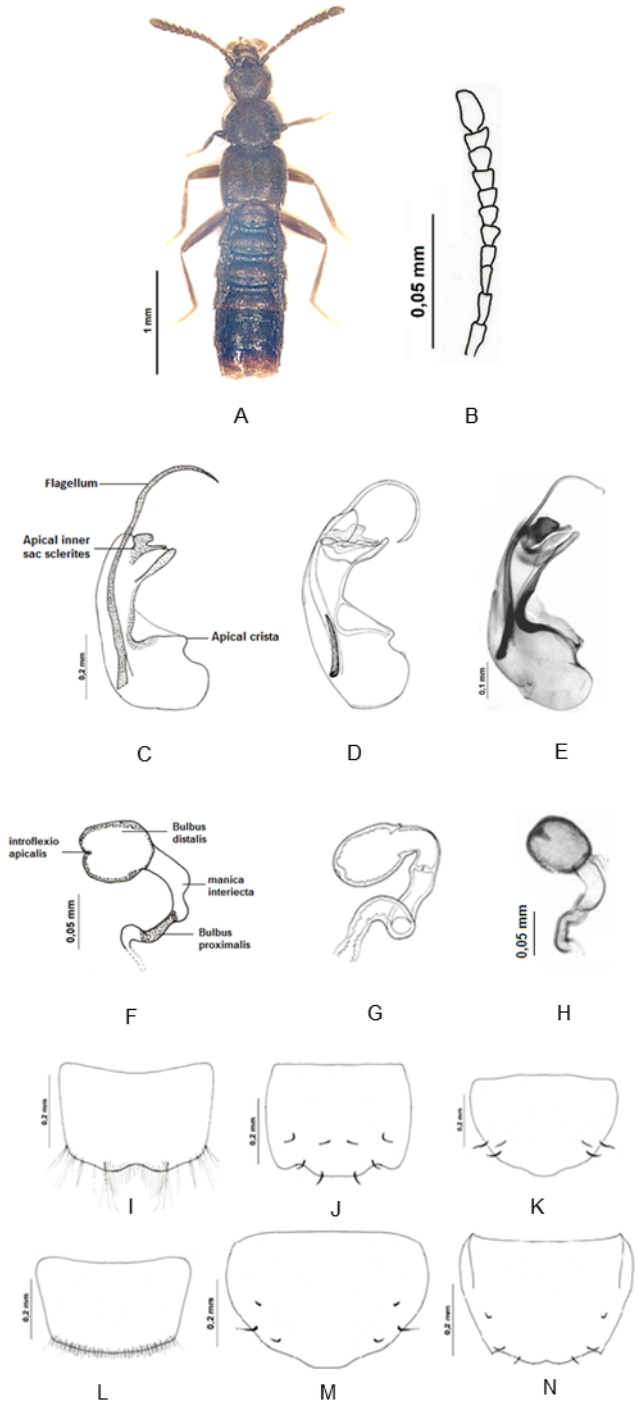

Figure 3. A) Habitus, B) antenna, C) aedeagus (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.), D) aedeagus (*Leptusa trapezuntis*) (Pace, 1989), E) aedeagus (*Leptusa flagrifera*) (Assing, 2009b), F) spermatheca (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.), G) spermatheca (*Leptusa trapezuntis*) (Pace, 1989), H) spermatheca (*Leptusa flagrifera*) (Assing, 2009b), I) male sternite VII (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.), J) male tergite VIII (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.), K) male sternite VIII (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.), L) female sternite VII (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.), M) female sternite VIII (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.), N) female tergite VIII (*Leptusa* (*Roubaliusa*) *giresunensis* sp. n.).

♂: sternite VII posterior margin in the middle weakly concave, with long setae both side of middle (Figure 3i); posterior margin of tergite VIII straight (Figure 3j); posterior margin of sternite VIII convex (Figure 3k); aedeagus about 0.5 mm long, with apically curved and long flagellum, median lobe with ventral process narrow at base, slightly convex on ventral side and apical crista swollen; apical inner sac sclerites rooster head-shape (Figure 3c).

♀: sternite VII posterior margin nearly straight; sternite VIII posterior margin slightly convex (Figure 3m); tergite VIII posterior margin weakly concave in the middle (Figure 3n); bulbus distalis of spermatheca wide, introflexio apicalis slightly curved; manica interiecta curved proximally, bulbus proximalis slightly curved as in Figure 3f.

Key to species:

1 Eyes shorter than postocular region in dorsal view 2

1' Eyes as long as postocular region in dorsal view; ♂: flagellum of the adeagus slightly curved, apically notched, apical inner sac not curved, ventral process of the median lobe slim; ♀: bulbus distalis of spermatheca narrow, bulbus proximalis of spermatheca slightly curved ..... *Leptusa flagrifera* Assing, 2009

2 ♂: flagellum of the adeagus distinctly curved, apical inner slightly curved, ventral process of the median lobe slim; ♀: bulbus distalis of spermatheca narrow, bulbus proximalis of spermatheca strongly curved ..... *Leptusa trapezuntis* Pace, 1989

2' ♂: flagellum of the adeagus slightly curved, apical inner sac curved in the middle, ventral process of the median lobe thick; ♀: bulbus distalis of spermatheca thick, bulbus proximalis of spermatheca sligthly curved ..... *Leptusa giresunensis* sp. n.

Leptusa is a staphylinid genera with numerous species that are small in size. In the taxonomic literature on *Leptusa*, morphological characters of habitus and reproductive organs are both important for distinguishing the species. Furthermore, variation in the male tergites VII-VIII and the male sternites VII-VIII may also be of taxonomic significance (Assing, 2002, 2007). This is particularly so for subgenera and species groups with rather uniform median lobes and spermatheca (Assing, 2007). Also, an identification key for *Leptusa* species of Turkey or/and Palearctic region is not available. Consequently, it can be difficult to diagnose the species. In this study, when comparing the new species with congeners species, it is difficult to see clear differences for habitus. So, as described above, aedeagus, spermatheca and secondary sexual characters were used to distinguish between the new species and related congeners.

Leptusa giresunensis sp. n. most closely resembles *L. trapezuntis* and *L. flagrifera.* When the habitus is compared with *L. trapezuntis* and *L. flagrifera*, there is no obvious difference. Clear differences are, however, evident in aedeagus and spermatheca. *Leptusa giresunensis* could be differentiated based on the following characteristics: Flagellum of *L. giresunensis* curved slightly, flagellum of *L. trapezuntis* is distinctly curved, apical of flagellum of *L. flagrifera* is notched; apical inner sac (rooster head-shape) curved distinctly in the middle in *L. giresunensis*, in *L. trapezuntis* slightly curved and in *L. flagrifera* not curved; in *L. giresunensis* median lobe ventral process (finger-shape) thicker than *L. trapezuntis* and *L. flagrifera* (Figure 3c,d,e). Bulbus distalis of spermatheca of *L. giresunensis* thicker than *L. trapezuntis* and *L. flagrifera*; in *L. trapezuntis* bulbus proximalis is distinctly curved, but in *L. giresunensis* and *L. flagrifera* slightly curved (Figure 3f,g,h). According to habitus characters: eyes about as long as postocular region in dorsal view in *L. flagrifera*, but eyes shorter than postocular region in dorsal view in *L. giresunensis* and *L. trapezuntis*; punctuations of pronotum barely visible, microreticulation distinct in *L. trapezuntis*, punctation fine and dense, barely noticeable in the pronounced microreticulation in *L. giresunensis* and *L. flagrifera*. In male sternite VII deeply concave in *L. flagrifera*, but in *L. giresunensis* weakly concave in the middle (Table 2).

Table 2. Characteristics of the species

Characters	*Leptusa giresunensis* sp. n.	*Leptusa trapezuntis*	*Leptusa flagrifera*
Aedeagus flagellum	Slightly curved	Distinctly curved	Slightly curved, apically notched
The apical inner sac (cock-shape)	Curved in the middle	Slightly curved	Not curved
Median lobe ventral process (finger-shape)	Thick	Slim	Slim
Bulbus distalis of spermatheca	Thick	Narrow	Narrow
Bulbus proximalis of spermatheca	Slightly curved	Strongly curved	Slightly curved
Eyes	Shorter than postocular region in dorsal view	Shorter than postocular region in dorsal view	As long as postocular region in dorsal view
Male sternite VII	Deeply concave	Not known	Weakly concave
Collection localities in Turkey and Iran	Giresun Province, Turkey	Ordu Province (Pace, 1989; Assing, 2003), Giresun and Gümüşhane Provinces (Assing, 2007), Turkey	Gilan Province, Iran (Assing, 2009b)
Zoogeographical distribution	Turkey	Turkey	Iran

Etymology

The name of the new species is derived from the name of Giresun Province of Turkey in which the type locality is situated (Figures 1&2).

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Original article (Orijinal araştırma)

Oribatid mite fauna of Kocaeli City Forest (Kocaeli, Turkey)[1]

Kocaeli Kent Ormanı (Kocaeli, Türkiye) Oribatid akar faunası

Merve YAŞA[2] **Şule BARAN[2*]**

Abstract

In this study, the oribatid mites collected from Kocaeli City Forest (Kocaeli, Turkey) between 2016-2017 were examined faunistically. In total 60 samples were collected from soil and litter. Berlese-Tullgren funnels were used for extraction of oribatid mites. Twenty-two species belonging to families Amerobelbidae, Astegistidae, Chamobatidae, Eniochthoniidae, Eremaeidae, Epilohmanniidae, Galumnidae, Gymnodamaeidae, Liacaridae, Micreremidae, Nanhermanniidae, Neoliodidae, Oppiidae, Oribatulidae, Parakalummidae, Protoribatidae, Scheloribatidae and Tectocepheidae were detected. One family (Parakalummidae), two genera (*Cultroribula* and *Masthermannia*) and four species [*Allogalumna integer* (Berlese, 1904), *Cultroribula bicultrata* (Berlese, 1905), *Masthermannia mammillaris* (Berlese, 1904), *Neoribates* (*N.*) *bulanovae* Grishina, 2009] are recorded for the first time in Turkey. Scanning electron microscopy images and geographical distributions of each species are provided.

Keywords: fauna, Kocaeli City Forest, new records, Oribatida, soil biodiversity

Öz

Bu çalışmada, 2016-2017 yılları arasında Kocaeli Kent Orman'ından toplanan oribatid akarlar faunistik bakımdan incelenmiştir. Toplam 60 örnek toprak ve döküntüden toplanmıştır. Oribatid akarların ayıklanması için Berlese-Tullgren huni düzeneği kullanılmıştır. Amerobelbidae, Astegistidae, Chamobatidae, Eniochthoniidae, Eremaeidae, Epilohmanniidae, Galumnidae, Gymnodamaeidae, Liacaridae, Micreremidae, Nanhermanniidae, Neoliodidae, Oppiidae, Oribatulidae, Parakalummidae, Protoribatidae, Scheloribatidae ve Tectocepheidae familyalarına ait 22 tür teşhis edilmiştir. Bir familya (Parakalummidae), iki cins (*Cultroribula* ve *Masthermannia*) ve dört tür [*Allogalumna integer* (Berlese, 1904), *Cultroribula bicultrata* (Berlese, 1905), *Masthermannia mammillaris* (Berlese, 1904), *Neoribates* (*N.*) *bulanovae* Grishina, 2009] Türkiye'de ilk kez kaydedilmiştir. Her bir türün morfolojik karakterlerine ait tarama elektron mikroskobu görüntüleri ve coğrafi dağılımları verilmiştir.

Anahtar sözcükler: fauna, Kocaeli Kent Ormanı, yeni kayıtlar, Oribatida, toprak biyoçeşitliliği

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Introduction

Oribatid mites are major constituents of soil biological diversity and they act as engineers of soil structure and indicators of soil health (Smol et al., 2001; Douglas, 2007). Oribatid mites tend to be concentrated in the surface litter layers of mineral soils. They are important due to their ecological roles such as fragmentation of organic materials, physical and chemical changes of organic substances by digestion and helping the distribution of bacterial and fungal spores (Norton & Behan-Pelletier, 2009; Sevimli & Baran, 2016). They consume different kinds of food such as living or dead parts of plants or fungal and bacterial microbes inhabiting in the litter, and the remains of other soil inhabitants (Behan &Hill 1978; Moore et al., 1988).

The life cycle of oribatids can take up to 7 years in extreme habitats but in boreal to temperate regions usually it takes 1 or 2 years, and they are considered K-strategists because of their life history characteristics (Sovik & Leinaas, 2003; Lindo & Visser, 2004; John et al., 2006; Norton & Behan-Pelletier, 2009).

There are nearly 11,000 known oribatid mite species in the world (Subías, 2018). About 250 species have been recorded in Turkey (Özkan et al., 1988, 1994; Erman et al., 2007; Bezci et al., 2018). Research on the oribatid mites of Turkey has been associated with specific regions of the country. Kocaeli is one of richest provinces of Turkey in terms of forest assets (44%), but there has been only one investigation of oribatid fauna in this province (Baran & Bilici, 2017). The aim of this study was to make contribution to the knowledge of the oribatid mite fauna of Turkey, which is currently quite limited.

Material and Method

The study area and sampling

Soil and litter samples were collected from Kocaeli City Forest (Figure 1) between December 2016 and November 2017 at five different stations (Table 1), every month between 12.00 and 15.00 h. Soil cores of 20 cm in diameter and about 10 cm depth was taken from the surface layer of soil. Each sample was placed in a labeled bag for examination in the laboratory. Samples were placed in Berlese-Tullgren funnels for about 7 d. The oribatid mites preserved in 70% ethanol bottles under the funnels were removed by pipette and needle under the stereomicroscope. Specimens were placed in lactic acid in hollow slides for examination by light microscopy.

Table 1. Details of collection sites

Station code	Elevation (m)	Coordinates
KKO1.1-12	408 m	40°49'51.88" N, 29°54'45.36" E
KKO2.1-12	412 m	40°49'50.72" N, 29°54'43.11" E
KKO3.1-12	412 m	40°49'50.25" N, 29°54'41.89" E
KKO4.1-12	419 m	40°49'49.24" N, 29°54'42.46" E
KKO5.1-12	416 m	40°49'50.05" N, 29°54'43.08" E

Figure 1. Map of the study area (Anonymous, 2018; Holt et al., 2013).

Scanning electron microscopy

Specimens for identification were examined in the scanning electron microscope. Specimens were cleaned for 12 h in soil cleaning detergent and placed in stubs. After the stubs were covered with gold in Quorum SC7620 Sputter Coater (Quorum Technologies, Newhaven, UK) at low voltage 5-15 kV according to size of specimens. The images were taken with a Philips XL 30 SFEG scanning electron microscope (FEI, Eindhoven, Netherlands).

Terminology

Terminology and taxa identification were according to Balogh & Balogh (1992), Weigmann (2006) and Norton & Behan-Pelletier (2009).

Results and Discussion

Twenty-two species belonging to 18 families of oribatid mites from Kocaeli City Forest (Kocaeli, Turkey) were determined. The diagnostic features for species that are new records for Turkey are detailed below.

Nanhermanniidae Sellnick, 1928

***Masthermannia mammillaris* (Berlese, 1904)**

Measurements. Average body length 389 µm and body width 177 µm (n = 2).

Diagnostic characters: Figure 2. Interlamellar, lamellar and notogastral setae T shaped, extremely long and flagellate. Notogaster with foveolate. Sensillus long, setiform and ciliate. Nine pairs of genital setae.

Material examined. KKO1.5, 2 specimens.

Distribution in Turkey. New genus record for Turkey.

General distribution. Pantropical and subtropical (Subías, 2018).

Remarks. This is the first record of genus *Masthermannia* for the Turkish fauna. This genus contains 10 species (Subías, 2018). This species is differentiated from all the other species of the genus by having long, setiform sensillus. The body length of this species is described as between 400-485 µm (Berlese, 1913; van der Hammen, 1959; Balogh & Mahunka, 1983; Weigmann, 2006) and the length of our specimen was close to the previously published dimensions of the species. In Europe this species reported from warm climates (Weigmann, 2006).

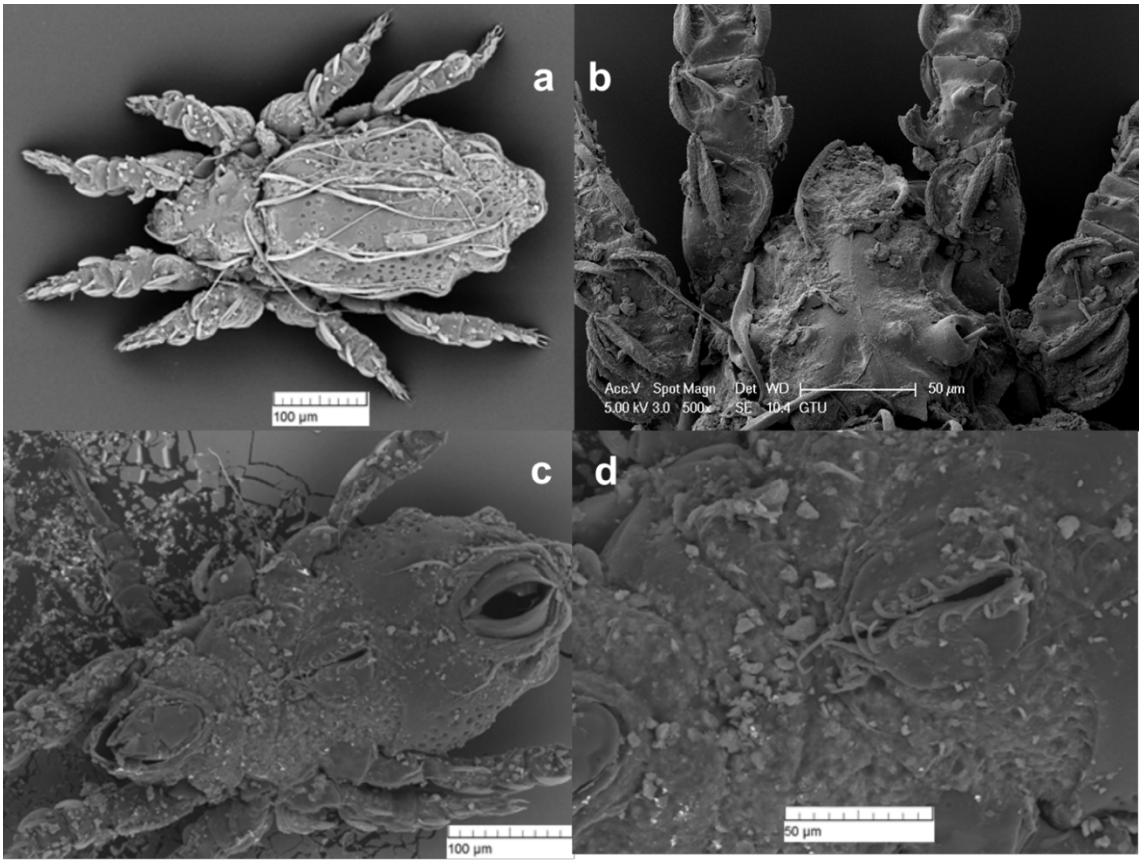

Figure 2. SEM images of *Masthermannia mammillaris*: a) dorsal view, b) prodorsum, c) ventral view and d) genital plate.

Astegistidae Balogh, 1961

***Cultroribula bicultrata* (Berlese, 1905)**

Measurements. Average body length 243 µm and body width 135 µm (n = 7).

Diagnostic characters. Figure 3. Rostrum with three very deep incisions. Legs with one claw. Interlamellar seta short and thin. Lamellar cuspis long and thin. Sensillus fusiform with long peduncle. Ten pairs of thin, setiform and short notogastral setae. Dorsal and ventral surface is smooth. Six pairs of genital setae and two pairs of anal setae present.

Material examined. KKO1.12, 5 specimens and KKO5.8, 2 specimens.

Distribution in Turkey. New family record for Turkey.

General distribution. Holarctic region (frequently Palearctic) (Subías, 2018).

Remarks. This is the first record of the genus *Cultroribula* for the Turkish fauna. *Cultroribula bicultrata* vary from the other species of genus by prolonged rostrum with long distal incisions. The body length of this species is described as between 225-250 µm (Bernini, 1969; Ghilarov & Krivolutsky 1975; Jacot, 1939; Balogh, 1943; Evans, 1952; Krivolutsky, 1962). Average body length of the Turkish specimens (243 µm) was in the range of the previously published dimensions for this species. This species has been reported from organic layers of forest soils (Weigmann, 2006).

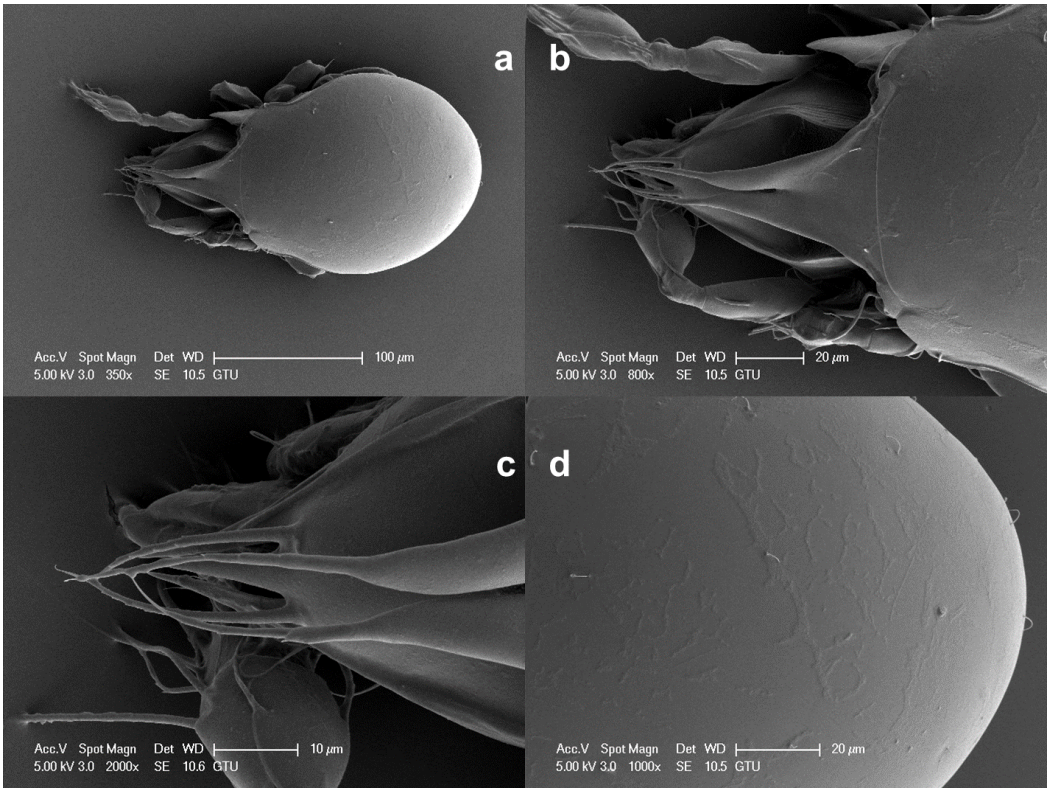

Figure 3. SEM images of *Cultroribula bicultrata* : a)dorsal view, b) prodorsum, c) rostrum and d) notogastral setae.

Parakalummidae Grandjean, 1936

***Neoribates* (*N.*) *bulanovae* Grishina, 2009**

Measurements. Average body length 894 µm and body width 705µm (n = 6).

Diagnostic characters. Figure 4. Ventral and dorsal sculpture smooth. Interlamellar seta bigger than lamellar and rostral seta. Rostral seta shorter than lamellar seta. Dorsosejugal suture oval, not distinct. Pteromorphs rounded, close to oval. Sensillus setiform and long. Five pairs of genital setae with thin and barbs. Large oribatids.

Material examined. KKO1.8, 3 specimens, KKO1.8, 1 specimen, KKO1.11, 1 specimen and KKO4.12, 1 specimen.

Distribution in Turkey. New record for Turkey.

General distribution. East Mediterranean (Subías, 2018).

Remarks. This is the first record of the family Parakalummidae for the Turkish fauna. *Neoribates* (*N.*) *bulanovae* differs from the other known species by larger size, rounded body shape and setiform sensillus. The body length of this species was described as 830-904 µm by Grishina & Vladimirova (2009). Average

body length of the Turkish specimens (894 µm) was in the range of the previously published dimensions of the species. *Neoribates* (*N.*) *bulanovae* was previously found in beech-oak forest in Ukraine (Grishina & Vladimirova, 2009). Our specimens were collected from soil under beech in a mixed forest.

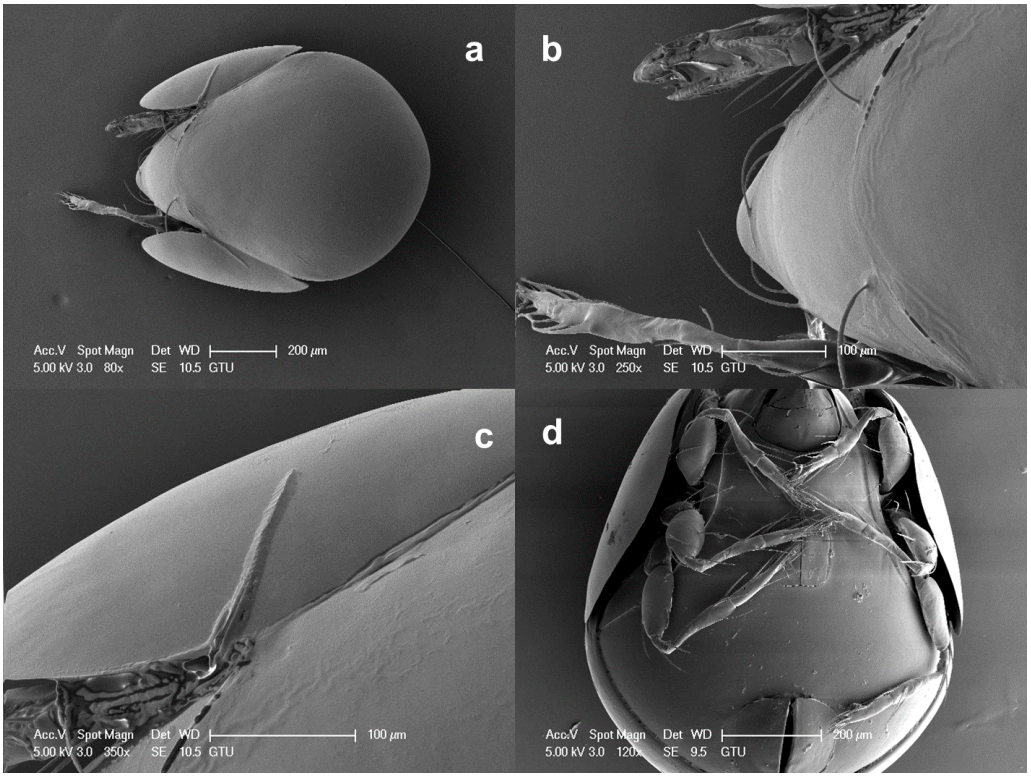

Figure 4. SEM images of *Neoribates* (*N.*) *bulanovae*: a) dorsal view, b) prodorsum, c) sensillus and d) ventral view.

Galumnidae Jacot, 1925

***Allogalumna integer* (Berlese, 1904)**

Measurements. Average body length 442 µm and body width 319 µm (n = 10).

Diagnostic characters. Figure 5. Sensillus with dilated head and lanceolate. Dorsosejugal suture medially broken. Dorsal and ventral surfaces are smooth. Rostral seta smooth. Interlamellar seta developed and long. *Aa* oval. Legs with three claws.

Material examined. KKO1.1-12 81 specimens, KKO2.3,6 specimens, KKO2.4, 1 specimen, KKO2.5 21 specimens, KKO2.6, 10 specimens, KKO2.7, 2 specimens, KKO2.8, 3 specimens, KKO2.9, 17 specimens, KKO2.10, 28 specimens, KKO2.11, 10 specimens, KKO2.12, 7 specimens, KKO3.1, 3 specimens, KKO3.4, 6 specimens, KKO3.5, 12 specimens, KKO3.6, 1 specimen, KKO3.7, 1 specimen, KKO3.8, 1 specimen, KKO3.9, 2 specimens, KKO3.10, 5 specimens, KKO3.11, 12 specimens, KKO3.12, 14 specimens, KKO4.1, 1 specimen, KKO4.4, 5 specimen, KKO4.5, 60 specimens, KKO4.6, 9 specimens, KKO4.7, 24 specimens, KKO4.8, 30 specimens, KKO4.9, 8 specimens, KKO4.10, 16 specimens, KKO4.11, 9 specimens, KKO4.12, 9 specimens, KKO5.2, 1 specimen, KKO5.4, 18 specimens, KKO5.5, 31specimens, KKO5.7, 20 specimens, KKO5.8, 10 specimens, KKO5.9, 13 specimens, KKO5.10, 25 specimens, KKO5.11, 6 specimens and KKO5.12, 51 specimens.

Distribution in Turkey. New record for Turkey.

General distribution. Centromeridional Europe (Subías, 2018).

Remarks. *Allogalumna* is known as cosmopolitan genus (Subías, 2018). Previously, *Allogalumna turkeyensis* Grobler, Bayram & Cobanoglu, 2004 (Grobler et al., 2004) was the only member of this genus previously recorded in Turkey. *Allogalumna integer* is similar to *Allogalumna iranica* Akrami, 2015 but it differs with ciliated lamellar seta and sensillus with dilated head (Akrami, 2015). The body length of this species was described as 440-600 µm (Berlese, 1904; Mihelcic, 1957). Average body length of the Turkish specimens (442 µm) was in the range of the previously published dimensions of the species. This species reported near ant nests (Berlese, 1904).

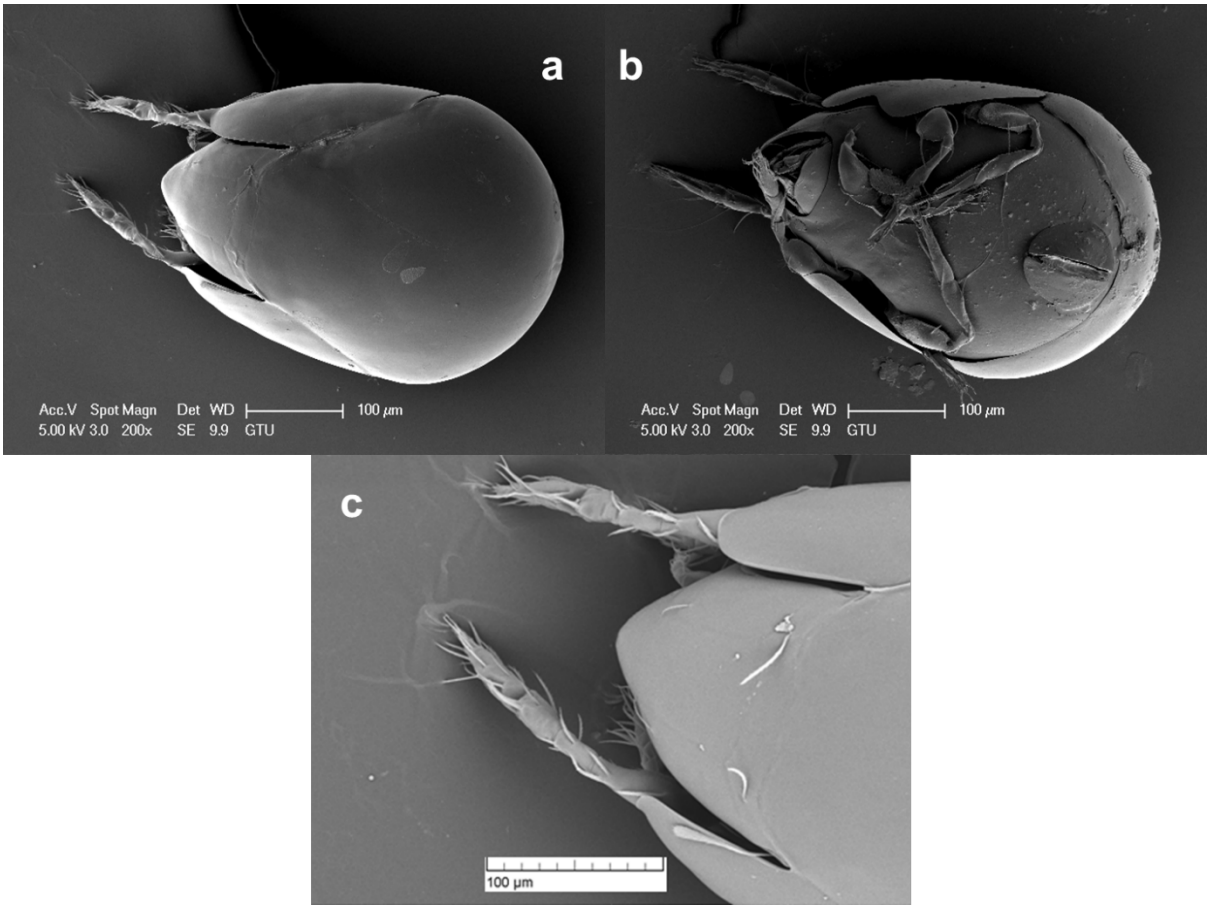

Figure 5. SEM images of *Allogalumna integer*: a) dorsal view, b) ventral view and c) prodorsum.

Species previously known in Turkey

Eniochthoniidae Grandjean, 1947

***Hypochthoniella minutissima* (Berlese, 1903) (Figure 6a)**

Measurements. Body length 362 µm and body width 178 µm.

Material examined. KKO1.1-12 36 specimens.

Distribution in Turkey. Bolu (Toluk et al., 2017).

General distribution. Cosmopolitan (Subías, 2018).

Epilohmanniidae Oudemans, 1923

***Epilohmannia* (*E.*) *cylindrica* (Berlese, 1904) (Figure 6b)**

Measurements. Body length 431 µm and body width 159 µm.

Material examined. KKO2.1-12 1 specimen, KKO3.1-12 7 specimens, KKO4.1-12 2 specimens, and KKO5.1-12 1 specimen.

Distribution in Turkey. Erzurum, Konya, Kayseri and Sakarya (Ayyıldız, 1988a; Dik et al., 1999; Toluk & Ayyıldız, 2008a; Baran et al., 2015).

General distribution. Cosmopolitan (Subías, 2018).

***Epilohmannia* (*E.*) *imreorum* Bayoumi & Mahunka, 1976 (Figure 6c)**

Measurements. Body length 698 µm and body width 291 µm.

Material examined. KKO1.1-12 4 specimens and KKO4.1-12 1 specimen.

Distribution in Turkey. Sakarya (Baran et al., 2015).

General distribution. Mediterranean (Subías, 2018).

Nanhermanniidae Sellnick, 1928

***Nanhermannia* (*N.*) *nana* (Nicolet, 1855) (Figure 6d)**

Measurements. Body length 545 µm and body width 245 µm.

Material examined. KKO4.1-12 2 specimens.

Distribution in Turkey. Samsun and Düzce (Ayyıldız et al., 1996; Sarial & Baran, 2013).

General distribution. Semicosmopolitan (Subías, 2018).

Neoliodidae Sellnick, 1928

***Platyliodes doderleini* (Berlese, 1883) (Figure 6e)**

Measurements. Body length 1095 µm and body width 553 µm.

Material examined. KKO4.1-12 2 specimens.

Distribution in Turkey. Çorum (Per, 2016).

General distribution. Southern Palearctic (Subías, 2018).

Gymnodamaeidae Grandjean, 1954

***Gymnodamaeus barbarossa* Weigmann, 2006 (Figure 6f)**

Measurements. Body length 525 µm and body width, 27 µm.

Material examined. KKO1.1-12 10 specimens, KKO2.1-12 89 specimens, KKO3.1-12 64 specimens, KKO4.1-12 8 specimens and KKO5.1-12 5 specimens.

Distribution in Turkey. Bolu (Toluk & Ayyildiz, 2014).

General distribution. Centromeridional Europe (Subías, 2018).

Liacaridae Sellnick, 1928

***Liacarus* (*L.*) *brevilamellatus* Mihelčič, 1955 (Figure 6g)**

Measurements. Body length 568 µm and body width 312 µm.

Material examined. KKO1.1-12 10 specimens, KKO2.1-12 4 specimens, KKO4.1-12 11 specimens, and KKO5.1-12 5 specimens.

Distribution in Turkey. Erzurum (Ocak et al., 2007; Akman et al., 2018).

General distribution. Southern Palearctic (Subías, 2018).

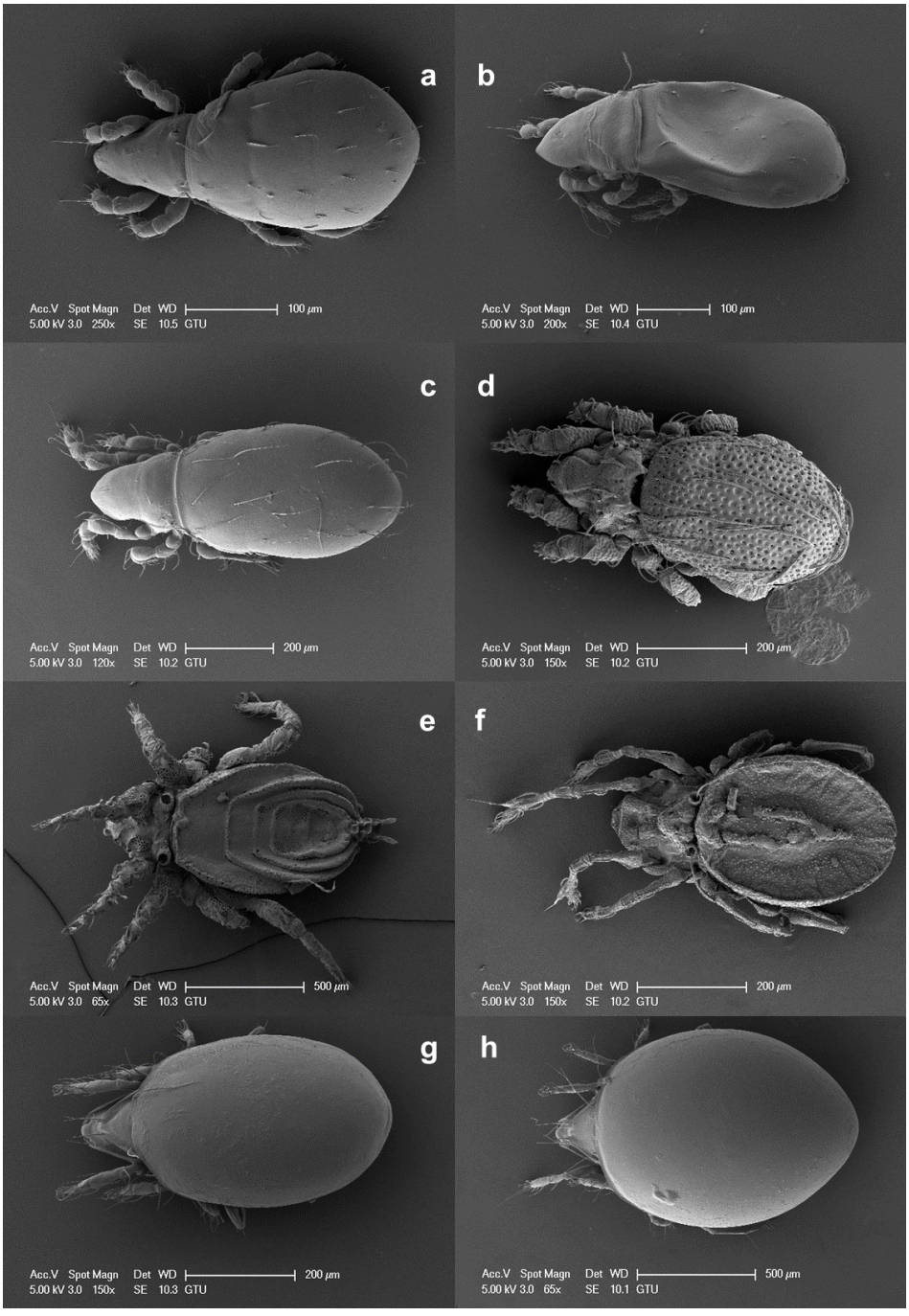

Figure 6. SEM images of a) *Hypochthoniella minutissima*, b) *Epilohmannia* (*E.*) *cylindrica*, c) *Epilohmannia* (*E.*) *imreorum*, d) *Nanhermannia* (*N.*) *nana*, e) *Platyliodes doderleini*, f) *Gymnodamaeus barbarossa*, g) *Liacarus* (*L.*) *brevilamellatus* and h) *Liacarus* (*L.*) *coracinus*.

***Liacarus* (*L.*) *coracinus* (Koch, 1841) (Figure 6h)**

Measurements. Body length 1285 µm and body width 814 µm.

Material examined. KKO1.1-12 12 specimens, KKO2.1-12 9 specimens, KKO3.1-12 10 specimens, KKO4.1-12 9 specimens and KKO5.1-12 15 specimens.

Distribution in Turkey. Giresun, Mersin, Ordu and Samsun, Trabzon (Grobler et al., 2003).

General distribution. Palearctic region (Subías, 2018).

Eremaeidae Oudemans, 1900

***Eremaeus hepaticus cordiformis* Grandjean, 1934 (Figure 7a)**

Measurements. Body length 631 µm and body width 368 µm.

Material examined. KKO1.1-12 33 specimens, KKO2.1-12 13 specimens, KKO3.1-12 23 specimens, KKO4.1-12 33 specimens and KKO5.1-12 31 specimens.

Distribution in Turkey. Bursa and Sakarya (Seniczak et al., 2013; Bezci et al., 2017)

General distribution. Southern Palearctic (Subías, 2018).

Amerobelbidae Grandjean, 1961

***Amerobelba decedens* Berlese, 1908 (Figure 7b)**

Measurements. Body length 689 µm and body width 413 µm.

Material examined. KKO1.1-12 2 specimens.

Distribution in Turkey. Sakarya (Baran & Şimşek, 2012).

General distribution. Palearctic (Centromeridional Europe) (Subías, 2018).

Oppiidae Sellnick, 1937

***Berniniella* (*B.*) *bicarinata* (Paoli, 1908) (Figure 7c)**

Measurements. Body length, 224 µm and body width, 94 µm.

Material examined. KKO1.1-12 95 specimens, KKO2.1-12 49 specimens, KKO3.1-12 49 specimens, KKO4.1-12 93 specimens and KKO5.1-12 234 specimens.

Distribution in Turkey. Yozgat (Toluk & Ayyildiz, 2008b).

General distribution. Palearctic region (Subías, 2018).

Tectocepheidae Grandjean, 1954

***Tectocepheus alatus* Berlese, 1913 (Figure 7d)**

Measurements. Body length 346 µm and body width 192 µm.

Material examined. KKO1.1-12 41 specimens, KKO2.1-12 38 specimens, KKO3.1-12 20 specimens, KKO4.1-12 56 specimens and KKO5.1-12 30 specimens.

Distribution in Turkey. Bolu (Toluk et al., 2017).

General distribution. Palearctic region (Subías, 2018).

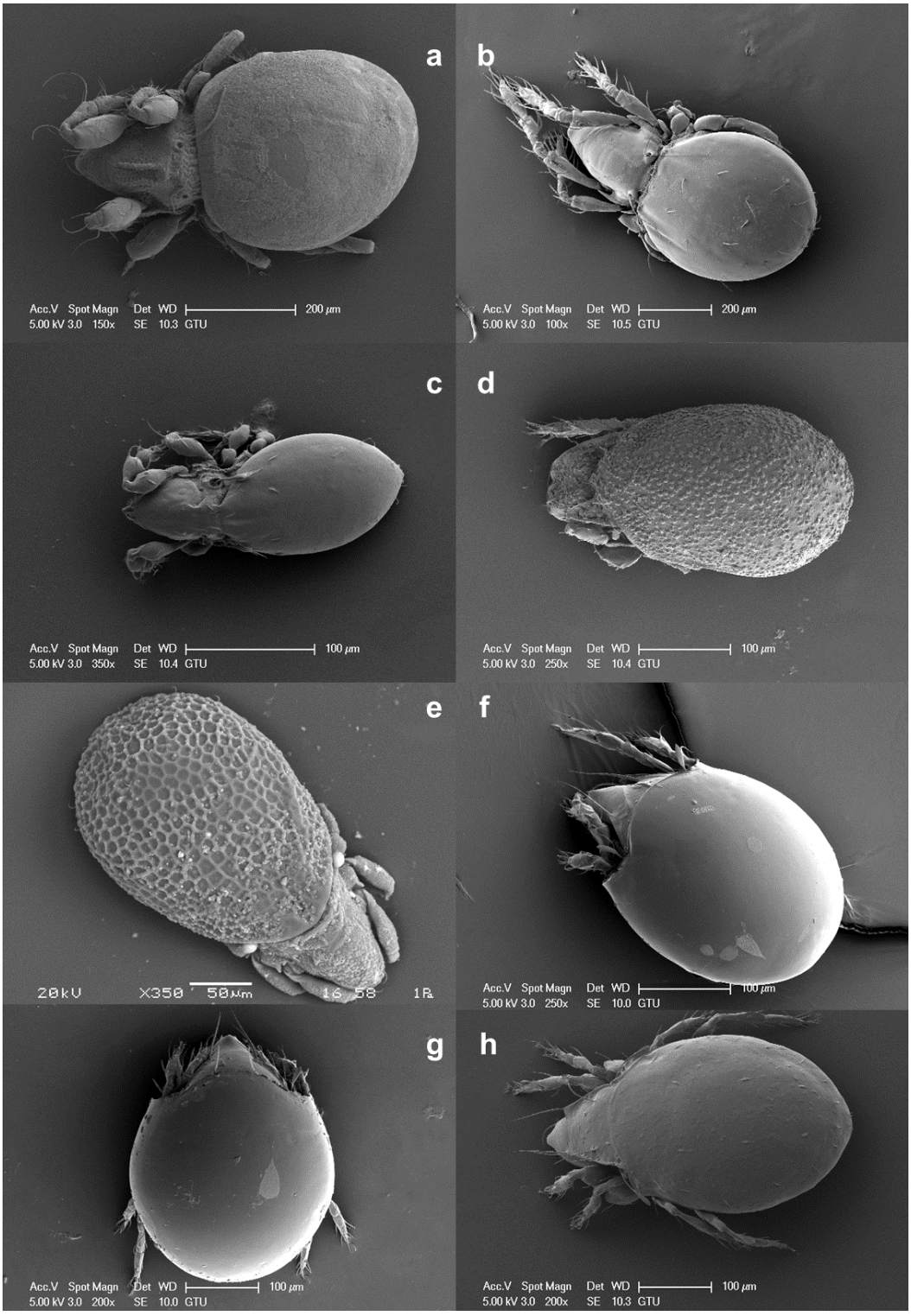

Figure 7. SEM images of a) *Eremaeus hepaticus cordiformis,* b) *Amerobelba decedens*, c) *Berniniella* (*B.*) *bicarinata*, d) *Tectocepheus alatus*, e) *Micreremus brevipes*, f) *Chamobates (Xiphobates) interpositus*, g) *Chamobates* (*Xiphobates*) *sergienkoae* and h) *Oribatula* (*Zygoribatula*) *frisiae*.

Micreremidae Grandjean, 1954

***Micreremus brevipes* (Michael, 1888) (Figure 7e)**

Measurements. Body length, 308 µm and body width, 166 µm.

Material examined. KKO1.1-12 5 specimens, KKO2.1-12 1 specimen and KKO3.1-12 2 specimens.

Distribution in Turkey. Sakarya (Baran, 2016).

General distribution. Palearctic region (Subías, 2018).

Chamobatidae Thor, 1937

***Chamobates (Xiphobates) interpositus* Pschorn-Walcher, 1953 (Figure 7f)**

Measurements. Body length 311 µm and body width 222 µm.

Material examined. KKO1.1-12 124 specimens, KKO2.1-12 176 specimens, KKO3.1-12 111 specimens, KKO4.1-12 112 specimens and KKO5.1-12 83 specimens.

Distribution in Turkey. Ankara (Grobler et al., 2004).

General distribution. Mediterranean (Subías, 2018).

***Chamobates (Xiphobates) sergienkoae* Shaldybina, 1980 (Figure 7g)**

Measurements. Body length 337 µm and body width 279 µm.

Material examined. KKO1.1-12 40 specimens, KKO2.1-12 36 specimens, KKO3.1-12 35 specimens, KKO4.1-12 43 specimens and KKO5.1-12 25 specimens.

Distribution in Turkey. Giresun (Bayartogtokh et al., 2002).

General distribution. Southern Palearctic (Subías, 2018).

Oribatulidae Thor, 1929

***Oribatula* (*Zygoribatula*) *frisiae* (Oudemans, 1900) (Figure 7h)**

Measurements. Body length 428 µm and body width 247 µm.

Material examined. KKO1.1-12 179 specimens, KKO2.1-12 219 specimens, KKO3.1-12 116 specimens, KKO4.1-12 195 specimens and KKO5.1-12 115 specimens.

Distribution in Turkey. Ankara (Grobler et al., 2005).

General distribution. Holarctic region (frequently Palearctic) (Subías, 2018).

Scheloribatidae Grandjean, 1933

***Scheloribates* (*S.*) *laevigatus* (Koch, 1835) (Figure 8a)**

Measurements. Body length 419 µm and body width 257 µm.

Material examined. KKO1.1-12 65 specimens, KKO2.1-12 196 specimens, KKO3.1-12 48 specimens, KKO4.1-12 104 specimens and KKO5.1-12 46 specimens.

Distribution in Turkey. Konya (Dik et al., 1999).

General distribution. Semicosmopolitan (Subías, 2018).

Protoribatidae J. & P. Balogh, 1984

***Protoribates* (*P.*) *capucinus* Berlese, 1908 (Figure 8b)**

Measurements. Body length 341 µm and body width 149 µm.

Material examined. KKO1.1-12 1 specimen, KKO2.1-12 2 specimens and KKO5.1-12 2 specimens.

Distribution in Turkey. Erzurum and Konya, (Ayyıldız, 1988b; Dik et al., 1999).

General distribution. Cosmopolitan (Subías, 2018).

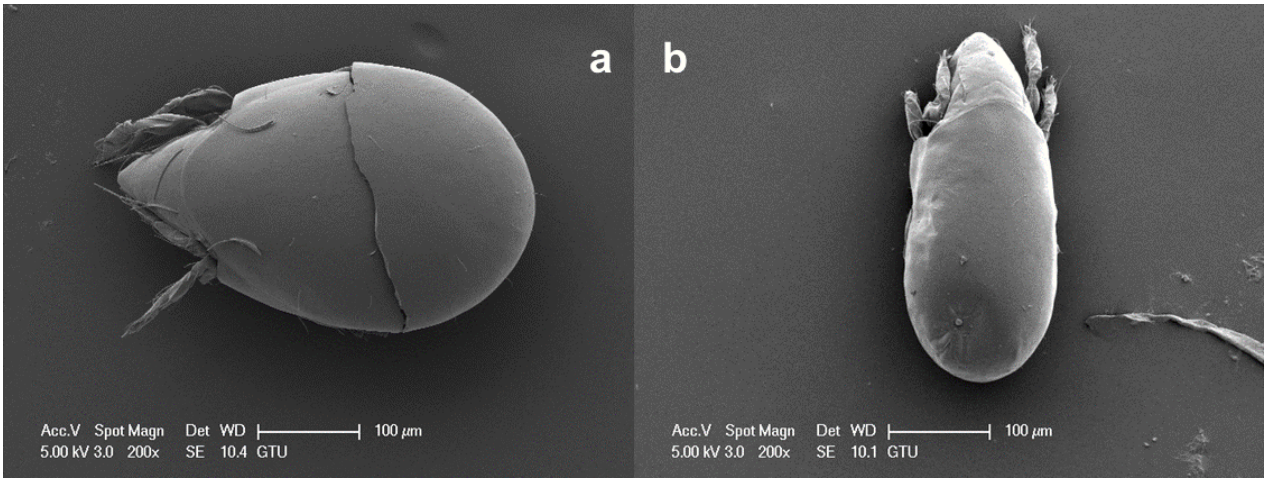

Figure 8. SEM images of a) *Scheloribates* (*S.*) *laevigatus* and b) *Protoribates* (*P.*) *capucinus*.

Conclusions

A total of 3,815 oribatid mites were allocated to 18 families and 22 species. One family (Parakalummidae), two genera (*Masthermannia* and *Cultroribula*) and four species [*M. mammillaris*, *C. bicultrata*, *N.* (*N.*) *bulanovae*, *A. integer*] are new records for Turkey. In the whole fauna, macropyline type oribatids represented 1.36% and brachypyline type oribatids 98.6% (29.2% picnonotic, 69.4% poronotic). Fifty percent of the Oribatid mites collected from Kocaeli City Forest have Palearctic distribution, 18.2% have Cosmopolitan, 13.6% have Mediterranean, 9.09% have Holarctic and 9.09% have semi cosmopolitan distribution.

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Original article (Orijinal araştırma)

Population development of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in vineyards of Çanakkale Province[1]

Çanakkale ili üzüm bağlarında *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae)'nin popülasyon gelişmesi

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Abstract

The spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is one of the important invasive pests of cultivated berry and stone fruits worldwide. The purpose of this study was to determine the population growth of *D. suzukii* in vineyards in Çanakkale Province from 2014 to 2017. The numbers of *D. suzukii* male and female individuals caught in the bait traps were recorded weekly. *Drosophila suzukii* population started to emerge from late September to February in all years. The maximum population of *D. suzukii* in the 2014-2015 sampling period was recorded on 29 November and 13 December 2014 at 153 individuals. In the 2015-2016 and 2016-2017 sampling periods, the maximum populations were 103 and 141 individuals recorded on 12 and 4 December, respectively. The results indicated that the *D. suzukii* population increased in the Çanakkale Province in three sampling years during November and December. These results are important as they provide information useful for the control of *D. suzukii* in vineyards in this province.

Keywords: Çanakkale, *Drosophila suzukii*, population development, vineyard, Turkey

Öz

Kanadı noktalı sirke sineği, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) dünyada meyve üretim alanlarında üzümsü ve çekirdekli meyvelerin önemli istilacı zararlılarından biridir. Bu çalışma ile 2014 ve 2017 yılları arasında Çanakkale ilindeki üzüm bağlarında *D. suzukii*'nin popülasyon gelişiminin belirlenmesi amaçlanmıştır. Örneklemeler 2014-2017 yılları arasında yem tuzakları kullanılarak haftada bir olacak şekilde yürütülmüştür. Tuzaklardan toplanan *D. suzukii* erkek ve dişi bireylerinin sayıları haftalık olarak sayılarak kaydedilmiştir. Üç yıl boyunca, *D. suzukii* popülasyonu eylül ayında ortaya çıkmaya başlamış ve şubat ayında sonlanmıştır. 2014-2015 örnekleme periyodunda *D. suzukii* popülasyonu, 29 Kasım ve 13 Aralık 2014 tarihlerinde toplam 153 erkek ve dişi birey ile en yüksek yoğunluğa ulaşmıştır. 2015-2016 ve 2016-2017 örnekleme döneminde, 12 Aralık ve 4 Aralık tarihlerinde sırası ile toplam 103 ve 142 birey olarak popülasyonun tepe noktasına ulaştığı saptanmıştır. Bu çalışmalar sonucunda *D. suzukii*' nin popülasyonunun Çanakkale ilinde kasım sonu ile aralık aylarında en yüksek noktaya ulaştığını göstermiştir. Çanakkale ili bağ alanlarında *D. suzukii*'nin mücadele zamanı hakkında bir fikir vermesi açısından bu sonuçlar oldukça önemlidir.

Anahtar sözcükler: Çanakkale, *Drosophila suzukii*, popülasyon gelişmesi, üzüm bağı, Türkiye

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Introduction

The worldwide increase in agricultural product trade with the advancement of international trade and technology has resulted in many invasive species rapidly spreading beyond their original range (Baser et al., 2015, 2018). Spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), from South Asia (India and Bangladesh), which has attracted significant attention as an invasive species in recent years, was first recorded as a pest of cherry (*Prunus* spp.) in Japan (Kanzawa, 1939). It was first found to have spread beyond its original range during the 1980s reaching Hawaii followed by North America (California, USA) and South America causing economic losses in fruits such as cherry, grape, peach and strawberry (Hauser, 2011). It was first recored in the Europe in Spain and Italy in 2008 (Raspi et al., 2011; Walsh et al., 2011; Calabria et al., 2012; Cini et al., 2014; Depra et al., 2014). *Drosophila suzukii* in now known to causes economic losses on stone fruits and berry fruits, such as blackberry, grape, mulberry, raspberry and strawberry (Beers et al., 2011; Bruck et al., 2011; Goodhue et al., 2011; Lee et al., 2011; Cini et al., 2012; Yu et al., 2013; Stewart et al., 2014; Ioriatti et al., 2015; Ögür et al., 2018).

Drosophila suzukii was first recorded in Turkey in August 2014 in strawberry in Erzurum Province (Orhan et al., 2016). It was then recorded during observations on fly species causing damage in Çanakkale Province in September 2014 in vineyards after which related studies were started (Özdamar & Kasap, 2017; Kasap & Özdamar, 2017).

The most important characteristic that distinguishes *D. suzukii* from other Drosophilidae species is its ability to oviposition during the pre-ripening period of the fruit with saw-shaped oviposition tubes (Baser et al., 2015; Ögür et al., 2018). The failure to detect infested fruits during and after the harvest period, hatching of the larvae after the harvest period, feeding on fruit tissue thereby damaging the fruit tissue and secondary infections (fungi and bacteria) in wounds inflicted during oviposition result in a decrease in marketable fruit, shortened shelf life and significant economic losses (Bolda et al., 2010; Walsh et al., 2010; Orhan et al., 2016; Önder et al., 2016; Tozlu et al., 2016; Ögür et al., 2018).

Çanakkale Province is an important area in Turkey for table and wine grape, and cherry production. Wine grape production in Turkey was 370 kt in 2004, increasing by 64% to 488 kt in 2017. Likewise, cherry production was 245 kt in 2014, increasing by about 2.5 times to 627 kt in 2017 (Anonymous, 2017). When production for the past 13 years is taken into consideration, the scale of potential damage that could be caused by *D. suzukii* cannot be ignored. Therefore, the purpose of this study was to determine the population development of *D. suzukii* in Çanakkale Province to help in minimizing the potential impact of this pest and to provide a baseline for future studies.

Material and Methods

Detection of *Drosophila suzukii* and field studies

The studies started in Çeşmealtı and Gümüşçay in Biga District of Çanakkale Province in August 2014 by hanging bait traps in vineyards for detecting *D. suzukii*. After the pest was detected, three vineyards were also selected in Çeşmealtı and Gümüşçay. Eight bait traps consisting of apple cider vinegar, wine and pure water were placed in each vineyard on 15 August. Observations were made weekly with trapped flies counted and placed in 70% ethanol. The traps were renewed every 15 d and data collection continued until the end of February 2017. The bait mixture (about 150 ml) was placed in 1.5 L plastic bottles with seven to eight holes of about 0.5 cm made in the upper half of the bottles as entry points for the flies (Figure 1). The traps were attached to the vines using wire ties.

Figure 1. Bait traps in the gardens to capture *Drosophila suzukii*.

Laboratory studies

The bait traps were collected weekly and brought to the ÇOMU Faculty of Agriculture Plant Protection Department Acarology Laboratory where the adult flies were collected using a sieve and then examined under a stereo microscope and gender recorded.

Results and Discussion

The first *D. suzukii* male individual was detected on 13 September 2014. The adult population continued to increase after this date and the highest number recorded was 153 individuals/trap on 29 November and 13 December 2014. The adult flies caught in the traps decreased from the end of December till the end of January. No adult flies were caught in the traps after that (Figure 2). A total of 1037 individuals were caught in all traps during 2014-2015 consisting of 514 females and 533 males. In 2015-2016, the traps were deployed on 30 August 2015 and the first *D. suzukii* adults were caught on 27 September (3♀ and 1♂). The number of adults caught in the traps increased after that date reaching 103 individuals/trap on 6 December. A total of 649 individuals were caught in all traps during 2015-2016 consisting of 303 females and 361 males (Figure 2). In 2016-2017, the traps were deployed in August with the first adults caught on 25 September (1♀ and 2♂). The number of adults caught in the traps increased after that date reaching 141 individuals/trap on 4 December. On 11 December, 124 individuals/trap were caught on 11 December and the population continued to decrease after that date. A total of 11 individuals/trap were caught on 29 January. No adults were caught after that (Figure 2). A total of 767 individuals were caught in all traps during 2016-2017 consisting of 370 females and 397 males. The female:male ratio was to be about 1:1 in all years (Figure 2).

The results showed that *D. suzukii* appeared in Çanakkale Province vineyards in mid-September, increasing during October and November, peaking in December and declining in January. Harvesting starts in Çanakkale Province vineyards in October. The increase of *D. suzukii* population after this date indicates that the pest feeds on fruit remaining in or near the vineyard and continues its life cycle by feeding on fruit such as nectarine and plum. It was found that *D. suzukii* may complete 15 generations/year under suitable conditions, with each cycle lasting about 10 d and remains active throughout the year under suitable conditions. It has also been reported that *D. suzukii* may feed on both ripe and unripe fruit particularly on apricot, blackberry, blueberry, cherry, peach, strawberry and other berry fruits (Anonymous, 2012). Baser et al. (2015) reported that *D. suzukii* populations continue throughout the year in southern Italy due to the suitable temperature and moisture, feeding on hosts such as blackberry, fig, grape, jujube, plum and rose hip, and reaching their maximum number in December. Orhan et al. (2016) reported *D. suzukii* in the Erzurum Province in August and September 2014 in strawberry fruit. Emiljanowicz et al. (2014) reported that *D. suzukii* lives for about 86 d at 22°C and 25% RH, with the longest life span of 154 d. They also report that females lays about 5.7 eggs/d totaling about 636 eggs over their entire life span. Also, the female:male ratio was about 1:1 and the net reproduction rate of a female (r_m value) was 0.179 females/female/d.

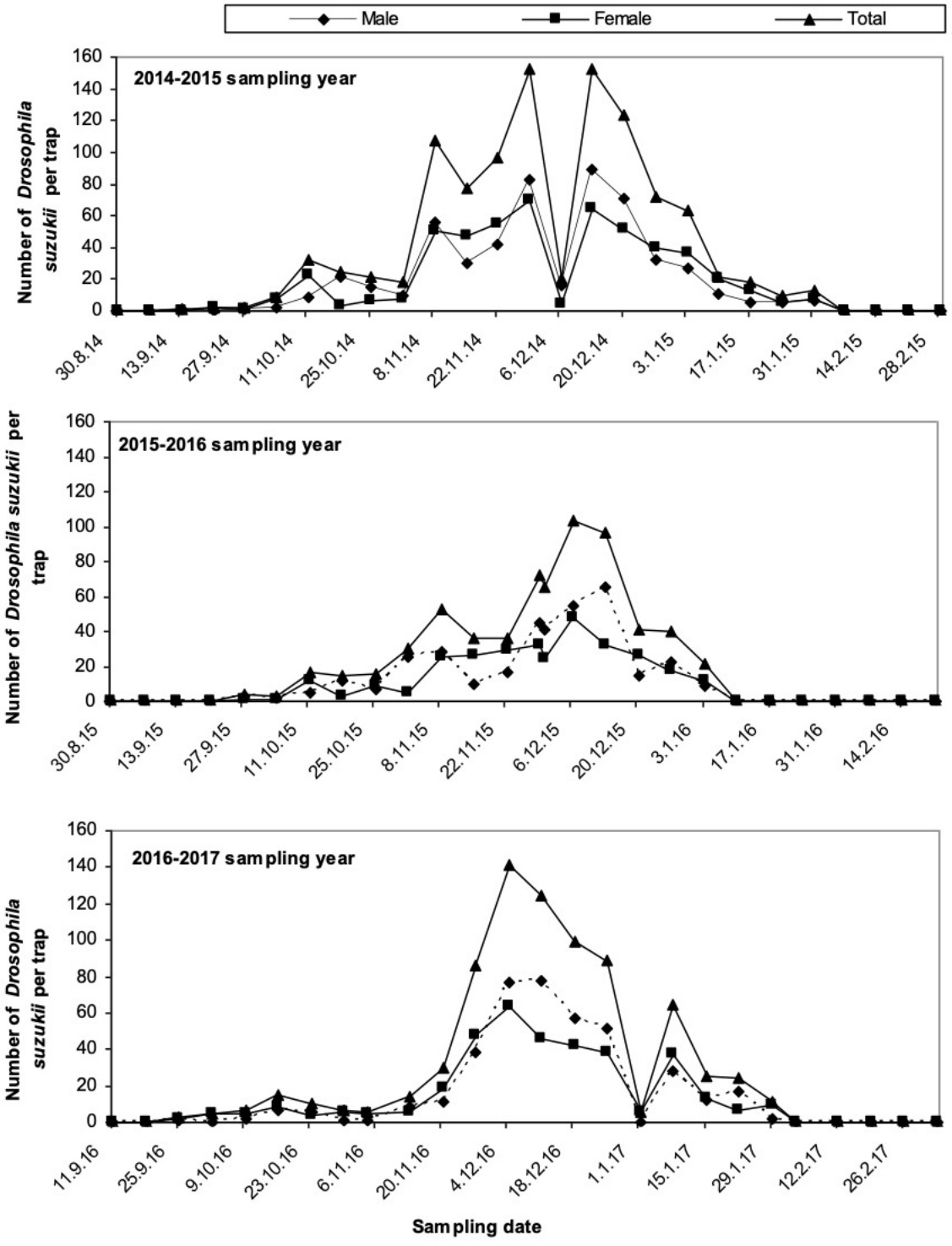

Figure 2. The population development of *Drosophila suzukii* in vineyards of Çanakkale Province in three growing seasons from 2014 to 2017.

Based on the present study and literature reports it is concluded that *D. suzukii* is an important pest for fruit such as cherry, grape, plum and strawberry throughout the year, and that the pest infests both damaged and healthy fruit. In Çanakkale Province, the population of the pest started to develop in September, reaching its maximum in December and continuing until January. It was noted that they primarily preferred healthy fruit during their development period, but that they can develop in damaged fruit when the availability of healthy fruit decreased.

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Original article (Orijinal araştırma)

Aphid (Hemiptera: Aphididae) species of the South Marmara Region of Turkey including the first record of *Dysaphis radicola meridialis* Shaposhnikov, 1964 for the aphid fauna of Turkey[1]

Güney Marmara Bölgesi'nden yaprakbitleri (Hemiptera: Aphididae) ile *Dysaphis radicola meridialis* Shaposhnikov, 1964'in Türkiye yaprakbiti faunası için ilk kaydı

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Abstract

This study aimed to determine aphid species, and to contribute new species and hosts, in Çanakkale and Balıkesir Provinces in the South Marmara Region of Turkey. Samples were taken from different host plants between March 2017 and November 2018. In total 74 aphid species were identified, including three subspecies from 34 genera belong to five subfamilies (Aphidinae, Calaphidinae, Chaitophorinae, Eriosomatinae and Lachninae) of the family Aphididae. Among these, *Dysaphis radicola meridialis* Shaposhnikov, 1964 collected from the roots of *Rumex* sp. (Polygonaceae) is a new subspecies record for the aphid fauna of Turkey from Çanakkale. The new host records for Turkey are *Ajuga orientalis* L. (Lamiaceae) on *Aulacorthum (Aulacorthum) solani* (Kaltenbach, 1843), *Cynoglossum creticum* Mill. (Boraginaceae) on *Acyrthosiphon (Acyrthosiphon) malvae* (Mosley, 1841) and *Tragopogon porrifolius* L. (Asteraceae) on *Trama (Neotrama) caudata* Del Guercio, 1909. Also, body measurements, diagnostic features, illustrations, distribution and biology are given for the new aphid records. With this contribution, the number of aphids in the fauna of Turkey has reached to 541 species and 14 subspecies.

Keywords: aphid, Balıkesir, Çanakkale, *Dysaphis radicola meridialis*, new host records

Öz

Bu çalışma Türkiye'nin Güneybatı Marmara bölgesinde yer alan Çanakkale ve Balıkesir illerinde bulunan yaprakbitlerinin belirlenmesi, yeni türler ve konukçu bitkiler ile katkıda bulunulmasını amaçlamaktadır. Örnekler Mart 2017 ve Kasım 2018 arasında farklı konukçu bitkiler üzerinden alınmıştır. Aphididae familyası içerisinde yer alan beş altfamilyaya (Aphidinae, Calaphidinae, Chaitophorinae, Eriosomatinae ve Lachninae) ait 34 cinse bağlı üç tanesi alttür olmak üzere toplam 74 yaprakbiti türü tanımlanmıştır. Tespit edilen türlerden *Rumex* sp. (Polygonaceae)'nin kök kısmından toplanan *Dysaphis radicola meridialis* Shaposhnikov, 1964 Çanakkale'den Türkiye yaprakbiti faunası için yeni alttür kaydı olarak verilmiştir. Konukçu bitkilerden *Ajuga orientalis* L. (Lamiaceae) türü *Aulacorthum (Aulacorthum) solani* (Kaltenbach, 1843); *Cynoglossum creticum* Mill. (Boraginaceae) türü *Acyrthosiphon (Acyrthosiphon) malvae* (Mosley, 1841) ve *Tragopogon porrifolius* L. (Asteraceae) türü *Trama (Neotrama) caudata* Del Guercio, 1909 için Türkiye'de yeni konukçu kayıtlarıdır. Ayrıca yeni yaprakbiti kaydının vücut ölçümleri, teşhis özellikleri, preparat resimleri, dağılımı ve biyolojisi verilmiştir. Bu güncel katkıyla birlikte Türkiye yaprakbiti faunasının sayısı 541 tür ve 14 alttüre ulaşmıştır.

Anahtar sözcükler: yaprakbiti, Balıkesir, Çanakkale, *Dysaphis radicola meridialis*, yeni konukçu kayıtları

[1] This manuscript is a part of the PhD thesis of the first author and a part of the study was presented as poster in VII. Turkish Plant Protection Congress with International Participation (14-17 November 2018, Muğla, Turkey).

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Introduction

Aphids (Hemiptera: Aphididae) are one of the most important insect groups that fed on plants in diverse habitats around the world. The family Aphididae currently consists of about 5000 species in 510 genera (Blackman & Eastop, 2018).

The first data on the aphid fauna of Turkey were obtained in the early part of the twentieth century with the reports of Trotter (1903), Fahringer (1922) and Houard (1922). Subsequently, the comprehensive study conducted by Bodenheimer & Swirski (1957) catalogued aphids from Middle East including 90 species from different regions of Turkey. Since then researchers such as Toros et al. (2002), Özdemir et al. (2005), Toper Kaygın et al. (2008), Görür et al. (2011; 2012), Özdemir & Barjadze (2015), Şenol et al. (2015) and Kök et al. (2016) have further contributed to our knowledge of the aphid fauna of Turkey. With recent new records (Görür et al., 2017), the aphid fauna of Turkey has reached 541 species and 13 subspecies belong to about 141 genera.

Turkey, situated at the junction between Europe and Asia, is one of the richest countries for flora and fauna due to its diverse climatical and topographical conditions. Despite this richness and characteristic features, knowledge of aphid fauna of Turkey is still limited compared to neighboring countries located the same zoogeographic region. For example, the aphid fauna of Greece, Iran and Georgia are known to consist of 335, 486 and 320 species, respectively, despite these countries having lower floristic diversity than Turkey (Barjadze et al., 2010; Rezwani, 2010; Margaritopoulos et al., 2013). More comprehensive studies are needed to reveal the biological richness of Turkey.

Çanakkale and Balıkesir Provinces area, called the South Marmara Region, which is an important link between Europe and Asia. The region has a Mediterranean climate and includes the Biga Peninsula, Edremit Gulf and Kaz Dağları (Ida Mountains) which are known to have a high degree of endemic floristic diversity. For example, there are about 800 plant taxa in the Kazdağı National Park and 68 of them are endemic to Turkey (Özhatay & Özhatay, 2005).

It is thought that aphid species found on other zoogeographical regions, especially in Europe, are likely to also occur in different regions of Turkey because of Turkey's position at the junction of Europe and Asia. Also, it is thought that global climate change may accelerate the migration of invasive aphid species between regions and continents (Kollar & Barta, 2016). This study aimed to determine aphid's species, and to contribute new species and hosts, in Çanakkale and Balıkesir Provinces in the South Marmara Region of Turkey. Also, the aim was to provide a details of host plants, body measurements, illustrations and diagnostic features of the new aphid records in order to support comprehensive and specific studies of aphids in Turkey.

Material and Method

Aphid sampling

Aphid were collected from their host plants in Çanakkale and Balıkesir Provinces of Turkey between March and November from 2017 to 2018 (Figure 1). Apterous and alate specimens from host plants were transferred with a soft brush (#00) to Eppendorf tubes containing 70% alcohol in sufficient numbers for laboratory study.

Figure 1. The sampling area of aphids in South Marmara region of Turkey (adapted from www.google.com/maps).

Collection, preparation and identification of aphid specimens

The collection and preparation of the specimens followed the method of Hille Ris Lambers (1950). Aphid species were determined using a LEICA DM 2500 microscope and LAS 4.1 version software according to Blackman & Eastop (2006; 2018). For new records, measurements of morphological characters, ratios of different body parts and chaetotaxy were made. Taxonomic status of all aphid species followed Favret (2018).

Abbreviations of morphological characters used in this study are: BL, body length; HW, head width; ANT, whole antenna length; ANT I, ANT II, ANT III, ANT IV, ANT V and ANT VI, antennal segments lengths; ANT III BD, antennal segment III basal diameter; LsH on ANT III, antennal segment III longest hair length; ANT VI base, antennal segment VI base length; ANT VI PT, processus terminalis of antennal segment VI; Urs (R IV+V), ultimate rostral segment length; HFem, hind femur length; HTib, hind tibia length; Ht I, hind tibia first segment length; Ht II, hind tibia second segment length; siph., siphinculi; Hairs on ABD tergite III, abdominal tergite segment III hair length; apt., apterous viviparous female; and alt, alate viviparous female.

Identification of all aphid species in this study were made by the senior author (ŞK), with confirmation of the new record of *Dysaphis radicola meridialis* Shaposhnikov, 1964 and some other species provided by Assoc. Prof. Dr. Shalva Barjadze (Institute of Zoology, Ilia State University, Tbilisi, Georgia). The host plants were identified by Assoc. Prof. Dr. Ersin Karabacak (Department of Biology, Faculty of Arts and Science, Çanakkale Onsekiz Mart University). Permanent slides of all aphid species collected have been deposited in the Department of Plant Protection, Faculty Agriculture, Çanakkale Onsekiz Mart University and Institute of Zoology, Ilia State University, Tbilisi, Georgia.

Results and Discussion

In total, 74 aphid species including three subspecies from 34 genera belong to five subfamilies, Aphidinae, Calaphidinae, Chaitophorinae, Eriosomatinae and Lachninae, of the Aphididae family were identified with their host plants from Çanakkale and Balıkesir Provinces in South Marmara Region of Turkey. Of these, *D. radicola meridialis*, is a new subspecies record for the aphid fauna of Turkey from Çanakkale Province. Also, its morphological features, detailed measurements of morphometric characters, illustrations, distribution and biology are detailed in this report. Of the 74 species collected, 72 belong to 33 genera from Çanakkale and 28 species belong to 16 genera from Balıkesir. Most of these species are

reported for the first time in both provinces as there have been relatively limited faunal studies of aphids in these provinces. Also, 96 host plant species belong to 37 families were found and are detailed below. Comparison of these data and that of previous studies revealed three new host records; *Ajuga orientalis* L. (Lamiaceae) for *Aulacorthum (Aulacorthum) solani* (Kaltenbach), *Cynoglossum creticum* Mill. (Boraginaceae) for *Acyrthosiphon (Acyrthosiphon) malvae* (Mosley) and *Tragopogon porrifolius* L. (Asteraceae) for *Trama (Neotrama) caudata* Del Guercio (Tuatay, 1988; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Özdemir et al., 2005; Eser et al., 2009; Akyürek et al., 2012; Sangün & Satar, 2012; Kuloğlu & Özder, 2017; Öztürk & Muştu, 2017). Taxonomic status, host plants, collection date and locality for the determined aphid species, with detailed taxonomic information of *D. radicola meridialis,* follow.

Family Aphididae

Subfamily Aphidinae

***Acyrthosiphon (Acyrthosiphon) lactucae* (Passerini, 1860)**

Material examined. Balıkesir, Edremit, 03.VI.2017, apt. 3♀♀, alt. 1♀, *Lactuca* sp. (Asteraceae); Çanakkale, Taşlıtarla, 31.V.2017, apt. 2♀♀, alt. 1♀, *Lactuca* sp. (Asteraceae).

***Acyrthosiphon (Acyrthosiphon) malvae* (Mosley, 1841)**

Material examined. Çanakkale, Umurbey, 01.V.2017, apt. 4♀♀, alt. 2♀♀, *Cynoglossum creticum* Mill. (Boraginaceae).

Comments. *Cynoglossum creticum* was not recorded as host plant for *A. malvae* in Turkey. The host plants determined in different regions of Turkey for *A. malvae* are given below.

Hosts in Turkey. This species has been reported on *Agrimonia* sp. (Rosaceae), *Geranium* sp. (Geraniaceae) and *Solanum* sp. (Solanaceae) (Tuatay & Remaudière, 1964; Çanakçıoğlu, 1975; Görür et al., 2009; Akyürek, 2013).

***Acyrthosiphon (Acyrthosiphon) pisum* (Harris, 1776)**

Material examined. Balıkesir, Gönen, 14.V.2017, apt. 4♀♀, alt. 2♀♀, *Medicago sativa* L. (Leguminosae); Susurluk, 14.V.2017, apt. 3♀♀, alt. 2♀♀, *M. sativa*; Çanakkale, Assos, 31.III.2017, apt. 4♀♀, alt. 2♀♀, *M. sativa*; Çan, 16.IV.2017, apt. 2♀♀, alt. 2♀♀, *M. sativa*; Eceabat, 06.V.2017, apt. 1♀, alt. 1♀, *M. sativa*.

***Aphis (Aphis) arbuti* Ferrari, 1872**

Material examined. Çanakkale, Yenice, 13.VI.2017, apt. 2♀♀, alt. 1♀, *Arbutus unedo* L. (Ericaceae).

***Aphis (Aphis) catalpae* Mamontova, 1953**

Material examined. Çanakkale, Central, 08.VI.2017, apt. 3♀♀, *Catalpa bignonioides* Walter (Bignoniaceae).

***Aphis (Aphis) craccivora* Koch, 1854**

Material examined. Balıkesir, Gönen, 27.IV.2017, apt. 2♀♀, alt. 1♀, *M. sativa*; İvrindi, 18.V.2018, apt. 1♀, alt. 1♀, *Amaranthus albus* L. (Amaranthaceae); Çanakkale, Biga, 12.V.2018, apt. 1♀, alt. 1♀, *Trifolium* sp. (Leguminosae); Central, 23.IV.2017, apt. 1♀, alt. 1♀, *Vicia faba* L. (Leguminosae); Central, 08.VI.2017, apt. 1♀, alt. 1♀, *Robinia pseudoacacia* L. (Leguminosae); Çiftlikköy, 22.IV.2018, apt. 1♀, alt. 1♀, *Trifolium stellatum* L. (Leguminosae); Musaköy, 21.VII.2017, apt. 1♀, alt. 1♀, *A. albus*; apt. 1♀, alt. 1♀, *Portulaca oleracea* L. (Portulacaceae); apt. 1♀, alt. 1♀, *Tribulus terrestris* L. (Zygophyllaceae); apt. 1♀, alt. 1♀, *Amaranthus retroflexus* L. (Amaranthaceae); Taşlıtarla, 24.V.2017, apt. 1♀, alt. 1♀, *Capsella rubella* Reut. (Brassicaceae); apt. 1♀, alt. 1♀, *M. sativa*.

***Aphis (Aphis) craccivora pseudacaciae* Takahashi, 1966**

Material examined. Çanakkale, Central, 02.VI.2017, apt. 3♀♀, alt. 1♀, *R. pseudoacacia*.

***Aphis (Aphis) fabae* Scopoli, 1763**

Material examined. Balıkesir, Havran, 26.IV.2018, apt. 1♀, alt. 1♀, *Chenopodium album* L. (Amaranthaceae); Susurluk, 14.V.2017, apt. 2♀♀, alt. 1♀, *Rumex* sp. (Polygonaceae); Çanakkale, Anzac Cove, 06.V.2017, apt. 1♀, alt. 1♀, *Rumex crispus* L. (Polygonaceae); Bayramiç, 16.IV.2018, apt. 2♀♀, alt. 1♀, *Rumex* sp.; Central, 20.V.2017, apt. 3♀♀, *Silybum marianum* (L.) Gaertn. (Asteraceae); Musaköy, 31.V.2017, apt. 3♀♀, *Fumaria* sp. (Papaveraceae); apt. 1♀, alt. 1♀, 16.V.2017, *Rumex* sp.; Taşlıtarla, 31.V.2017, apt. 2♀♀, alt. 1♀, *C. album*.

***Aphis (Aphis) fabae mordvilkoi* Börner & Janich 1922**

Material examined. Çanakkale, Central, 02.VI.2017, apt. 5♀♀, *Philadelphus coronarius* L. (Hydrangeaceae).

***Aphis (Aphis) frangulae* Kaltenbach, 1845**

Material examined. Çanakkale, Yolindi, 12.V.2018, apt. 2♀♀, alt. 2♀♀, *Lysimachia atropurpurea* L. (Primulaceae).

***Aphis (Aphis) gossypii* Glover, 1877**

Material examined. Balıkesir, Central, 23.V.2018, apt. 2♀♀, alt. 1♀, *Malus domestica* Borkh. (Rosaceae); Çanakkale, Central, 22.IV.2017, apt. 2♀♀, alt. 1♀, *Hibiscus syriacus* L. (Malvaceae); Central, 02.VI.2017, apt. 2♀♀, alt. 1♀, *C. bignonioides*; Eceabat, 14.XII.2017, apt. 2♀♀, alt. 1♀, *Chrysanthemum* sp. (Asteraceae); Musaköy, 24.V.2017, apt. 2♀♀, alt. 1♀, *Crepis* sp. (Asteraceae); 31.V.2017, apt. 2♀♀, *M. domestica*; 31.V.2017, apt. 1♀, alt. 1♀, *Urtica urens* L. (Urticaceae); Umurbey, 01.V.2017, apt. 2♀♀, alt. 2♀♀, *Veronica* sp. (Plantaginaceae).

***Aphis (Aphis) hederae* Kaltenbach, 1843**

Material examined. Çanakkale, Central, 27.IV.2017, apt. 4 ♀♀, *Hedera helix* L. (Araliaceae).

***Aphis (Aphis) nerii* Boyer de Fonscolombe, 1841**

Material examined. Balıkesir, Central, 12.VI.2017, apt. 2♀♀, alt. 2♀♀, *Nerium oleander* L. (Apocynaceae); Çanakkale, Dardanos, 24.V.2017, apt. 2♀♀, *N. oleander*; Kepez, 20.05.2018, apt. 2♀♀, N. *oleander*; Taşlıtarla, 24.V.2017, apt. 4♀♀, alt. 2♀♀, *Cynanchum acutum* L. (Apocynaceae).

***Aphis (Aphis) pomi* De Geer, 1773**

Material examined. Çanakkale, Central, 08.VI.2017, apt. 4♀♀, alt. 2♀♀, *Malus floribunda* Siebold ex Van Houtte (Rosaceae).

***Aphis (Aphis) punicae* Passerini, 1863**

Material examined. Çanakkale, Central, 20.V.2017, apt. 5♀♀, *Punica granatum* L. (Lythraceae).

***Aphis ruborum* (Börner, 1932)**

Material examined. Çanakkale, Gallipoli Peninsula, 7.IV.2018, apt. 4♀♀, alt. 2♀♀, *Rubus* sp. (Rosaceae). Musaköy, 29.III.2017, apt. 3♀♀, alt. 1♀, *Rubus caesius* L.

***Aphis (Aphis) rumicis* Linnaeus, 1758**

Material examined. Balıkesir, Susurluk, 14.V.2017, apt. 4♀♀, alt. 1♀, *R. crispus*; Çanakkale, Biga, 12.V.2018, apt. 4♀♀, alt. 1♀, *Rumex pulcher* L. (Polygonaceae).

***Aphis (Aphis) sambuci* Linnaeus, 1578**

Material examined. Çanakkale, Central, 15.IV.2017, apt. 5♀♀, *Sambucus nigra* L. (Adoxaceae).

***Aphis (Aphis) solanella* Theobald, 1914**

Material examined. Balıkesir, Susurluk, 14.V.2017, apt. 6♀♀, alt. 2♀♀, *Matricaria* sp. (Asteraceae); Çanakkale, Çıplak, 31.III.2017, apt. 3♀♀, alt. 1♀, *U. urens*; Musaköy, 16.V.2017, apt. 4♀♀, *Rumex* sp. (Polygonaceae); 16.V.2017, apt. 3♀♀, alt. 1♀, *Papaver rhoeas* L. (Papaveraceae); 31.V.2017, apt. 2♀♀, *C. album*; 21.VII.2017, apt. 4♀♀, *Solanum americanum* Mill. (Solanaceae); Taşlıtarla, 24.V.2017, apt. 2♀♀, *Cirsum* sp. (Asteraceae).

***Aphis (Aphis) spiraecola* Patch, 1914**

Material examined. Balıkesir, Central, 10.VI.2017, apt. 3♀♀, alt. 1♀, *Prunus avium* L. (Rosaceae); Çanakkale, Central, 02.VI.2017, apt. 4♀♀, *Spiraea* x *vanhouttei* (Briot) Zabel (Rosaceae); 08.VI.2017, apt. 4♀♀, alt. 1♀, *Viburnum opulus* L. (Adoxaceae); 12.V.2018, apt. 2♀♀, alt. 1♀, *Pittosporum tobira* (Thunb.) W.T. Aiton (Pittosporaceae); 20.V.2018, apt. 2♀♀, *N. oleander*); 15.06.2018, apt. 3♀♀, alt. 1♀, *Chrysanthemum* sp. (Asteraceae) ; Dardanos, 16.V.2017, apt. 2♀♀, alt. 1♀, *P. avium*; 24.V.2017, apt. 1♀, alt. 1♀, *N. oleander*; 31.V.2017, *apt.* 2♀♀, alt. 1♀, *Viburnum tinus* L. (Adoxaceae).

***Aphis (Aphis) umbrella* (Börner, 1950)**

Material examined. Balıkesir, Gönen, 10.VI.2018, apt. 3♀♀, alt. 2♀♀, *Malva* sp. (Malvaceae); Çanakkale, Gallipoli Peninsula, 7.IV.2018, apt. 3♀♀, alt. 1♀, *M. sylvestris* L.; Kepez, 04.IV.2017, apt. 3♀♀, alt. 1♀, *Malva* sp.

***Aphis (Aphis) vallei* Hille Ris Lambers & Stroyan, 1959**

Material examined. Çanakkale, Küçükanafarta, 06.V.2017, apt. 6♀♀, *Euphorbia rigida* M. Bieb. (Euphorbiaceae).

***Aphis (Aphis) viticis* Ferrari, 1872**

Material examined. Çanakkale, Central, 11.V.2018, apt. 3♀♀, *Vitex agnus-castus* L. (Lamiaceae); Gallipoli Peninsula, 06.V.2017, apt. 2♀♀, alt. 2♀♀, *V. agnus-castus* L.

***Aulacorthum (Aulacorthum) solani* (Kaltenbach, 1843)**

Material examined. Balıkesir, Ida Mountains, 13.V.2017, apt. 4♀♀, alt. 2♀♀, *A. orientalis*.

Comments. *Aulacorthum solani* was not recorded as host plant for *A. solani* in Turkey. The host plants determined in different regions of Turkey for *A. solani* are given below. Both adult apterous-alate female and nymph of *A. solani* were found under the leaves of *A. orientalis,* which is quite hairy-dusty and distributed in foothills of Ida Mountains including in both Çanakkale and Balıkesir Provinces.

Hosts in Turkey. *Antirrhinum* sp. (Plantaginaceae)*, Begonia semperflorens* Link & Otto (Begoniaceae)*, Canna indica* L. (Cannaceae), *Cydonia oblonga* Mill. (Rosaceae)*, Dianthus anatolicus* Boiss. and *Dianthus barbatus* L. (Caryophyllaceae)*, Hydrangea macrophylla* (Thunb.) Ser. (Hydrangeaceae), *Lactuca* sp. (Asteraceae), *Lycopersicum esculentum* L. (Solanaceae)*, N. oleander*, *Rubus* sp. (Rosaceae); *Taraxacum scaturiginosum* G. Hagl. (Asteraceae)*, Tulipa gesneriana* L. (Liliaceae), *Veronica anagalloides* Guss. (Scrophulariaceae); *Viburnum orientale* Pall (Adoxaceae); *Yucca flamentosa* L. (Asparagaceae) (Tuatay, 1988; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Eser et al., 2009; Akyürek et al., 2012; Sangün & Satar, 2012; Kuloğlu & Özder, 2017; Öztürk & Muştu, 2017).

***Brachycaudus (Thuleaphis) amygdalinus* (Schouteden, 1905)**

Material examined. Çanakkale, Central, 15.IV.2017, apt. 2♀♀, alt. 2♀♀, *Prunus cerasifera* Ehrh. (Rosaceae); Ezine, 22.IV.2017, apt. 3♀♀, alt. 1♀, *Prunus persica* (L.) (Rosaceae).

***Brachycaudus (Prunaphis) cardui* (Linnaeus, 1758)**

Material examined. Balıkesir, Balya, 12.VI.2017, apt. 2♀♀, alt. 1♀, *Cirsium* sp. (Asteraceae); Çanakkale, Biga, 12.V.2018, apt. 3♀♀, alt. 2♀♀, *Carduus pycnocephalus* L. (Asteraceae); Kepez, 04.IV.2017, apt. 3♀♀, alt. 1♀, *Cynara* sp. (Asteraceae); Taşlıtarla, 24.V.2017, apt. 4♀♀, *Cirsium* sp. (Asteraceae).

***Brachycaudus (Brachycaudus) helichrysi* (Kaltenbach, 1843)**

Material examined. Balıkesir, Susurluk, 14.V.2017, apt. 4♀♀, alt. 2♀♀, *Matricaria* sp. (Asteraceae); Çanakkale, Central, 27.IV.2017, apt. 6♀♀, alt. 1♀, *Calendula officinalis* L. (Asteraceae); Çiftlikköy, 22.IV.2018, apt. 5♀♀, *Carduus pycnocephalus* L.(Asteraceae) ; Taşlıtarla, 12.IV.2017, apt. 5♀♀, alt. 3♀♀, *Prunus domestica* L. (Rosaceae)

***Brachyunguis (Brachyunguis) tamaricis* (Lichtenstein, 1886)**

Material examined. Çanakkale, Central, 5.VI. 2017, apt. 5♀♀, alt. 3♀♀, *Tamarix* sp. (Tamaricaceae).

***Brevicoryne brassicae* (Linnaeus, 1758)**

Material examined. Balıkesir, Susurluk, 14.V.2017, apt. 4♀♀, alt. 2♀♀, *Brassica nigra* (L.) K. Koch (Brassicaceae); Çanakkale, Assos, 31.III.2017, apt. 3♀♀, alt. 2♀♀, *Brassica oleracea* L. (Brassicaceae); Halileli, 05.XII.2017, apt. 2♀♀, alt. 1♀, *B. oleracea*; Taşlıtarla, 24.V.2017, apt. 5♀♀, *Capsella rubella* Reut. (Brassicaceae).

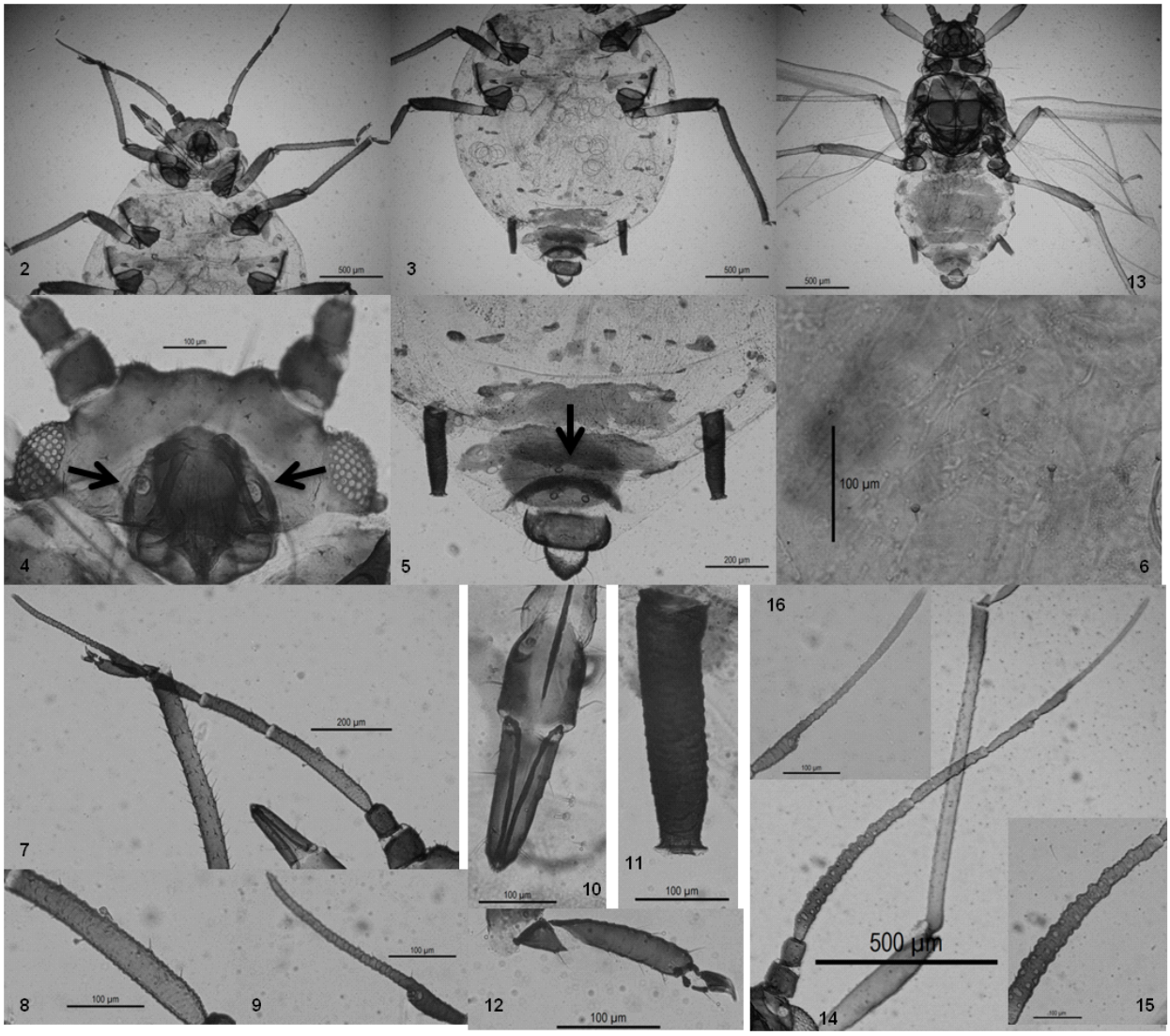
***Dysaphis (Pomaphis) plantaginea* (Passerini, 1860)**

Material examined. Çanakkale, Central, 11.V.2017, 4♀♀, alt. 1♀, *M. domestica*.

***Dysaphis radicola meridialis* Shaposhnikov, 1964**

Dysaphis radicola meridialis collected from root of *Rumex* sp. (Polygonaceae) is a new subspecies record for the aphid fauna of Turkey from Çanakkale.

Description. Color in living specimens is greenish gray to dark gray with waxy-powdered appearance. Color of apterous viviparous female specimens on slide; ANT I and II brown or dark brown, ANT III and IV pale or dusky, ANT V and base of ANT VI brown or dark brown, PT of ANT VI pale or dusky; head dusky; legs pale brown, brown or dark brown; coxa dark, trochanter dusky, femur dark brown with paler base, tibia pale or dusky with dark brown apices; II and III segments of rostrum generally pale, while URS dusky or brown; siphinculi and cauda dark. Alate viviparous females have a widely patch on ABD Tergite II between V. Body of apterous viviparous female elliptical (Figures 2 and 3); BW 0.62-0.68 x BL. ANT PT 2.67-2.95 x ANT VI Base. Antennal tubercle weakly developed. ANT III, IV and V of apterous female without secondary rhinaria while ANT III, IV and V of alate female 46-47, 11-12, 0 on secondary rhinaria, respectively (Figures 7, 8, 14 and 15). The number of hairs of antennal segments: ANT I 4-6, ANT II 2-3 and ANT III 9-11. Longest hairs on ANT III 0.014-0.015 mm, 0.50-0.52 x ANT BD III. Dorsum of head, thorax and abdomen reticulate. Marginal tubercle always present ABD Tergite I-VII; Spinal tubercles present on prothorax (1) and ABD tergite 7 (1) and 8 (2) (Figures 4 and 5). Rostrum about reaches to the hind coxae, URS 1.43-1.47 x HT II, 4 hairs present on URS (Figure 10). Hairs on ABD tergite III are 0.010-0.012 mm, shorter than ANT BD III. Siphunculi dark, cylindrical, narrowed to the apex and slightly sigmoid (Figure 11), siph. 1.89 x 2.22 cauda. Cauda escutcheon shape, its length 0.66-0.80 x width (Table 1).



Figures 2-16. *Dysaphis radicola meridialis:* 2,3 - body of apterous viviparous female; 4,5 - Spinal tubercles on head and ABD tergite 7 and 8 of apterous female; 6 - Hairs on ABD tergite 3 of apterous female; 7 - ANT segments of apterous female; 8,9 - ANT III and PT and base of ANT VI of apterous female; 10 - URS of apterous female; 11 - Siph. of apterous female; 12 - HT I and HT II of apterous female; 13 - body of alate viviparous female; 14 - ANT of alate female; 15,16 - seconder rhinaria on ANT III-IV and PT of ANT VI of alate female.

Material examined. To identify this new subspecies for the aphid fauna of Turkey, six apterous viviparous females and four alate viviparous females on three slides were examined and measurements made (Table 1). Specimens of this species were collected from the Biga District of Çanakkale, 40°15'17.1" N 27°13'12.5" E, 16.V.2017.

Table 1. The morphometric data (mm) of *Dysaphis radicola meridialis* from Çanakkale

Morphometric characters		*Dysaphis radicola meridialis*	
		Apterous female (n=6)	Alate female (n=4)
Length of body parts (mm)	BL	2.652-2.900	2.089-2.490
	HW	0.507-0.577	0.493-0.515
	ANT	1.135-1.193	1.526-1.542
	ANT I	0.083-0.092	0.081-0.082
	ANT II	0.074-0.081	0.079-0.080
	ANT III	0.300-0.383	0.490-0.501
	ANT III BD	0.027-0.030	0.026-0.028
	LsH on ANT III	0.014-0.015	0.011-0.012
	ANT IV	0.161-0.214	0.247-0.267
	ANT V	0.125-0.163	0.160-0.172
	ANT VI	0.376-0.414	0.446-0.463
	ANT VI base	0.100-0.115	0.106-0.109
	ANT VI PT	0.275-0.299	0.337-0.357
	Urs (R IV+V)	0.194-0206	0.183-0.200
	H Fem	0.542-0.656	0.643-0.701
	H Tib	0.951-1.110	1.203-1.291
	Ht I	0.041-0.051	0.042-0.043
	Ht II	0.132-0.140	0.135-0.136
	Siph.	0.246-0.274	0.210-0.229
	Cauda length	0.111-0.145	0.125-0.139
	Cauda width	0.160-0.180	0.135-0.147
	Hairs on ABD tergite III	0.010-0.012	0.009-0.010
Number of setae on various body parts	Ant I	4-6	4
	Ant II	2-3	2-3
	Ant III	9-11	6-7
	Urs (R IV+V)	4	4
	Cauda	5-6	4-6
Number of seconder rhinaria on antenal segments	Ant III	0	46-47
	Ant IV	0	11-12
	Ant V	0	0

Table 1. (Continued)

Morphometric characters		*Dysaphis radicola meridialis*	
		Apterous female (n=6)	Alate female (n=4)
Ratios of various body parts (mm)	Whole antenna / Body	0.40-0.69	0.62-0.73
	Pt / Base	2.67-2.95	3.09-3.53
	Pt / Ant III	0.78-0.92	0.67-0.73
	Urs / Ht II	1.43-1.47	1.36-1.47
	Siph. / Ant III	0.72-0.85	0.42-0.47
	Siph. / Body length	0.09-0.10	0.09-0.10
	Siph. / Cauda	1.89-2.22	1.65-1.68
	Siph. / Hind femur	0.42-0.47	0.33-0.34
	Cauda length / Cauda width	0.66-0.80	0.92-0.95
	LsH on Ant III / BD III	0.47-0.54	0.39-0.42

An identification keys the species of the genus of *Dysaphis* Börner, 1931 for apterous females found worldwide (After Blackman & Eastop, 2018);

1. Cauda helmet-shaped and bearing 4-8 hairs 2

- Cauda tongue-shaped, finger-shaped, rounded, bluntly triangular and bearing more than 8 hairs…….....other genera (including *Acyrthosiphon*, *Aphis*, *Brachyunguis* and *Macrosiphum*)

2. Spinal tubercles (STu) usually present on head and ABD tergite 8, or 7 and 8. Marginal tubercles (MTu) usually present. Siph. without subapical annular incision 3

- STu absent, and MTu sporadically present. Siph. with subapical annular …….....…..other genus (*Brachycaudus cardui* and *B. helichrysi*)

3. STu present on all segments from head to ABD tergite 8 4

- STu only on head and ABD tergite 8, or 7 and 8 5

4. Hairs on ANT III and ABD tergite 1-5 fine-pointed, on ANT III longer than 45 µm, 2 or more × ANT BD III. R IV+V 1.55-1.70 × HT II ..... *Dysaphis rumecicola*

- Hairs on ANT III and ABD tergite 1-5 with blunt apices, on ANT III being less than 45 µm long, less than 2 × BD III. R IV+V 1.3-1.6 × HT II ..... *Dysaphis emicis*

5. Siph. 1.2-1.8 × cauda. Longest hairs on ABD tergite 3: 24-39 µm long, as long as or longer than ANT BD III ..... *Dysaphis foeniculus*

- Siph. 2.0-2.5 × cauda. Longest hairs on ABD tergite 3: 8-23 µm long, shorter than ANT BD III 6

6. Longest hairs on ANT III 20-40 µm long, 0.9-1.5 × BD III ..... *Dysaphis radicola* s. str.

- Longest hairs on ANT III 8-13 µm long, 0.4-0.7 × BD III ..... *Dysaphis radicola meridialis*

Host plant: Apterous females and nymphs of *D. radicola meridialis* were collected from the roots of *Rumex* sp. (Polygonaceae).

Distribution. Armenia, Austria Hungary, Azerbaijan, Belarus, Britain, Bulgaria, Czech Republic, Denmark, France, Georgia, Great Greece, Iran, Italy, Japan. Lithuania, Moldova, Netherlands, Poland, Portugal, Romania, Russia, Slovakia, South Korea, Spain, Sweden and Ukraine (Stekolshchikov, 2006).

Biology. Spring generations of *D. radicola* cause the rolling of and red galls on the lateral margins of the leaves of *Malus* spp. such as *M. domestica*, *M. orientalis and M. sylvestris*. While their fundatrix (first hatched aphid females following winter) occur in galls formed by a longitudinal down folding of the tip of leaf, fundatrigeniae live in galls formed by a downward enrolling of the margin of the leaf. Alate migrants arise from the second generation and migrate to stem and leaf bases near the root of *Rumex* spp. from early May to mid-June in the northern areas (Leningrad Province) and the southern areas (North Caucasus, Crimea). Subsequently, this species remigrates to *Malus* from late August to early November in the northern areas (Sweden, Leningrad Province) and in the southern areas (North Caucasus, Crimea). While the first males appear a few days later than gynoparae forms, the first oviparous females on leaves of *Malus* spp. were reported early September in Leningrad Province (Russian Federation) and in late October in the North Caucasus (Stekolshchikov, 2006).

Comments. *Dysaphis radicola* was initially identified by Mordvilko (1897) as a result of examination on numerous apterous and alate females collected from *R. crispus* in Poland in 1895. Afterwards, Shaposhnikov (1964) described the southern populations of this species as a subspecies, *Dysaphis radicola meridialis* Shaposhnikov, 1964, with short hairs on the body. However, after numerical analysis, it was suggested that *D. radicola meridialis* is a synonym of *D. radicola radicola* (Mordvilko, 1897). However, there are still differences of opinion between authors on this subject. Therefore, it was reported as *D. radicola meridialis* as in Blackman & Eastop (2018). This subspecies is newly recognized in Turkey. *Dysaphis* consists of 14 species in Turkey. These species are *Dysaphis affinis* (Mordvilko, 1928), *D. crataegi* (Kaltenbach, 1843), *D. devecta* (Walker, 1849), *D. emicis* (Mimeur, 1935), *D. foeniculus* (Theobald, 1923), *D. lauberti* (Börner, 1940), *D. tulipae* (Boyer de Fonscolombe, 1841), *D. (Cotoneasteria) microsiphon* (Nevsky, 1929), *D. (Pomaphis) aucupariae* (Buckton, 1879), *D. (Pomaphis) pavlovskyana* Narzikulov, 1957, *D. (Pomaphis) plantaginea* (Passerini, 1860), *D. (Pomaphis) pyri* (Boyer de Fonscolombe, 1841), *D. (Pomaphis) reaumuri* (Mordvilko, 1928) and *D. (Pomaphis) sorbi* (Kaltenbach, 1843) (Görür et al., 2012).

***Hayhurstia atriplicis* (Linnaeus, 1761)**

Material examined. Çanakkale, Musaköy, 31.V.2017, apt. 6♀♀, alt. 2♀♀, *C. album*.

***Hyalopterus amygdali* (Blanchard, 1840)**

Material examined. Balıkesir, Central, 14.V.2017, apt. 1♀, alt. 1♀, *Prunus. armeniaca* L. (Rosaceae); apt. 6♀♀, alt. 3♀♀, *Prunus dulcis* (Mill.) D. A. Webb and *Prunus cerasifera*, Çanakkale-Central,11.V.2017.

***Hyalopterus pruni* (Geoffroy, 1762)**

Material examined. Balıkesir, Balya, 13.V.2017; apt. 2♀♀, alt. 2♀♀, *Phragmites australis* (Cav.) Trin. ex Steud. (Poaceae); Çanakkale, Taşlıtarla, 16.V.2017, apt. 4♀♀, alt. 2♀♀, *P. domestica*.

***Hyperomyzus (Hyperomyzus) lactucae* (Linnaeus, 1758)**

Material examined. Balıkesir, Ida Mountains, 13.VI.2017, apt. 2♀♀, alt. 2♀♀, *Sonchus* sp. (Asteraceae); Çanakkale, Kepez, 04.IV.2017, apt. 2♀♀, alt. 2♀♀, *Sonchus* sp.

***Liosomaphis berberidis* (Kaltenbach, 1843)**

Material examined. Balıkesir, Central, 12.VI.2018, apt. 5♀♀, *Berberis thunbergii* DC. (Berberidaceae); Çanakkale, Central, 01.V.2017, apt. 5♀♀, alt. 2♀♀, *B. thunbergii*; 15.IV.2017, apt. 2♀♀, alt. 1♀, *Berberis aquifolium* Pursh.

***Macrosiphoniella (Macrosiphoniella) sanborni* (Gillette, 1908)**

Material examined. Çanakkale, Eceabat, 14.XII.2017, apt. 5♀♀, alt. 2♀♀, *Chrysanthemum* sp. (Asteraceae).

***Macrosiphum (Macrosiphum) euphorbiae* (Thomas, 1878)**

Material examined. Çanakkale, Central, 10.VI.2017, apt. 5♀♀, alt. 1♀, *Petunia* sp. (Solanaceae); Eceabat, 08.IV.2017, apt. 2♀♀, alt. 1♀, *Rosa* sp. (Rosaceae).

***Macrosiphum (Macrosiphum) funestum* (Macchiati, 1885)**

Material examined. Çanakkale, Yenice, Ida Mountains, 13.VI.2017, apt. 5♀♀, alt. 2♀♀, *Rubus* sp.

***Macrosiphum (Macrosiphum) rosae* (Linnaeus, 1758)**

Material examined. Balıkesir, Edremit, 13.VI.2017, 2♀♀, alt. 1♀, *Rosa* sp. (Rosaceae); Çanakkale, Biga, Hacıköy, 12.V.2018, 4♀♀, alt. 2♀♀, *Scabiosa* sp. (Caprifoliaceae); Eceabat, 08.IV.2017, apt. 5♀♀, *Rosa* sp.

***Myzus (Myzus) cerasi* (Fabricius, 1775)**

Material examined. Çanakkale, Musaköy, 13.V.2017, apt. 3♀♀, alt. 2♀♀, *P. avium*; Taşlıtarla, 19.V.2018, apt. 5♀♀, alt. 3♀♀, *P. avium.*

***Myzus (Nectarosiphon) persicae* (Sulzer, 1776)**

Material examined. Balıkesir, Edremit, 10.VI.2017, apt. 4♀♀, alt. 3♀♀, *Prunus persica* (Rosaceae); Çanakkale, Central, 02.VI.2017, apt. 2♀♀, alt. 1♀, *C. bignonioides*; Halileli, 05.XII.2017, apt. 5♀♀, alt. 3♀♀, *B. oleracea*; Lâpseki, 16.V.2017, apt. 2♀♀, alt. 2♀♀, *P. persica*.

***Ovatus (Ovatus) insitus* (Walker, 1849)**

Material examined. Çanakkale, Gallipoli Peninsula, 7.IV.2018, apt. 4♀♀, alt. 2♀♀, *C. oblonga*.

***Rhodobium porosum* (Sanderson, 1900)**

Material examined. Çanakkale, Eceabat, 16.IV.2017, apt. 3♀♀, alt. 1♀, *Rosa* sp.

***Rhopalosiphum maidis* (Fitch, 1856)**

Material examined. Çanakkale, Bayramiç, 26.IX.2017, apt. 3♀♀, *Zea mays* L. (Poaceae); Ezine, Akköy, 22.IV.2017, apt. 5♀♀, *Triticum aestivum* L. (Poaceae); Musaköy, 21.VII.2017, apt. 5♀♀, *Setaria* sp. (Poaceae).

***Schizaphis* (*Schizaphis*) *graminum* (Rondani, 1847)**

Material examined. Çanakkale, Musaköy, 21.VII.2017, apt. 3♀♀, alt. 2♀♀, *Sorghum* sp. (Poaceae); 21.VII.2017, apt. 4♀♀, *Setaria* sp. (Poaceae).

***Sitobion (Sitobion) avenae* (Fabricius, 1775)**

Material examined. Balıkesir, Balya, 10.V.2017, apt. 4♀♀, *T. aestivum*; Çanakkale, Batak Plain, 15.IV.2018, apt. 5♀♀, alt. 1♀, *T. aestivum*; Belen, 22.IV.2018, apt. 3♀♀, alt. 2♀♀, *Poa* sp. (Poaceae); Biga, 12.V.2018, apt. 2♀♀, alt. 2♀♀, *Dactylis glomerata* L. (Poaceae); Biga, Gerlengeç, 12.V.2018, apt. 4♀♀, alt. 2♀♀, *Hordeum bulbosum* L. (Poaceae).

***Sitobion (Sitobion) fragariae* (Walker, 1848)**

Material examined. Balıkesir, Ida Mountains, 13.V.2017, apt. 3♀♀, alt. 2♀♀, *Anthoxanthum odoratum* L. (Poaceae); apt. 5♀♀, alt. 1♀, *D. glomerata*; Çanakkale, Büyükanafarta, 06.V.2017, apt. 3♀♀, alt. 2♀♀, *Bromus arvensis* L. (Poaceae); apt. 4♀♀, alt. 2♀♀, *Hordeum murinum* L. (Poaceae).

***Uroleucon (Uromelan) jaceae aeneum* (Linnaeus, 1758)**

Material examined. Çanakkale, Biga, 12.V.2018, apt. 5♀♀, alt. 3♀♀, *Carlina* sp. (Asteraceae).

***Uroleucon (Uroleucon) sonchi* (Linnaeus, 1767)**

Material examined. Balıkesir, Balya, 18.V.2017, apt. 3♀♀, alt. 2♀♀, *Sonchus* sp. (Asteraceae); Çanakkale, Küçükanafarta, 06.V.2017, apt. 4♀♀, alt. 2♀♀, *Sonchus* sp.

***Wahlgreniella arbuti* (Davidson, 1910)**

Material examined. Çanakkale, Ida Mountains, 13.VI.2017, apt. 4♀♀, alt. 2♀♀, *Arbutus. Unedo* L. (Ericaceae).

Subfamily Calaphidinae

***Chromaphis juglandicola* (Kaltenbach, 1843)**

Material examined. Balıkesir, Gönen, 12.VI.2018, alt. 5♀♀, *Juglans regia* L. (Juglandaceae); Çanakkale, Musaköy, 16.V.2017, alt. 6♀♀, *J. regia*.

***Eucallipterus tiliae* (Linnaeus, 1758)**

Material examined. Çanakkale, Central, 20.V.2017, alt. 4♀♀, *Tilia tomentosa* Moench (Malvaceae).

***Myzocallis (Myzocallis) carpini* (Koch, 1855)**

Material examined. Çanakkale, Yenice, Ida Mountains, 13.VI.2017, alt. 6♀♀, *Carpinus betulus* L. (Betulaceae).

***Therioaphis (Pterocallidium) trifolii* (Monell, 1882)**

Material examined. Balıkesir, Gönen, 17.VII.2018, apt. 2♀♀, alt. 4♀♀, *M. sativa*; Çanakkale, Taşlıtarla, 14.VII.2017, apt. 2♀♀, alt. 2♀♀, *M. sativa*.

***Tinocallis (Sappocallis) saltans* (Nevsky, 1929)**

Material examined. Çanakkale, Central, 27.IV.2017, alt. 6♀♀, *Ulmus minor* Mill. (Ulmaceae).

Subfamily Chaitophorinae

***Chaitophorus leucomelas* Koch, 1854**

Material examined. Balıkesir, Balya, 13.V.2017, alt. 4♀♀, *Populus* sp. (Salicaceae); Çanakkale, Gallipoli Peninsula, 06.V.2017, alt. 3♀♀, *Populus* sp. (Salicaceae).

***Chaitophorus niger* Mordvilko, 1929**

Material examined. Çanakkale, Central, 22.IV.2017, apt. 5♀♀, *Salix alba L.* (Salicaceae).

***Chaitophorus tremulae* Koch, 1854**

Material examined. Çanakkale, Anzac Cove, 06.V.2017, alt. 5♀♀, *Populus* sp. (Salicaceae).

Subfamily Eriosomatinae

***Baizongia pistaciae* (Linnaeus, 1767)**

Material examined. Çanakkale, Ezine, 05.XII.2017, apt. 2♀♀, alt. 6♀♀, *Pistacia terebinthus* L. (Anacardiaceae).

***Patchiella reaumuri* (Kaltenbach, 1843)**

Material examined. Çanakkale, Central, 01.V.2017, apt. 5♀♀, *Tilia Tomentosa* Moench (Malvaceae).

***Pemphigus* sp.**

Material examined. Çanakkale, Gallipoli Peninsula, 06.V.2017, apt. (fundatrix) 3♀♀, *Populus* sp. (Salicaceae)

Comments. In this study, three apterous fundatrix of this species were obtained from galls on a poplar tree. The main identification keys for aphids forming galls or pseudogalls on *Populus* spp. are based on alate females, and apterous females in a rare case. Therefore, the identification of aphids based on the fundatrix could not be performed, and the species given as *Pemphigus* sp. in this study.

***Pemphigus* (*Pemphigus*) *immunis* Buckton, 1896**

Material examined. Çanakkale, Dardanos, 31.V.2017, alt. 5♀♀, *Populus* sp. (Salicaceae).

***Periphyllus obscurus* Mamontova, 1955**

Material examined. Çanakkale, Yenice, 13.VI.2017, apt. 5♀♀, *Acer campestre* L. (Sapindaceae).

***Tetraneura* (*Tetraneura*) *caerulescens* (Passerini, 1856)**

Material examined. Çanakkale, Central, 10.V.2017, alt. 4♀♀, *Ulmus* sp. (Ulmaceae).

***Tetraneura* (*Tetraneurella*) *nigriabdominalis* Sasaki, 1899**

Material examined. Çanakkale, Central, 20.V.2017, alt. 5♀♀, *Ulmus minor* Mill. (Ulmaceae).

***Tetraneura* (*Tetraneura*) *ulmi* (Linnaeus, 1758)**

Material examined. Çanakkale, Umurbey, 01.V.2017, apt. 6♀♀, *Alopecurus* sp. (Poaceae).

Subfamily Lachninae

***Cinara* (*Cinara*) *brauni* Börner, 1940**

Material examined. Balıkesir, Ida Mountains, 13.V.2017, apt. 3♀♀, *Pinus nigra* subsp. *pallasiana* (Lamb.) Holmboe sp. (Pinaceae).

***Cinara (Cinara) cedri* Mimeur, 1936**

Material examined. Çanakkale, Central, 08.VI.2017, apt. 5♀♀, *Cedrus deodara* (Roxb. ex D. Don) G. Don (Pinaceae).

***Cinara* (*Cupressobium*) *fresai* Blanchard, 1939**

Material examined. Çanakkale, Central, 15.IV.2017, apt. 5♀♀, *Cupressus arizonica* Greene (Cupressaceae); 22.IV.2017, apt. 4 ♀♀, *Juniperus sabina* L. (Cupressaceae).

***Cinara* (*Cupressobium*) *oxycedri* Binazzi, 1996**

Material examined. Çanakkale, Gallipoli Peninsula, 7.IV.2018, apt. 6♀♀, *Juniperus oxycedrus* L. (Cupressaceae).

***Cinara* (*Cinara*) *pini* (Linnaeus, 1758)**

Material examined. Çanakkale, Bayramiç, 26.IX.2017, apt. 6♀♀, alt. 2♀♀, *Pinus* sp. (Pinaceae).

***Cinara* (*Cupressobium*) *tujafilina* (Del Guercio, 1909)**

Material examined. Çanakkale, Central, 02.VI.2017, apt. 6♀♀, *Platycladus orientalis* (L.) Franco (Cupressaceae).

***Trama* (*Neotrama*) *caudata* Del Guercio, 1909**

Material examined. Çanakkale, Umurbey, 01.V.2017, apt. 8♀♀, *T. porrifolius*.

Comments. *Trama porrifolius* subsp. *longirostris* has not been recorded as host plant for *T. caudata* in Turkey. The apterous females and nymphs of *T. caudata* were collected from roots of the plant. The host plants determined in different regions of Turkey for *T. caudata* are given below.

Hosts in Turkey. This species was reported on *Cirsium arvense* (L.) Scop. and *C. pycnocephalus* from Elmadağ and Gölbaşı in Ankara (Özdemir et al., 2005).

The present and other studies show that the comprehensive regional faunal studies of aphids are important for reporting new records and to better understand their relationship with host plants. In Turkey, despite its location between Europe and Asia, and its floristic and faunistic richness, the aphid fauna of Turkey is still inadequately known compared neighboring countries in the same zoogeographic region. For example, the aphid fauna of Greece, Iran and Georgia includes 335, 486 and 320 species, respectively, despite having less floristic diversity than Turkey (Barjadze at al., 2010; Rezwani, 2010; Margaritopoulos et al., 2013). These data clearly show that local faunal studies should be conducted in different habitats to increase the knowledge of aphid species of Turkey. In this regard, there are many regions of Turkey that still need to be studied to add to the studies of aphids already conducted in some regions (Toros et al., 2002; Özdemir et al., 2005; Toper Kaygın et al., 2008; Görür et al., 2011; Özdemir & Barjadze, 2015; Şenol et al., 2015; Kök et al., 2016; Görür et al., 2017). Further local faunal studies are needed to increase the knowledge of aphid species in Turkey. Consequently, we conclude that the faunal studies examining aphid and their taxonomic characters and features in all regional areas of Turkey should be conducted. Also, the large number of aphids and their host plants from South Marmara Region should be a useful guide to other researchers undertaking detailed taxonomic and faunal studies of aphids in different parts of Turkey.

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Original article (Orijinal araştırma)

Success of DNA extraction and PCR amplification from dry pinned sand bees (*Andrena* spp. Fabricius, 1775) using newly-designed primers

İğnelenerek kurutulmuş kum arılarından (*Andrena* spp. Fabricius, 1775) DNA eldesi ve yeni tasarlanmış primerler kullanıldığında PCR amplifikasyonu başarısı

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Abstract

The suitability of dry pinned museum specimens for DNA extraction of sand bees (*Andrena* spp. Fabricius, 1775) (Hymenoptera: Andrenidae) and the effectiveness of existing and new primers used in DNA analysis of specimens for future studies were evaluated. A total 256 specimens were analyzed, including 222 dry pinned bee specimens representing 37 subgenera and 101 species and 34 ethanol-preserved specimens belonging to 21 species. Several different protocols were tested for DNA extraction, and DNA was extracted from almost all of the specimens. The samples preserved in ethanol had the highest quality DNA. Of 31 primer sets tested for amplification of the DNA, 14 of them were newly designed or redesigned. The amplified sequence length ranged from 130 to 1571 bp. DNA from 32 specimens belonging to 25 species was successfully amplified at three to four loci. This study demonstrates the importance of storage conditions for specimens possibly destined for later DNA extraction, and for selecting suitable primers when dealing with older bee specimens. Some primers can be diagnostically informative provided appropriate gene regions are used.

Keywords: *Andrena*, DNA, molecular, museum specimens, sand bees

Öz

İğnelenerek kurutulmuş kum arısı müze örneklerinin (*Andrena* spp. Fabricius, 1775) (Hymenoptera: Andrenidae) DNA ekstraksiyonu için uygunluğu incelenmiş ve gelecekteki çalışmalarda DNA analizi amacıyla yeni primerlerin etkinliği değerlendirilmiştir. Otuz yedi altcins ve 101 türü temsil eden 222 iğnelenmiş kuru arı örneği ve etanol içerisinde saklanmış 21 türe ait 34 arı örneği olmak üzere toplamda 256 örnek analiz edilmiştir. DNA ekstraksiyonu için birkaç farklı protokol denenmiş ve örneklerin tamamından DNA izole edilmiştir. En yüksek kaliteli DNA etanol içerisinde saklanan örneklerden elde edilmiştir. DNA amplifikasyonunda test edilen 31 primerden 14 tanesi ya yeni ya da yeniden tasarlanmıştır. Primerlerin sekans uzunluğu 130 ile 1571 bp arasında değişkenlik göstermiştir. Yirmi beş türe ait 32 örneğin üç-dört DNA lokusu başarılı bir şekilde çoğaltılmıştır. Bu çalışma, gelecekte DNA elde edilme ihtimali olan arı örneklerinin saklama koşullarının ve uygun primer seçiminin önemini ortaya koymuştur. Bazı primerler uygun gen bölgelerinin kullanılması şartıyla tür teşhislerini yapmaya yarayacak bilgileri sağlayabilirler.

Anahtar sözcükler: *Andrena*, DNA, moleküler, müze örnekleri, kum arıları

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Introduction

Bees (Apoidea: Hymenoptera) have an important role as pollinators in natural ecosystems and for many valuable crops (Klein et al., 2007). Because of their significance in agriculture and nature, many phylogenetic and evolutionary studies have been conducted with particular emphasis on wild bees (Praz et al., 2008; Rehan et al., 2010; Danforth et al., 2013; Schmidt et al., 2015). Molecular methods provide useful information regarding diagnostics for bee species, bee diversity, phylogeny, ecology, behavior, patterns of bee-host plant association and eusociality (Danforth et al., 2013; Hedtke et al., 2013; Woodard et al., 2015).

Freshly collected specimens are preferable for obtaining sufficient high-quality DNA for further scientific analysis. However, the destruction of native habitats means that museum specimens provide the only available samples for rare or otherwise difficult to acquire species (Schander & Halanychi, 2003). Also, entomological museum collections are rich repositories of insect fauna and provide historical data on the genetics, distribution and diversity of bee species (Strange et al., 2009). Unfortunately, the use of museum specimens can be challenging due to various factors including DNA degradation, contamination, and uncertainty related to specimen collection and preservation (Hernandez-Triana et al., 2014).

Dry insect specimens are usually held in museum collections, constituting over a million species. They are potentially a source of DNA. DNA sequence data from such specimens can provide useful information for both phylogenetic inference and taxonomic identification (Gilbert et al., 2007). DNA extracted from museum specimens has been helpful in the context of molecular-based identification of different bee species, as well as being a useful source of information for understanding the recent shifts in population structure, particularly regarding population declines of native pollinator species (Andersen & Mills, 2012).

The sand bee (*Andrena* spp. Fabricius, 1775) genus is presumed to be the largest genus of bees with over 1500 species described (Dubitzky et al., 2010). Information obtained from DNA of sand bees specimens held in museum collections is highly valuable because cryptic variation is common in the genus, and accurate identification using morphological methods is so challenging (Schmidt et al., 2015).

In this study, i) the effectiveness of DNA extraction protocols for dry pinned specimens of *Andrena* species compared to ethanol-preserved specimens were examined; and ii) new primer sets were designed for PCR amplification of targeted loci as a tool to obtain useful amplicons from samples with potentially fragmented DNA. Different mitochondrial and nuclear gene regions of the DNA were chosen that would be useful for analyzing the phylogeny of *Andrena* species. For this research, we used specimens of 101 bee species previously identified based on morphological methods.

Material and Methods

A total of 256 specimens were obtained for use from the Wild Bee Museum of Turkey (TUYAM) in Adnan Menderes University, Aydin, Turkey (Table 2). Of these, 222 of the specimens were dry pinned museum specimens of various *Andrena* bee species which were collected between 2004 and 2011 from different regions of Turkey. Collection and morphological identification of the bees were previously conducted by the senior author and Erwin Scheuchl. The bee specimens belong to 101 species from 37 subgenera. For purposes of comparison, a further 34 bee specimens that had been preserved in ethanol (96%) and belonging to 21 different species were included. All the ethanol-preserved specimens were collected in 2014 by the senior author from different areas in Turkey. All experiments were conducted at the Southeastern Fruit and Tree Nut Research Laboratory, USDA-ARS, Byron, Georgia, USA between 2014 and 2015.

DNA extraction

Qiagen DNeasy Blood and Tissue Kits (Quiagen, Redwood City, CA, USA) were used to extract DNA from the sand bee specimens following the manufacturer's protocol with three modifications (Nishiguchi et al., 2002; Ward, 2009; Crane, 2011). Nonetheless, due to difficulties with DNA extraction from certain specimens, a 2x cetyltrimethylammonium bromide (CTAB) DNA extraction method was implemented for

six bee species. The 2x CTAB DNA extraction method was that of Danforth (2013). This CTAB method takes 3 d to complete. Briefly, the DNA extraction procedure was as follows (with buffers AE, AL, ATL, AW1, AW2 and TE from the Qiagen kit). Individual dry pinned specimens were placed in Petri dishes and separated into three sections (head, thorax and abdomen) using a pair of fine forceps. The wings and legs were removed from the thorax. The bee samples preserved in 96% ethanol were washed with TE buffer for 10 min, dried on filter paper at room temperature for 30 min, and placed in a 2 ml microcentrifuge tube in a freezer at -20°C overnight. A single body part (head, thorax or abdomen) was used for DNA extraction and the remaining body parts were retained as a voucher and for further use if required. A bashing bead to crush the sample was placed in the 2 ml microcentrifuge tube with the bee body part and the sample homogenized using a Qiagen TissueLyser at 200 Hz for 1-2 min. In the second method tested, 20 samples were homogenized with a plastic pestle until they were finely ground. Proteinase K (20 µl) and buffer AL (200 µl) were added and mixed thoroughly by vortexing; the mixture was incubated at 54-56°C for 3-4 h or overnight in a water bath. The sample was vortexed for 15 s. A further 200 µl of buffer AL was added to the sample, and mixed thoroughly by vortexing. Ethanol (96-100%) (200 µl) was added and the mixture again vortexed thoroughly. The mixture was pipetted into the DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 min. The flow-through was discarded. The DNeasy Mini spin column was placed in a new 2 ml collection tube and 500 µl buffer AW1 was added, and the tubes were centrifuged at 8000 rpm for 1 min. The flow-through was discarded. The DNeasy Mini spin column was placed in a new 2 ml collection tube, 500 µl buffer AW2 was added, and centrifuged for 3 min at 14,000 rpm. The flow-through was discarded. The DNeasy Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and 100 µl buffer AE was pipetted directly onto the DNeasy membrane. The tubes and column were incubated at room temperature for 1 min, and subsequently centrifuged for 1 min at 8000 rpm to elute the DNA from the column. The elution was repeated once as described in previous step.

Three steps in the protocol were changed as in previous studies. In the first variation, the samples were incubated with buffer ATL and proteinase K at 56°C overnight (Nishiguchi et al., 2002). After adding a further 200 µl AL buffer, the samples were incubated at 65°C for 15 min. Then, 200 µl ethanol was added and the mixture incubated at 4°C for 1 h. Buffer AE was added to tube at 70°C. The second variation to the protocol was as described by Ward (2009), who added 180 µl buffer AL to microcentrifuge tube including the sample. The mixture was incubated 56°C for 4 h in a water bath. The tube was shaken briefly every 45-60 min. The third variations were as follows. The bee body part was placed in a 1.5 ml microcentrifuge tube and 60 µl PBS (phosphate buffered saline), 40 µl proteinase K and 200 µl buffer AL were added and mixed thoroughly by vortexing, and incubated at 56°C for 4 h in a water bath (Crane, 2011). A Nanodrop spectrophotometer was used to quantify the DNA in each sample. Extracted DNA was stored at -20°C until it was used in the PCR.

PCR amplification of DNA with primers

An adequate amount of the PCR reagent mix (without adding the sample DNA) was prepared and stored in an ice bucket for each cohort of PCR reactions, with sufficient additional mix for two samples; one as a negative control and the other as spare reagent in case of pipetting errors. The reagent mix was vortexed thoroughly.

The following reagents were used in each 10 µl PCR mix: 5 µl PCR Master Mix (Promega PCR Master Mix, 2X), containing 50 units/ml of Taq DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400µM of nucleotides (dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP, 3mM MgCl2), 1 µl forward primer, 1 µl reverse primer and 2 µl deionize water.

The reagent mix (9 µl) was added to each PCR tube and 1 µl of the sample was added (10 ng DNA per reaction). Dilutions of DNA samples were made based on the results from the Nanodrop measurements. The negative control contained 1 µl of ddH_2O. The tubes were placed in the PCR machine, and the appropriate PCR program (initial denaturation for 3 min at 94°C; 36 cycles of 1 min denaturation at 94°C, 1 min annealing ranging between 40 and 60°C and 1 min elongation at 72°C, and a final extension for 5 min at 72°C) was run for the marker being amplified. The annealing temperatures for each primer set are presented in Table 1. The tubes were removed from the PCR machine and stored in a refrigerator.

Seventeen primer sets were initially screened in this study. Eleven of them amplify mitochondrial gene regions and six of them amplify nuclear gene regions. However, these primers failed to produce amplicons with many samples, particularly with dry pinned bee samples. So, new primers were designed for these sand bee samples. BLAST, Primer-BLAST and Primer3 programs were used for designing new primers (Ye et al., 2012). In addition, six primers previously described in the literature were redesigned according to sequence alignments data of *Andrena* spp. or other Andrenidae species in Genbank (NCBI-NIH, MD, USA). Subsequently, an additional 14 primer sets were tested. These were newly designed or redesigned primer sets; two amplifying mitochondrial gene regions and 12 amplifying nuclear gene regions. Thus, a total of 31 primer sets were tested in this study (Table 1), with sequence lengths ranging from 130 to 1571 bp.

Table 1. Primer sets used to amplify DNA of *Andrena* species

PRIMERS		DNA sequence (bp)	Annealing temperature (°C)	References
	MITOCHONDRIAL DNA PRIMERS			
mitochondrial cytochrome oxidase I (COI)				
mtD8	For 5'-CCACATTTATTTTGATTTTTTGG-3'	853	48	Dubitzky, 2005
mtD12	Rev 5'-TCCAATGCACTAATCTGCCATATTA-3'			
AP-L-2176	For 5'-GGTACAGGTTGAACTGTTTACCC-3'	521	40	Koulianos & Schmid-Hempel, 2000
AP-H-2650	Rev 5'-TCCGACTGTAAATAAGTGATGTGCTC-3'			
LCO1490	For 5'-GGTCAACAAATCATAAAGATATTGG-3'	710	45-50	Reemer et al., 2008
HCO2198	Rev 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'			
LepFl	For 5′-ATTCAACCAATCATAAAGATAT-3'	350	45-51	Hebert et al., 2004
LepR2	Rev 5'-CTTATATTATTTATTCGTGGGAAAGC-3'			
CO1-2166F	For 5'-GGAGGATTTGGTAATTTTTTAATTCC-3'	226	45	Francoso & Arias, 2013
CO1-2386R	Rev 5'-GAAAAAATTGTAAAATCAAC-3'			
Uni-MinibarF1	For 5'-TCCACTAATCACAARGATATTGGTAC-3'	130	46-53	Meusnier et al., 2008
Uni-MinibarR1	Rev 5'-GAAAATCATAATGAAGGCATGAGC-3'			
AndCOI-F1	For 5'-TTGCYATATGAGCAGGCATAGTCG-3'	631	51	New
COIand-R1	Rev 5'-TTGGTATARAATDGGRTCTCCWCCT-3'			
AndCOI-F2	For 5'-GAGCCGGAATAATTGGTGCC-3'	615	53	New
COIand-R2	Rev 5'-GGATCGGATCTCCACCTCCTA-3'			
mitochondrial COI-COII				
Jack	For 5'-AGATCACTTGAATGATCACAAAAT-3'	695	55	Larkin et al., 2006
Barb	Rev 5'-CCACAAATTTCTGAACATTGACCA-3'			
mitochondrial cytochrome oxidase b (Cyt b)				
cb1	For 5'-TATGTACTACCATGAGGACAAATATC-3'	429	50	Rehan et al., 2010
cb2	Rev 5'-ATTACACCTCCTAATTTATTAGGAAT-3'			

Table 1. continued

PRIMERS		DNA sequence (bp)	Annealing temperature (C°)	References
MITOCHONDRIAL DNA PRIMERS				
mitochondrial 12S rRNA				
12Sa	For 5' TGGGATTAGATACCCCACTAT-3'	428	50	Cameron&Williams, 2003
12SLR	Rev 5'-YYTACTATGTTACGACTTAT-3'			
mitochondrial 16S rRNA				
16S-F	For 5'-TTATTCACCTGTTTATCAAAACAT-3'	600	50	Ramirez et al., 2010
16S-R	Rev 5'-TATAGATAGAAACCAATCT-3'			
16SWb	For 5'-CACCTGTTTATCAAAAACAT-3'	500	48	Hines et al., 2006
874-16SlR	Rev 5'-TATAGATAGAAACCAATCTG-3'			
NUCLEAR DNA PRIMERS				
28S rRNA				
Bel28S	For 5'-AGAGAGAGTTCAAGAGTACGTG-3'	690	60	Danforth et al., 2006
Mar28Srev	Rev 5'-TAGTTCACCATCTTTCGGGTCCC-3'			
And28S-F	For 5'-GAGATTCAKCGTCRACGAGGCT-3'	669	60	New
28Sand-R	Rev 5'-TGACCAGGCATAGTTCACCA-3'			
EF-1 α F1 copy				
EF-1For2	For 5'-AAGGAGGC[C/G]CAGGAGATGGG-3'	457	52	Schwarz et al., 2004
EF-1Rev2	Rev 5'-[T/C]TC[G/C]AC[T/C]TTCCATCCGTACC-3'			
EF-1 *α* F2 copy				
For1-deg	For 5'-GYATCGACAARCGTACSATYG-3'	1571	52	Danforth et al., 1999
F2-Rev1	Rev 5'-AATCAGCAGCACCTTTAGGTGG-3'			
F2-ForH	For 5′-GGRCAYAGAGATTTCATCAAGAAC-3′	720	54	Hines et al., 2006
F2-RevH2	Rev 5′- TTGCAAAGCTTCRKGATGCATTT-3′			
HaF2For1-And	For 5'-GGGYAAGGGWTCCTTCAARTACGC-3'	1080	59	redesigned (Danforth et al., 1999)
F2-rev1-And	Rev 5'-AATCRGCAGCACCYTTGGGTGG-3'			
AndEF-F1	For 5'-TTACBGGYACMTCACARGCTGACT-3'	700	60	New
EFand-R1	Rev 5'-CACGRCCGACTRGTACTGTTC-3'			
AndEF-F2	For 5'-TGAGACGTGGTTACGTAGCAG-3'	538	52	New
EFand-R2	Rev 5'-GGGAACTCTTGGAAAGCCTCA-3'			

Table 1. continued

	PRIMERS	DNA sequence (bp)	Annealing temperature (°C)	References
	NUCLEAR DNA PRIMERS			
Opsin				
Opsin For3 (mod)	For 5'-TTCGAYAGATACAACGTRATCGTNAARGG-3'	639	56	Michez et al., 2009
Opsin Rev (mod)	Rev 5'-ATANGGNGTCCANGCCATGAACCA-3'			
Opsin For3 (mod)-And	For 5'-TTCGACAGATACAACGTRATYGTMAARGG-3'	610	58	redesigned (Michez et al., 2009)
OPSand-R1	Rev 5'-TCGAATATGCCCGACGTGTT-3'			New
AndOPS-F2	For 5'-TTCTCTCTGGGCTGGACAAT-3'	708	51	New
OPSand-R2	Rev 5'-AACAGYGCAGCTCGATACTT-3'			
ArgK				
F	For 5'-GTTGACCAAGCYGTYTTGGA-3'	860	48	Hines et al., 2006
R	Rev 5'-CATGGAAATAATACGRAGRTG-3'			
Wingless				
wgColletFor-And	For 5'-CACGTGTCBTCGGRAATGAGRCAGGA-3'	670	59	redesigned (Almeida & Danforth, 2009)
Lep wg2a-Rev	Rev 5'-ACTICGCARCACCARTGGAATGTRCA-3'			Almeida & Danforth, 2009
AndWNG-F	For 5'-ATCGGGTACGGGTTCAAGTT-3'	653	59	New
WNGand-R	Rev 5'-GTCACCTCCTGCGTCYTGTA-3'			
CAD				
ApCADfor4-And	For 5'-TGGAARGARGTBGAATTCGAAGTGAACGC-3'	684	51	redesigned (Danforth et al., 2006)
CADand-R	Rev 5'-TTCACTACCGCAGCAATCTG-3'			New
AndCAD-F	For 5'-GCTATCCSCTGGCWTACGTAGCT-3'	720	60	New
ApCADrev4a-And	Rev 5'-GGCCAYTCCGCAGCCACHGTGTCTATYTG YTTCACC-3'			redesigned (Danforth et al., 2006)
RNA polymerase II (Pol II)				
polfor2a-And	For 5'-GGAGAACTKGTGATGGGTATACTTTG-3'	587	59	redesigned (Danforth et al., 2006)
polrev2a-And	Rev 5'-AGGTACGARTTYTCAACGAATCCTCT-3'			
AndPOL-F	For 5'-AAATGACGAAGAGGGACGTG-3'	723	50	New
POLand-R	Rev 5'-CGCAAGCGATAACCTGAGAG-3'			

Agarose gel electrophoresis

After the PCR reaction was compete, 5 µl from each sample was run on a 1.5% agarose minigel against a standard size marker (Bionexus Hi-Lo™ DNA marker, Oakland, CA, USA). The sizes of the

amplicons were compared against the molecular weight marker to confirm whether the PCR reaction had amplified the target region. PCR products were purified using the QIAquick Purification Kit (Qiagen).

Results and Discussion

Several different protocols including the Qiagen DNeasy Blood and Tissue Kit, and three modifications of its protocol (Nishiguchi et al., 2002; Ward, 2009; Crane, 2011), and 2x CTAB DNA (Danforth, 2013) were compared for extraction of DNA from sand bees. The Qiagen kit was the most effective method especially for the dry pinned specimens. DNA was extracted from almost all tested specimens. However, the fresher, more recently collected ethanol-preserved samples clearly had better quality DNA compared with the dry pinned specimens. DNA was obtained from both sexes. There was no significant difference in the quantity and quality of DNA extracted from female and male bees (Tables 2 and 3).

Table 2. Quantification and quality of DNA of *Andrena* species obtained from dry pinned specimens

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA (ng/µl)	260/280	260/230
1001A	F	*Aciandrena*	*Andrena aciculata* Morawitz, 1886	all body	Qiagen	3	45.4	1.93	1.75
1001B	M			all body	Qiagen	3	17.0	1.60	1.22
1002A	M	*Aciandrena*	*Andrena lamiana* Warncke, 1965	Head, Thx	Qiagen	3	3.8	2.26	1.19
1101A	F	*Aenandrena*	*Andrena aeneiventris* Morawitz, 1872	4 Legs	Qiagen	4	1.5	2.25	0.55
1101B	F			all body	Qiagen	3	57.1	1.90	1.67
1101C	F			all body	Qiagen	3	35.3	1.84	1.74
1102A	F	*Aenandrena*	*Andrena bisulcata* Morawitz, 1877	4 Legs	Qiagen	4	11.2	1.41	0.88
				Thorax	Nishiguchi et al., 2002	over night	3639.5	1.43	0.60
1102B	F			all body	Qiagen	3	128.4	1.95	1.87
1102C	F			all body	Qiagen	3	108.1	1.97	1.70
1103A	F	*Aenandrena*	*Andrena hystrix* Schmiedeknecht, 1883	4 Legs	Qiagen	4	6.6	1.42	0.61
				Head	Nishiguchi et al., 2002	over night	2.2	1.68	1.49
1103B	F			all body	Qiagen	3	42.8	1.57	0.78
1103C	F			all body	Qiagen	3	47.3	1.66	1.09
1201A	F	*Brachyandrena*	*Andrena colletiformis* Morawitz, 1874	3 Legs	Qiagen	4	8.7	1.42	0.79
1201B	F			all body	Qiagen	3	10.7	1.83	1.07
1201C	M			all body	Qiagen	3	11.3	1.98	1.35
1301A	F	*Campylogaster*	*Andrena lateralis* Morawitz, 1876	Thx, abd	Qiagen	3	18.8	1.56	0.63
1401A	F	*Carandrena*	*Andrena falcinella* Warncke, 1969	all body	Qiagen	3	86.7	1.89	1.72
1401B	F			all body	Qiagen	3	42.6	1.91	2.00
1402A	F	*Carandrena*	*Andrena purpureomicans* Alfken, 1935	all body	Qiagen	3	17.7	1.63	1.15
1402B	M			all body	Qiagen	3	16.0	1.74	1.28
1402C	M			Head, Thx	Qiagen	3	11.0	1.97	0.96
1501A	F	*Charitandrena*	*Andrena hattorfiana* (Fabricius, 1775)	Thx, abd	Qiagen	3	151.3	1.89	1.73
1501B	F			Thx, abd	Qiagen	3	16.3	1.69	0.99
1601A	F	*Chlorandrena*	*Andrena cinerea* Brulle, 1832	all body	Qiagen	3	48.9	1.80	0.91
1601B	F			all body	Qiagen	3	73.7	1.88	1.63
1602A	F	*Chlorandrena*	*Andrena cinereophila* Warncke, 1965	all body	Qiagen	3	15.9	1.73	0.62
1602B	F			all body	2XCTAB	2	473.9	1.37	0.91
1602C	F			Thx, abd	Qiagen	3	42.7	1.78	0.99
1602D	F			all body	Qiagen	3	60.9	1.91	2.11

Table 2. (Continued)

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA (ng/µl)	260/280	260/230
1603A	F	*Chlorandrena*	*Andrena clypella* Strand, 1921	all body	Qiagen	3	126.2	1.90	1.82
1603B	F			all body	2XCTAB	2	934.7	1.40	0.62
1604A	F	*Chlorandrena*	*Andrena exquisita* Warncke, 1975	all body	Qiagen	3	66.1	1.85	1.41
1604B	F			all body	2XCTAB	2	756.5	1.40	0.56
1604C	F			all body	Qiagen	3	34.9	1.63	1.04
1604D	F			all body	Qiagen	3	181.3	1.94	1.74
1605A	F	*Chlorandrena*	*Andrena humabilis* Warncke, 1965	Thx, abd	Qiagen	3	27.9	1.74	1.01
1605B	F			all body	Qiagen	3	38.4	1.77	1.17
1606A	F	*Chlorandrena*	*Andrena humilis* Imhoff, 1832	all body	Qiagen	3	74.2	1.87	1.51
1606B	F			all body	2XCTAB	2	419.8	1.06	0.93
1606D	M			Head, Thx	Qiagen	3	44.5	1.87	1.13
1607A	F	*Chlorandrena*	*Andrena orientana* Warncke, 1965	all body	Qiagen	3	234.9	1.97	2.06
1607B	F			all body	2XCTAB	2	1086.7	1.47	0.77
1608A	F	*Chlorandrena*	*Andrena panurgimorpha* Mavromoustakis, 1957	all body	Qiagen-insects	15	61.1	1.77	1.48
1608B	F			all body	2XCTAB	2	1332.1	1.38	0.64
1608C	F			all body	Qiagen	3	111.1	1.96	2.10
1608D	M			Head, Thx	Qiagen	3	39.3	1.76	1.37
1701A	F	*Chrysandrena*	*Andrena hesperia* Smith, 1853	all body	Qiagen	3	225.9	1.96	1.82
1701B	F			all body	Qiagen	3	347.2	2.01	2.23
1702A	F	*Chrysandrena*	*Andrena merula* Warncke, 1969	all body	Qiagen	3	20.0	1.67	0.78
1702B	F			all body	Qiagen	3	69.5	1.95	1.69
1702C	F			all body	Qiagen	3	41.8	1.84	1.08
1801A	F	*Cordandrena*	*Andrena cordialis* Morawitz, 1877	all body	Qiagen	3	51.4	1.67	0.90
1801B	F			all body	Qiagen	3	40.0	1.49	0.83
1801C	F			all body	Qiagen	3	151.5	1.89	1.93
1802A	F	*Cordandrena*	*Andrena cypria* Pittioni, 1950	all body	Qiagen	3	164.7	1.91	1.67
1802B	F			all body	Qiagen	3	117.9	1.95	2.11
1901A	F	*Didonia*	*Andrena nasuta* Giraud, 1863	Thx, legs	Qiagen	3	59.8	1.81	1.14
2001A	F	*Euandrena*	*Andrena bicolor* Fabricius, 1775	all body	Qiagen	3	14.6	1.55	0.75
2001B	F			Thx, abd	Qiagen	3	253.5	1.79	1.83
2002A	F	*Euandrena*	*Andrena glabriventris* Alfken, 1935	all body	Qiagen	3	148.1	1.93	1.54
2002B	F			Thx, abd	Qiagen	3	116.6	1.95	1.93
2101A	F	*Holandrena*	*Andrena labialis* (Kirby, 1802)	all body	Qiagen	3	150.1	1.73	1.14
2101B	F			Thx, abd	Qiagen	3	84.8	1.81	1.48
2101C	F			Abdomen	Qiagen	3	17.5	1.55	0.65
2101D	M			Head, Thx	Qiagen	3	36.4	1.77	0.90
2102B	F	*Holandrena*	*Andrena variabilis* Smith, 1853	all body	Qiagen	3	15.4	1.51	0.60
2102C	M			Thx, abd	Qiagen	3	132.1	1.93	1.83
2103A	F	*Holandrena*	*Andrena wilhelmi* Schuberth, 1995	all body	Qiagen	3	27.3	1.37	0.53
2103B	F			Thx, abd	Qiagen	3	116.5	1.91	1.62
2103C	F			all body	Qiagen	3	35.8	1.64	0.55
2201A	F	*Hyperandrena*	*Andrena bicolorata* (Rossi, 1790)	all body	Qiagen	3	133.2	1.76	1.20
2201B	F			Thx, legs	Qiagen	3	83.1	1.90	1.92

Table 2. (Continued)

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA (ng/µl)	260/280	260/230
2301A	F	*Larandrena*	*Andrena medioxima* Warncke, 1975	Thx, abd	Qiagen	3	69.9	1.95	1.73
2302A	F	*Larandrena*	*Andrena sericata* Imhoff, 1866	all body	Qiagen	3	26.0	1.63	0.77
2302B	F			all body	Qiagen	3	204.1	1.97	2.05
2302C	M			all body	Qiagen	3	23.2	1.81	1.37
2401A	F	*Lepidandrena*	*Andrena gamskrucki eburnea* Warncke, 1975	all body	Qiagen	3	193.4	2.00	1.92
2401C	M			Head, Thx	Qiagen	3	47.9	1.91	1.55
2402A	F	*Lepidandrena*	*Andrena gamskrucki impasta* Warncke, 1975	Thx, abd	Qiagen	3	541.4	2.07	2.14
2501A	F	*Leucandrena*	*Andrena mistrensis* Grünwaldt, 2005	all body	Qiagen	3	123.9	1.76	1.38
2502A	F	*Leucandrena*	*Andrena parviceps* Kriechbaumer, 1873	Thx, abd	Qiagen	3	187.3	1.81	1.89
2601A	F	*Melanapis*	*Andrena fuscosa* Erichson, 1835	Thx, legs	Qiagen	3	331.9	2.01	1.87
2701A	F	*Melandrena*	*Andrena albopunctata* (Rossi, 1792)	Thx, legs	Qiagen	3	139.7	1.9	2.02
2701B	F			all body	Qiagen	3	290.3	1.92	1.87
2702A	F	*Melandrena*	*Andrena atrotegularis* Hedicke, 1923	Thx, legs	Qiagen	3	17.2	1.83	0.63
2703A	F	*Melandrena*	*Andrena danuvia* Stöckhert, 1950	Thx, legs	Qiagen	3	61.3	1.81	1.43
2703B	F			Thx, abd	Qiagen	3	135.4	1.85	1.43
2704A	M	*Melandrena*	*Andrena fuscocalcarata* Morawitz, 1877	Head, Thx	Qiagen	3	209.5	1.98	2.01
2705A	F	*Melandrena*	*Andrena limata* Smith, 1853	all body	Qiagen	3	125.9	0.44	0.37
2705B	F			all body	Qiagen	3	70.4	1.73	1.12
2705C	F			Thx, abd	Qiagen	3	79.4	1.76	1.40
2705D	F			Thx, abd	Qiagen	3	158.6	1.80	1.17
2706A	F	*Melandrena*	*Andrena morio* Brullè, 1832	Thx, legs	Qiagen	3	204.7	1.85	1.41
2706B	F			Thx, abd	Qiagen	3	76.5	1.68	0.94
2706C	F			Thx, abd	Qiagen	3	79.8	1.43	0.59
2706D	F			Thx, abd	Qiagen	3	47.1	1.39	0.72
2707A	F	*Melandrena*	*Andrena nigroaenea candiae* Strand, 1915	Thx, abd	Qiagen	3	29.1	1.42	0.55
2707B	F			Thx, abd	Qiagen	3	30.7	1.29	0.59
2707C	F			Thx, abd	Qiagen	3	32.1	1.47	0.62
2707D	M			Head, Thx	Qiagen	3	51.7	1.57	0.79
2708A	F	*Melandrena*	*Andrena nitidemula* Scheuchl & Hazir, 2012	Thx, legs	Qiagen	3	63.7	1.92	1.44
2709A	F	*Melandrena*	*Andrena pyropygia* Kriechbaumer, 1873	Thx, abd	Qiagen	3	29.8	1.53	0.49
2801A	F	*Melittoides*	*Andrena curiosa* (Morawitz, 1877)	Thx, legs	Qiagen	3	43.2	1.53	0.54
2901A	F	*Micrandrena*	*Andrena virgata* Warncke, 1975	all body	Qiagen	3	71.9	1.91	1.63
3001A	F	*Nobandrena*	*Andrena anatolica* Alfken, 1935	all body	Qiagen	3	218.5	1.97	1.86
3001C	F			all body	Qiagen	3	122.2	1.91	1.65

Table 2. (Continued)

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA (ng/µl)	260/280	260/230
3002A	F	*Nobandrena*	*Andrena athenensis* Warncke, 1965	all body	Qiagen	3	96.5	1.83	1.27
3002B	F			Thx, abd	Qiagen	3	219.0	1.91	2.23
3003A	F	*Nobandrena*	*Andrena nobilis* Morawitz, 1874	all body	Qiagen	3	138.9	1.78	1.37
3003B	F			Thx, abd,	Qiagen	3	274.5	1.95	1.96
3004A	M	*Nobandrena*	*Andrena probata* Warncke, 1973	Head, Thx	Qiagen	3	106.3	1.94	1.60
3101A	F	*Notandrena*	*Andrena langadensis* Warncke, 1965	Thx, abd	Qiagen	3	95.3	1.90	1.79
3102A	M	*Notandrena*	*Andrena recurvirostra* Warncke, 1975	Head, Thx	Qiagen	3	19.9	1.72	0.88
3103A	F	*Notandrena*	*Andrena ungeri* Mavromoustakis, 1952	all body	Qiagen	3	23.8	1.78	1.57
3103B	F			all body	Qiagen	3	72.9	1.83	1.34
3201A	F	*Opandrena*	*Andrena schencki* Morawitz, 1866	all body	Qiagen	3	52.2	1.60	0.80
3201B	F			all body	Qiagen	3	61.5	1.71	1.10
3201C	F			Thx, abd	Qiagen	3	53.8	1.69	1.07
3201D	F			Thx, abd	Qiagen	3	111.4	1.83	1.41
3301A	F	*Orandrena*	*Andrena garrula* Warncke, 1966	all body	Qiagen	3	54.0	1.86	1.70
3301B	F			all body	Qiagen	3	121.3	1.83	1.64
3401A	F	*Parandrenella*	*Andrena crispa* Warncke, 1975	all body	Qiagen	3	117.2	1.84	1.45
3401B	F			all body	Qiagen	3	153.1	1.92	1.85
3402A	F	*Parandrenella*	*Andrena dentiventris* Morawitz, 1874	all body	Qiagen	3	109.5	1.92	2.05
3402B	F			all body	Qiagen	3	83.8	1.99	2.00
3403A	F	*Parandrenella*	*Andrena figurata* Morawitz, 1866	all body	Qiagen	3	60.1	1.90	1.70
3403B	F			all body	Qiagen	3	81.8	1.94	1.84
3501A	F	*Plastandrena*	*Andrena bimaculata* (Kirby, 1802)	Thx, abd	Qiagen	3	43.3	1.38	0.60
3501B	F			Thx, abd	Qiagen	3	26.3	1.78	1.07
3501C	M			all body	Qiagen	3	23.6	1.56	0.65
3502A	F	*Plastandrena*	*Andrena pilipes* Fabricius, 1781	Thx, abd	Qiagen	3	34.3	1.53	0.72
3502B	F			Thx, abd	Qiagen	3	143.9	1.90	1.85
3502C	M			Head, Thx	Qiagen	3	69.6	1.90	1.53
3502D	M			Head, Thx	Qiagen	3	35.9	1.92	1.32
3601A	F	*Poecilandrena*	*Andrena crassana* Warncke, 1965	all body	Qiagen	3	106.0	1.94	1.70
3602A	F	*Poecilandrena*	*Andrena efeana* Scheuchl & Hazir, 2012	Thx, abd	Qiagen	3	98.3	1.86	1.52
3603A	F	*Poecilandrena*	*Andrena hybrida* Warncke, 1975	all body	Qiagen	3	119.3	1.95	1.89
3604A	F	*Poecilandrena*	*Andrena labiata* Fabricius, 1781	all body	Qiagen	3	45.4	1.92	1.66
3604B	M			Head, Thx	Qiagen	3	8.7	1.42	0.67
3605A	F	*Poecilandrena*	*Andrena laticeps* Morawitz, 1877	all body	Qiagen	3	108.4	1.91	1.79
3605B	F			all body	Qiagen	3	53.6	1.89	1.55
3605C	M			Head, Thx	Qiagen	3	28.4	1.95	1.11
3606A	F	*Poecilandrena*	*Andrena semirubra* Morawitz, 1876	all body	Qiagen	3	75.1	1.89	1.33
3607A	F			all body	Qiagen	3	97.6	1.93	1.88

Table 2. (Continued)

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA (ng/µl)	260/280	260/230
3701A	F	*Poliandrena*	*Andrena kriechbaumeri* Schmiedeknecht, 1883	all body	Qiagen	3	140.1	1.93	1.86
3701B	F			all body	Qiagen	3	69.7	1.89	1.14
3701C	M			Head, Thx	Qiagen	3	33.9	1.90	1.19
3702A	F	*Poliandrena*	*Andrena limbata* Eversmann, 1852	all body	Qiagen	3	27.7	1.62	0.67
3702B	F			Thx, abd	Qiagen	3	27.5	1.37	0.51
3703A	F	*Poliandrena*	*Andrena polita* Smith, 1847	Thx, abd	Qiagen	3	160.3	1.98	2.15
3703C	M			all body	Qiagen	3	35.5	1.85	1.45
3801A	F	*Proxiandrena*	*Andrena alutacea* Stoeckhert, 1942	all body	Qiagen	3	118.4	1.88	1.74
3801B	F			all body	Qiagen	3	258.5	1.96	1.85
3901A	F	*Ptilandrena*	*Andrena glidia* Warncke, 1965	all body	Qiagen	3	30.4	1.61	0.84
3902A	F	*Ptilandrena*	*Andrena vetula* Lepeletier, 1841	all body	Qiagen	3	204.7	1.92	1.83
4001A	F	*Scitandrena*	*Andrena scita* Eversmann, 1852	Thx, abd	Qiagen	3	36.4	1.53	0.73
4001B	F			Thx, abd	Qiagen	3	55.8	1.80	1.33
4001C	F			Thx, abd	Qiagen	3	32.7	1.48	0.56
4001D	M			Head, Thx	Qiagen	3	69.3	1.83	1.45
4101A	F	*Simandrena*	*Andrena combinata* (Christ, 1791)	Thx, abd	Qiagen	3	80.9	1.66	1.04
4101B	F			all body	Qiagen	3	22.8	1.66	1.15
4102A	F	*Simandrena*	*Andrena dorsata* (Kirby, 1802)	all body	Qiagen	3	102.2	1.87	1.57
4102D	M			Head, Thx	Qiagen	3	41.4	1.96	1.53
4103A	F	*Simandrena*	*Andrena lepida* Schenck, 1861	all body	Qiagen	3	227.9	1.90	2.06
4103D	F			all body	Qiagen	3	142.0	1.90	1.55
4104A	F	*Simandrena*	*Andrena transitoria* Morawitz, 1871	Thx, abd	Qiagen	3	24.5	1.47	0.80
4104B	F			Thx, abd	Qiagen	3	105.8	1.79	1.23
4104C	M			Head, Thx	Qiagen	3	34.9	1.87	1.86
4201A	F	*Thysandrena*	*Andrena ranunculorum* Morawitz, 1877	Thx, abd	Qiagen	3	175.6	1.79	1.41
4301A	F	*Trachandrena*	*Andrena haemorrhoa* (Fabricius, 1781)	all body	Qiagen	3	164.0	1.91	1.84
4401A	F	*Truncandrena*	*Andrena asiatica* Friese, 1921	all body	Qiagen	3	44.0	1.49	0.77
4401B	F			Thx, abd	Qiagen	3	30.8	1.53	0.86
4401D	M			Head, Thx	Qiagen	3	173.8	2.05	2.22
4402A	F	*Truncandrena*	*Andrena caneae* Strand, 1915	Thx, abd	Qiagen	3	179.1	2.00	2.06
4402B	M			Head, Thx	Qiagen	3	60.6	2.05	1.76
4403A	F	*Truncandrena*	*Andrena combusta* Morawitz, 1876	all body	Qiagen	3	33.2	1.58	0.89
4403B	F			Thx, abd	Qiagen	3	81.8	1.88	1.38
4404A	F	*Truncandrena*	*Andrena medeninensis usura* Warncke, 1967	all body	Qiagen	3	171.9	1.92	1.72
4404B	F			all body	Qiagen	3	111.2	1.89	1.54
4405A	F	*Truncandrena*	*Andrena optata* Warncke, 1975	all body	Qiagen	3	316.8	1.86	1.84
4405D	M			Head, Thx	Qiagen	3	34.4	1.67	0.77
4406A	F	*Truncandrena*	*Andrena roseotincta* Warncke, 1975	all body	Qiagen	3	93.7	1.83	1.20
4406C	F			Thx, abd	Qiagen	3	99.1	1.95	2.13
4407A	F	*Truncandrena*	*Andrena schmiedeknechti* Magretti, 1883	all body	Qiagen	3	147.7	1.95	1.94
4407D	M			Head, Thx	Qiagen	3	106.1	1.91	1.47

Table 2. (Continued)

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA (ng/µl)	260/280	260/230
4408A	F	*Truncandrena*	*Andrena seitzi* Alfken, 1935	Thx, abd	Qiagen	3	88.8	1.81	1.55
4408B	F			all body	Qiagen	3	95.5	1.82	1.59
4409A	F	*Truncandrena*	*Andrena truncatilabris* Morawitz, 1877	all body	Qiagen	3	32.0	1.40	0.69
4409B	F			all body	Qiagen-insects	15	54.4	1.64	0.99
4409C	F			all body	Qiagen	3	337.8	1.99	2.08
4409D	M			Head, Thx	Qiagen	3	45.1	1.91	1.62
4409F	M			Head, Thx	Qiagen	3	15.7	1.79	0.79
4410A	F	*Truncandrena*	*Andrena ulula* Warncke, 1969	all body	Qiagen	3	47.9	1.70	1.02
4410B	F			all body	Qiagen	3	97.1	1.88	1.73
4411A	F	*Truncandrena*	*Andrena urfanella* Scheuchl & Hazir, 2012	all body	Qiagen	3	58.5	1.73	1.08
4411B	F			all body	Qiagen	3	106.1	1.81	1.51
4411C	F			Thx, abd	Qiagen	3	96.1	1.86	1.47
4501A	F	*Ulandrena*	*Andrena cantiaca* Warncke, 1975	all body	Qiagen	3	140.1	1.93	1.81
4501D	M			Head, Thx	Qiagen	3	51.7	1.89	1.45
4502A	F	*Ulandrena*	*Andrena crecca* Warncke, 1965	Thx, abd	Qiagen	3	97.3	1.46	0.71
4502B	F			Thx, abd	Qiagen	3	77.4	1.91	1.85
4502C	M			Head, Thx	Qiagen	3	44.0	1.83	1.02
4503A	F	*Ulandrena*	*Andrena elegans* Giraud, 1863	Thx, legs	Qiagen	3	100.6	1.85	1.74
4503C	M			Head,Thx	Qiagen	3	108.4	1.60	1.19
4504A	F	*Ulandrena*	*Andrena fulvitarsis* Brullè, 1832	all body	Qiagen	3	120.7	1.89	1.71
4504B	F			all body	Qiagen-insects	15	44.3	0.95	0.70
4504E	M			Head, Thx	Qiagen	3	59.7	1.71	1.02
4505A	M	*Ulandrena*	*Andrena heinrichi* Grünwaldt, 2005	Head, Thx	Qiagen	3	129.8	1.89	1.41
4506A	F	*Ulandrena*	*Andrena neocypriaca* Mavromoustakis, 1956	all body	Qiagen	3	80.4	1.90	2.07
4506B	F			all body	Qiagen	3	42.5	1.90	1.56
4507A	F	*Ulandrena*	*Andrena osychniukae* Osytshnjuk, 1977	all body	Qiagen	3	71.5	1.97	1.52
4507B	M			all body	Qiagen	3	71.5	1.60	1.43
4507C	M			Head, Thx	Qiagen	3	26.6	1.66	1.16
4601A	F	*Zonadrena*	*Andrena flavipes* Panzer, 1799	all body	Qiagen-insects	15	114.5	1.76	1.51
4601B	F			Thx, abd	Qiagen	3	17.4	1.25	0.58
4601E	F			all body	Qiagen	3	135.7	0.59	0.52
4601F	F			all body	Ward, 2009	3	6.5	1.79	1.11
				Head, Thx	Crane, 2011	3	28.0	1.79	1.43
				Abdomen	Nishiguchi et al., 2002	over night	912.8	1.42	0.61
4601G	F			Head	Qiagen	4	82.8	1.96	2.02
4601H	M			Head, Thx	Qiagen	3	27.2	1.88	0.92
4601K	M			all body	Ward, 2009	4	24.3	1.97	1.12
				Head, Thx	Crane, 2011	4	0.4	4.95	0.13
4602A	F	*Zonadrena*	*Andrena gazelle* Friese, 1922	all body	Qiagen	3	42.4	1.74	1.19
4602B	F			Thx, abd	Qiagen	3	23.1	1.44	0.60

Thx: Thorax; Abd: Abdomen

Table 3. Quantification and quality of DNA obtained from ethanol (96%) preserved specimens of *Andrena* species

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA	260/280	260/230
1501X	F	*Charitandrena*	*Andrena hattorfiana* (Fabricius, 1775)	Thorax	Qiagen	4	284.4	1.59	1.06
1501X	F			Abdomen	Qiagen	4	373.1	1.47	0.77
1501X	F			Legs	Qiagen	4	17.0	1.45	0.63
1501Y	F			Thx, legs	Qiagen	3	456.1	2.16	2.25
1501Z	F			Thx, legs	Qiagen	3	392.1	2.14	2.19
1701X	F	*Chrysandrena*	*Andrena hesperia* Smith, 1853	Thx, abd	Qiagen	3	422.4	2.14	2.28
1701Y	F			Abdomen	Qiagen	3	216.4	2.12	2.22
1701Y	F			Thorax	Qiagen	3	111.4	2.14	2.19
1702X	F	*Chrysandrena*	*Andrena merula* Warncke, 1969	Thx, abd	Qiagen	3	471.7	2.14	2.13
1801X	F	*Cordandrena*	*Andrena cordialis* Morawitz, 1877	Thx, abd	Qiagen	3	307.7	2.06	1.75
1901X	F	*Didonia*	*Andrena nasuta* Giraud, 1863	Thorax	Qiagen	3	274.4	2.16	2.25
1901Y	F			Thx, legs	Qiagen	3	399.9	2.17	2.26
2403X	F	*Lepidandrena*	*Andrena curvungula* Thomson, 1870	Thorax	Qiagen	3	481.8	2.13	2.20
2501X	F	*Leucandrena*	*Andrena mistrensis* Grünwaldt, 2005	Thx, legs	Qiagen	3	281.3	2.12	2.13
2702X	F	*Melandrena*	*Andrena atrotegularis* Hedicke, 1923	Thorax	Qiagen	3	351.4	2.08	1.92
2703X	F	*Melandrena*	*Andrena danuvia* Stöckhert, 1950	Thorax	Qiagen	3	363.5	2.09	1.59
2703Y	F			Thx, legs	Qiagen	3	437.0	2.14	2.19
2703Z	F			Thx, legs	Qiagen	3	464.5	2.11	2.16
2710X	F	*Melandrena*	*Andrena thoracica* (Fabricius, 1775)	Thx, legs	Qiagen	3	695.1	2.12	2.26
3003X	F	*Nobandrena*	*Andrena nobilis* Morawitz, 1874	Thorax	Qiagen	3	353.6	2.15	2.16
3003Y	F			Thx, legs	Qiagen	3	512.3	2.14	2.28
3201X	F	*Opandrena*	*Andrena schencki* Morawitz, 1866	Thx, legs	Qiagen	3	379.9	2.07	1.91
3502X	M	*Plastandrena*	*Andrena pilipes* Fabricius, 1781	Thx, legs	Qiagen	3	84.8	2.03	2.09
3703X	F	*Poliandrena*	*Andrena polita* Smith, 1847	Thx, abd	Qiagen	3	564.9	2.13	2.21
3703Y	F			Thx, abd	Qiagen	3	413.3	2.14	2.19
3703Z	F			Abdomen	Qiagen	3	441.8	2.13	2.22
3703Z	F			Thorax	Qiagen	3	222.4	2.14	2.07
3703Z	F			Legs	Qiagen	3	25.0	2.20	1.36

Table 3. (Continued)

Code	Sex	Subgenus	Species	Material	DNA extraction method	Water bath incubation period (h)	Nanodrop		
							DNA	260/280	260/230
4001X	F	*Scitandrena*	*Andrena scita* Eversmann, 1852	Thorax	Qiagen	3	398.5	2.13	2.22
4103X	F	*Simandrena*	*Andrena lepida* Schenck, 1861	Thx, abd	Qiagen	3	474.4	2.15	2.24
4401X	F	*Truncandrena*	*Andrena asiatica* Friese, 1921	Thorax	Qiagen	3	386.5	2.13	2.25
4401Y	F			Thx, legs	Qiagen	3	351.5	2.15	2.24
4401Z	M			Thx, legs	Qiagen	3	133.2	2.04	1.84
4407X	F	*Truncandrena*	*Andrena schmiedeknechti* Magretti, 1883	Thx, legs	Qiagen	3	350.3	2.16	2.26
4502X	F	*Ulandrena*	*Andrena crecca* Warncke, 1965	Thorax	Qiagen	3	359.3	2.16	2.27
4502Y	F			Thx, legs	Qiagen	3	268.4	2.14	2.12
4601X	F	*Zonandrena*	*Andrena flavipes* Panzer, 1799	3 Legs	Qiagen	4	10.0	1.68	0.58
4601X	F			Thorax	Qiagen	4	250.6	1.59	0.91
4601X	F			Abdomen	Qiagen	4	692.4	1.90	1.48
4601X	F			Legs	Qiagen	4	28.0	1.44	0.67
4601Y	F			Thx, abd	Qiagen	3	278.6	2.15	2.18
4602X	F	*Zonandrena*	*Andrena gazella* Friese, 1922	Thx, legs	Qiagen	3	220.9	2.14	2.02

Thx: Thorax; Abd: Abdomen

DNA of all ethanol preserved samples was amplified with most of the primers tested (with the exception of primer pairs Uni-MinibarF1/Uni-MinibarR1, 12Sa/12SLR, ArgK-F/R, AndCOI-F1/COIand-R1 and AndCOI-F2/COIand-R2). The PCR was successful for almost all ethanol samples, whereas the amplification of DNA from only a few of the dry pinned specimens was achieved. The primers were selected that worked for both ethanol preserved and dry pinned specimens. These were used for further studies. The DNA samples were amplified by five primer sets (AndWNG-F/WNGand-R, polfor2a-And/polrev2a-And, Bel28S/Mar28Srev, And28S-F/28Sand-R, AndEF-F1/EFand-R1) and for four loci (wingless wnt-1 gene, RNA polymerase II, 28S ribosomal RNA, elongation factor 1 alpha F2 copy). DNA from 32 specimens belonging to 25 species and representing three to four loci was successfully amplified. Of these, 28 were ethanol-preserved specimens. Four of them (*Andrena gamskrucki impasta* Warncke, 1975*, A. fuscosa* Erichson, 1835*, A. gamskrucki eburnea* Warncke, 1975*, A. semirubra* Morawitz, 1876) were dry pinned specimens (Figure 1).

Preservation methods can have a direct effect on the quality and quantity of the DNA extracted from insect specimens. This study demonstrates that good quality DNA can be readily extracted and amplified from samples of sand bees preserved in ethanol. However, obtaining PCR amplifiable DNA from dry pinned specimens was difficult. None of the primers tested were reliable for amplifying DNA from the dry pinned specimens. However, five primers worked for about 2% of the dry specimens and they would be potentially useful for phylogenetic analyses. There are other factors that can affect the success of DNA extraction resulting in suitable quality DNA for PCR amplification from dry pinned specimens, including bee size, time since collection, DNA degrading contaminants, kill method and marker allele size.

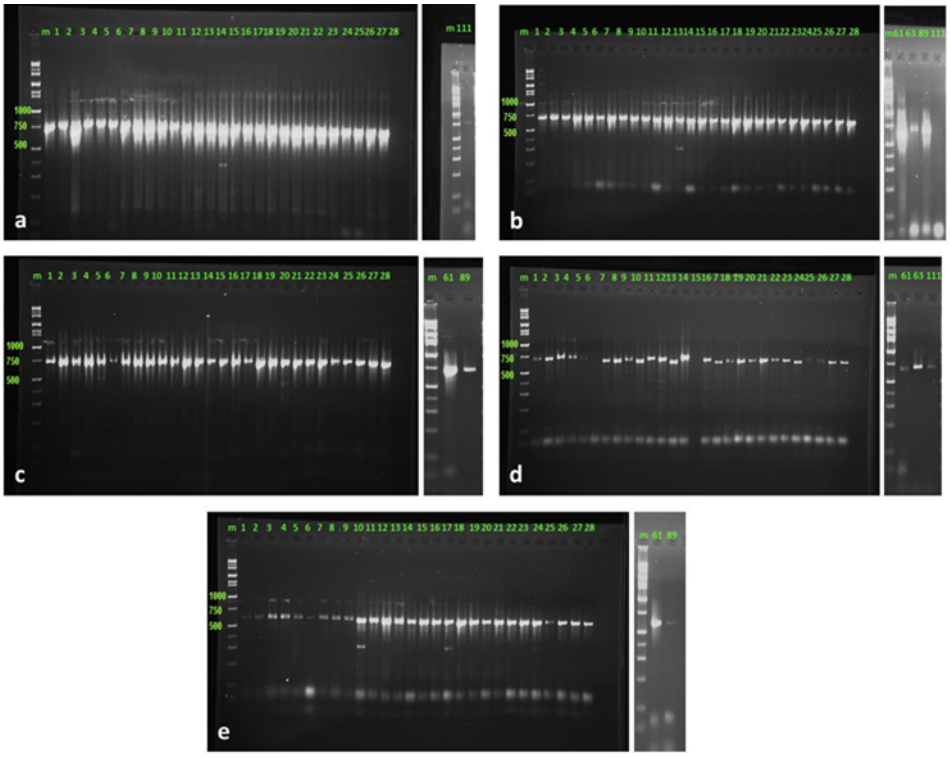

Figure 1. Agarose gel images showing products of PCR amplification of different primers for DNA sequence in *Andrena* species: a) Bel28S/Mar28Srev (~750 bp); b) And28S-F/28Sand-R (700 bp); c) AndEF-F1/EFand-R1(750 bp); d) AndWNG-F/WNGand-R (653 bp); e) polfor2a-And/polrev2a-And (587 bp) (lanes 1 to 28 contain DNA of ethanol preserved specimens; lanes 61 (2402A), 63 (2601A), 89 (2401A), 111 (3607A) contain DNA of dry pinned specimens).

Andersen & Mills (2012) reported that specimen size significantly affected the total amount of extracted DNA from a braconid parasitoid specimen. Strange et al. (2009) suggested that pinned bumble bee specimens from museum collections are suitable for population genetic studies because of their large size. In our study, the size of bee specimens ranged from small (5 mm) to medium (18 mm). The better quality DNA was obtained from larger bees, for example *A. albopunctata* (Rossi, 1792), *A. fuscosa* Erichson, 1835, *A. fuscocalcarata* Morawitz, 1877 (Table 2). The result could be associated with both the number of cells and the thickness of exoskeleton layer. The cuticle, which is an extracellular layer that covers the complete external surface of insects and acts both as a skeleton for muscle attachment and as a protective barrier. The thickness of cuticular layer varies from a few micrometers to a few millimeters, depending upon the insect species (Andersen, 2009). Strange et al. (2009) hypothesized that sclerotization of the bumble bees in museum collections helped preserve the genetic material. It is known that insect body size is strongly correlated with cuticle thickness (Peeters et al., 2017). Considering this information, it can be suggested that the bigger bees have a thicker cuticle, which protect the cells more. This hypothesis is supported by our data for sand bees.

If no preservation steps are taken, time since death has a negative effect on the likelihood of successful DNA amplification. DNA in museum specimens generally becomes degraded and the quality and quantity remaining often precludes molecular genetic studies (Gilbert et al., 2007; Zimmermann et al., 2008; Strange et al., 2009). In our study 222 dry pinned specimens of *Andrena* bees were collected between 2004-2011. DNA was extracted from almost all the dry pinned specimens that were screened. However, successful DNA amplification was possible for only four of these specimens. Notably, in our study, two of these samples, *Andrena gamskrucki impasta* (2402A) and *A. gamskrucki eburnea* (2401A), were relatively

recent collections (2011), but other specimens collected at the same time either did not contain sufficient DNA, or the DNA was not of sufficient quality. However, DNA amplification was successful with two older museum specimens, *A. fuscosa* (2601A) and *A. semirubra* (3607A), collected in 2007. Even if DNA amplification success decreases with the time since collection, at least some successful results would have been expected for specimens collected in 2008, 2009 or 2010. It is likely that the failure to amplify DNA from the oldest specimens is likely due to postmortem degradation of DNA. However, the reason why DNA was not amplified from most of the younger specimens is unknown. It is possible that the killing methods and storage conditions of these samples may have resulted in degradation of all of the DNA. Although time is important, it is likely to be only one of the factors.

Insect specimens are usually killed with ethyl acetate, ethyl alcohol, formalin or cyanide depending on the taxon, the method of collection and the choice of collector. Specimen labels usually do not include details of the killing agent used, or the length of exposure to the killing agent. Due to these factors, DNA extraction and amplification is unlikely to be successful from all dry specimens. Also, different collection and storage conditions affect the quantity and quality of DNA (Gilbert et al., 2007). In our study, all of the specimens were killed using ethyl acetate. There are few studies that have focused on the effect of the ethyl acetate on quality of DNA. Dillon et al. (1996) suggested that specimens killed in ethyl acetate vapor had fragmented DNA that gave consistently low yields when extracted and that could not be successfully amplified. Consequently, we consider that ethyl acetate is not a suitable killing reagent if the aim is to perform molecular studies on insects. Whereas, Willows-Munro & Schoeman (2015) claimed that there was no evidence that DNA degradation depended on killing method, including use of ethyl acetate. Therefore, further research is needed to clarify this issue.

Andersen & Mills (2012) suggested that short fragments of 28S and COI genes were sufficient for species identification, and for examining within species genetic diversity. They examined DNA extraction from museum specimens of parasitic Hymenoptera (Braconidae). In their study, several primer combinations of various length were tested, but these did not amplify fragments longer than 150 bp. In our study, 31 primer sets were tested. Of these, 13 amplified mitochondrial gene regions and 18 amplified nuclear gene regions. Five primers were selected that work for both ethanol preserved bee specimens and a few of the dry pinned specimens (AndWNG-F/WNGand-R, polfor2a-And/polrev2a-And, Bel28S/Mar28Srev, And28S-F/28Sand-R, AndEF-F1/EFand-R1) for four loci (wingless wnt-1 gene, RNA polymerase II, 28S ribosomal RNA, Elongation factor 1 alpha F2 copy). Sequence lengths were 653, 587, 690, 669 and 700 bp, respectively. Shorter amplicon sequence lengths were also tested (130, 226 and 350 bp) but these failed to give useful results. Accordingly, we contend that shorter sequence length is not effective in DNA amplification for dry museum specimens.

PCR can be influenced by many conditions including the template DNA preparation and reaction conditions, and primer design (Ye et al., 2012). The primer design is an important step to ensure successful PCR. In this study, 14 primer sets were newly designed or modified based on reported primer sequence in the literature. Twelve of these resulted in successful amplification of chosen nuclear gene regions. These primers are useful for entomologists intending to use bee specimens for systematic studies.

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Original article (Orijinal araştırma)

Contact toxicity of pine, laurel and juniper essential oils to spirodiclofen-resistant and -susceptible *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) populations

Çam, defne ve ardıç eterik yağlarının spirodiklofen'e dirençli ve hassas *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) popülasyonlarına kontak toksisitesi

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Abstract

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) causes significant losses in agricultural production because of it is a polyphagous pest and develops resistance to pesticides in a short time. This study was conducted in 2017-2018 in Isparta, Turkey. Contact toxicities of pine, laurel and juniper essential oils to different developmental stages of a highly spirodiclofen-resistant and a spirodiclofen-susceptible population of *T. urticae* were investigated. The was aim was to contribute to the development of alternative methods to control in resistant populations. The essential oil solutions were applied using a spray tower at 100 kPa to the leaf surface at 1.2-1.6 mg/cm^2. The experiments were conducted with three replicates, with 15 individuals each treatment replicate. The highest mortality in adults was 100% for pine, juniper and laurel oil in the susceptible population, and 59.5% for pine oil, 57.5% for laurel oil and 51.2% for juniper oil in the spirodiclofen-resistant population. In addition, the highest mortality for nymphs was 81.6% for pine oil, 95.2% for laurel oil, 95.7% for juniper oil in the susceptible population, and 50.0% for pine oil, 56.3% for laurel oil and 58.0% for juniper oil in the spirodiclofen-resistant population. In toxicity tests on egg the highest mortality was lower than 55.0% in both populations. As a result, the three essential oils were showed to have a significant effect in the *T. urticae* population with high level resistance to spirodiclofen.

Keywords: essential oil, juniper, laurel, pine, spirodiclofen, *Tetranychus urticae*

Öz

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) polifag bir zararlı olması ve pestisitlere kısa sürede direnç geliştirmesinden dolayı tarımsal üretim alanlarında önemli kayıplara neden olmaktadır. Bu çalışmada 296 kat spirodiclofen dirençli ve hassas *T. urticae* popülasyonlarında çam, defne ve ardıç uçucu yağlarının zararlının farklı dönemleri üzerindeki kontakt toksisiteleri araştırılmıştır. Denemeler 2017-2018 yılları arasında yürütülmüştür. Bu çalışmada dirençli popülasyonlarda alternatif mücadele yöntemlerinin geliştirilmesine katkı sağlanması amaçlanmıştır. Uçucu yağ çözeltileri ilaçlama kulesi yardımıyla 100 kPa basınçta yaprak yüzeyine 1.2-1.6 mg/cm^2 olacak şekilde püskürtülmüştür. Denemelerde her doz için 3 tekerrür ve her tekerrürde 15 birey kullanılmıştır. Uçucu yağların *T. urticae* popülasyonlarının her ikisinde de larva ve erginler üzerindeki en yüksek etkileri 20 ml/l konsantrasyonda ve 96. saat sonunda elde edilmiştir. Erginlerde en yüksek etki hassas popülasyon için, çam, ardıç ve defne yağında %100; spirodiclofen dirençli popülasyonda ise çam yağında %59.5, defne yağında %57.5, ardıç yağında %51.2 olarak belirlenmiştir. Larvalarda ise en yüksek etki hassas popülasyon için, çam yağında %81.6, defne yağında %95.2, ardıç yağında %95.7; spirodiclofen dirençli popülasyonda ise çam yağında %50.0, defne yağında %56.3, ardıç yağında %58.0 olarak belirlenmiştir. Toksisite testlerinde yumurtalar üzerindeki en yüksek ölüm oranları her üç uçucu yağ için de %55.0'ten düşük bulunmuştur. Sonuç olarak spirodiclofen'e yüksek seviyede dirençlibir *T. urticae* popülasyonunda bu üç uçucu yağın kayda değer bir etkisi olduğu bulunmuştur.

Anahtar sözcükler: uçucu yağ, ardıç, defne, çam, spirodiklofen, *Tetranychus urticae*

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Introduction

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) is a major pest that feeds on about 1000 plants from 250 families around the world (Migeon & Dorkeld, 2011). To control this pest, chemical methods including the insecticide/acaricide applications with varying modes of action and formulation are used (Pavlidi et al., 2017). However, due to its parthenogenetic reproduction and short life cycle, *T. urticae* can develop resistance to pesticides after a few applications. For this reason, the research on alternative control methods for *T. urticae* has gained importance in recent years. As an alternative to synthetic pesticides some compounds extracted from plants are being investigated. Essential oils are the most important herbal products that can be used for pest control due to their monoterpene and diterpene content. Essential oils and their components are effect insect physiology, behavior and biology (Singh & Upadhyay, 1993). Also, it has been shown that essential oils have insecticidal, acaricidal, ovicidal, antifeedant and repellent effects on many harmful species (Govindarajan et al., 2016; Reddy et al., 2016). In comparison to synthetic pesticides, essential oils are substances that have minimal effect on human and non-target organisms, on ecological balance and do not lead to resistance development in the pests (Isman et al., 2011).

Pine oil and its components are widely used in the cosmetics, perfumery and foods industry as raw material and aroma (Yang et al., 2010). Laurel oil, usually obtained by hydrodistillation and steam distillation, is used in the cosmetic industry and in the treatment of some diseases in medicine (Hafizoglu & Reunanen, 1993). Juniper oil has diuretic and antiseptic effects due to containing terpene hydrocarbons, such as sabinene, thujone, α-pinene, myrcene and limonene (Pepeljnjak et al., 2005). There have been some studies on the effects of pine, laurel and juniper essential oils on insects. Shaaya et al. (1997) evaluated the fumigant effect of laurel essential oil against to the pests, *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae), *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae). Karci & Isıkber (2007) report that the fumigant effects of pine, laurel and juniper essential oils on *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) eggs is low.

The mechanism of the contact effect of the essential oil and their components in insects is on the octopamine system. Octopamine receptors have a role in the central nervous system, such as neurotransmitters, neurohormones and neuromodulators. When an essential oil or its components contact the body of the insect, it is recognized by the octopamine system, which is followed by hyperactivity, the heart rate accelerates and excessive stress occurs in legs and abdomen and then the insect rapidly falls to ground and dies. These symptoms are reported to be generated by octopamine receptors (Kostyukovsky et al., 2002).

Spirodiclofen is located within the spirocyclictetronicacaricide group (in Group 23 of the IRAC MoA list). Spirodiclofen, and is efficacious against pest mite species such as Tetranychidae. It reduces fertility and the number of eggs deposited by females (IRAC, 2019).

The aim of this study was to contribute to the development of alternative control methods for resistant *T. urticae* populations. The contact toxicity of different concentrations of pine, laurel and juniper essential oils on different developmental stages of spirodiclofen-resistant and spirodiclofen-susceptible populations of *T. urticae* were investigated.

Material and Methods

Populations and rearing

Spirodiclofen-resistant and spirodiclofen-susceptible populations of *T. urticae* were used in this study (conducted in 2017-2018 in Isparta, Turkey). The susceptible population of *T. urticae* was obtained

in 2001 from Rothamsted Experimental Station (UK) and long-term rearing has continued in a climate room. The spirodiclofen-resistant population of *T. urticae* was collected from a pepper greenhouse in which intensive chemical control was applied in 2017. *Tetranychus urticae* populations were rearing on bean plants at 26±2ºC, 50-60% RH, and 16:8 h L:D photoperiod conditions in a controlled climate room.

Acaricide and essential oils

A commercial acaricide, Envidor® SC 240 (Bayer Crop-Science), with spirodiclofen as the active ingredient was used.

The names, scientific names and families of the pine, laurel and juniper oils used in the study are given in Table 1. Pine, laurel and juniper oils were provided by a commercial supplier, Botalife Company (Turkey). The essential oils had been obtained by hydro-Clevenger method and the green parts of the plants was used. The components of the essential oils were not determined.

Table 1. Plant essential oils tested

Essential oils		
Name	Scientific name	Family
Pine oil	*Pinus sylvestris*	Pinaceae
Laurel oil	*Laurus nobilis*	Lauraceae
Juniper oil	*Juniperus oxycedrus*	Cupressaceae

Acaricide bioassay for spirodiclofen

LC_{50} values of susceptible and resistant *T. urticae* populations were determined in order to determine to spirodiclofen resistance ratio. The spirodiclofen dose that gives 90% mortality was chosen as the first dose. Seven doses were prepared by two-fold serial dilution from the first dose. All the bioassay tests were conducted as a control plus seven spirodiclofen doses, all with three replicates. To provide sufficient humidity, 3-cm diameter bean leaf discs were placed into 9 cm diameter Petri dishes which had a saturated cotton in the base. Twenty-five adult female individuals were transferred onto the leaf discs. Two mL of the acaricide doses were applied by spray tower at a pressure of 100 kPa. Only pure water was applied to the control. Mortality assessment was made after 24 h.

Contact toxicity tests for essential oils

In contact toxicity tests, the Miresmailli et al. (2006) method was used for adults and nymphs, and Badawy et al. (2010) method for eggs. The same stage, eggs, nymphs or adults, were used. In the experiments, 15 adult female individuals were transferred to a prepared Petri dish as described above. Concentrations of 1, 5, 10 and 20 mL/l of pine, laurel and juniper essential oils were used. The essential oils were dissolved in 0.3% Tween 20 solution prepared with purified water. For the control, only the Tween solution was used. All the experiments were conducted with three replicates each containing 15 individuals. The essential oil concentrations were sprayed onto the leaf surface at 1.2-1.6 mg/cm^2 at 100 kPa using a spray tower (Mansour et al., 1986). Mortality assessments for nymphs and adults were made after 24, 48 and 96 h. For eggs, the assessments were made when all eggs had hatched in the control.

Statistical analysis

LC_{50} values were calculated in the POLO computer package program (LeOra Software, 1994). The spirodiclofen resistance ratio was determined by the ratio of the LC_{50} value of the resistant and susceptible populations. The mortality percentages from the contact toxicity experiments were calculated using the Abbott formula (Abbott, 1925). The data were arcsin transformed. The contact effects of

essential oils on the nymph and adult stages of the pest were analyzed using three-way ANOVA. In order to determine the toxic effect of the essential oils on the eggs, two-way ANOVA was used for essential oil and concentration data. Tukey multiple comparison test was used to compare the differences between the means.

Results

Resistance ratio in *Tetranychus urticae* greenhouse population

LC_{50} values determined against spirodiclofen for the field population and for the susceptible population are given in Table 2. A high level (296-fold) of spirodiclofen-resistance was determined for the *T. urticae* population collected from pepper greenhouse.

Table 2. LC_{50} values determined against spirodiclofen in resistant and susceptible populations of *Tetranychus urticae*

Population	n*	Slope±SE	LC_{50} (mg a. i l^{-1})	R**
Resistant	605	2.35±0.37	210 (143-324)	296
Susceptible	607	2.72±0.46	0.71 (0.30-0.99)	-

*: number of individuals used in the experiment;
**: resistance ratio.

Contact toxicity of the essential oils on *Tetranychus urticae* populations

Contact toxicity on adult

The contact toxicity on the adults in the susceptible and resistant populations of essential oils are given in Table 3. The effects on adults for all concentrations of essential oils were found to be higher in the susceptible population than the resistant population. Highest mortality of adults with the essential oils was at 20 mL/l after 96 h. In the susceptible population, 100% mortality was observed at 20 mL/l after 96 h for all three essential oils. In the resistant population, the mortality was 59.5% for pine oil, 57.5% for laurel oil and 51.2% for juniper oil.

Contact toxicity on nymph

The contact toxicity on the nymph in the susceptible and resistant populations of essential oils are given in Table 4. The contact toxicity of essential oils on the nymphs of *T. urticae* were found to be similar to the adults. For both populations, when the concentrations of essential oils increased, the mortality on nymphs increased. However, the mortality of the resistant nymphs was found to be lower than the susceptible nymphs. According to the concentrations of 20 mL/l of essential oils after 96 h counting results the mortality was 92.6%, 95.2% and 98.4% for pine oil, laurel oil and juniper oil, respectively, in the susceptible population, and 50.0%, 56.3%, and 58.0% for pine oil, laurel oil and juniper oil, respectively, in the resistant population.

Table 3. Contact toxicity of essential oils on adults of susceptible and resistant populations of *Tetranychus urticae*

Time (hour)	Concentration (ml/l)	Mortality (%)					
		Pine		Laurel		Juniper	
Susceptible							
24	1	24.49±0.45	aD*	4.45±0.25	cE	10.64±0.33	bF
	5	34.69±0.25	bC	8.89±0.85	cD	48.94±2.15	aD
	10	48.98±0.65	bC	8.89±0.55	cD	68.09±1.45	aC
	20	77.55±0.48	bB	46.67±0.24	cB	100.00±0.65	aA
48	1	97.73±1.15	aA	11.36±1.65	bD	20.00±0.65	bE
	5	97.73±0.23	aA	15.91±1.45	cD	66.67±0.95	bC
	10	100.00±0.65	aA	27.27±0.89	cC	82.22±0.75	bB
	20	100.00±0.45	aA	90.91±0.48	aA	100.00±0.23	aA
96	1	100.00±0.25	aA	30.95±0.88	bC	37.78±0.35	bD
	5	100.00±0.55	aA	30.95±0.25	bC	95.56±1.75	aA
	10	100.00±1.25	aA	90.48±0.65	aA	100.00±0.25	aA
	20	100.00±0.63	aA	100.00±0.75	aA	100.00±0.45	aA
Resistant							
24	1	0.00±0.00	bE	0.00±0.00	bE	2.13±0.35	aF
	5	0.00±0.00	cE	2.33±0.45	bD	6.38±0.55	aE
	10	2.17±0.25	bD	2.33±0.65	bD	8.51±0.75	aD
	20	4.35±0.55	bD	13.95±1.25	aC	10.64±1.45	aD
48	1	7.50±0.75	aD	2.33±1.35	cD	4.65±1.75	bE
	5	25.00±0.56	aC	4.35±0.75	cD	10.25±0.65	bD
	10	30.00±0.68	aC	9.30±0.25	cC	16.28±0.33	bC
	20	42.50±0.25	aB	34.38±2.15	bB	16.28±0.70	cC
96	1	29.73±1.45	aC	7.50±1.10	bC	20.93±0.65	aC
	5	40.54±0.70	aB	42.50±0.75	aB	23.46±1.15	bC
	10	45.95±0.65	bB	60.00±0.44	aA	39.53±0.45	bB
	20	59.46±0.60	aA	57.50±0.78	aA	51.16±0.95	aA

*: Different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to essential oils and application doses, respectively ($p<0.05$).

Table 4. Contact toxicity of essential oils on nymphs of susceptible and resistant populations of *Tetranychus urticae*

Time (hour)	Concentration (ml/l)	Mortality (%)		
		Pine	Laurel	Juniper
Susceptible				
24	1	13.95±1.25 bE*	22.92±0.75 aE	20.41±0.45 aE
	5	53.49±0.25 bC	64.58±0.46 aC	67.35±0.75 aC
	10	55.81±0.55 bC	70.83±0.85 aB	69.39±0.15 aC
	20	62.79±0.85 bB	79.17±0.43 aB	79.59±0.65 aB
48	1	18.60±0.88 cD	29.79±0.54 bE	36.73±0.48 aD
	5	67.44±0.68 aB	70.21±0.64 aB	71.43±0.35 aB
	10	74.42±0.15 bB	76.60±0.25 bB	83.67±0.13 aB
	20	76.74±0.78 bB	82.98±1.45 aA	87.76±0.78 aB
96	1	23.68±0.65 cD	47.62±1.74 aD	36.96±0.36 bD
	5	68.42±0.35 bB	83.33±1.58 aA	78.26±0.98 aB
	10	81.58±0.25 bA	85.71±0.35 bA	91.30±0.25 aA
	20	92.58±1.35 aA	95.24±0.75 aA	98.41±0.35 aA
Resistant				
24	1	6.00±0.25 bE	10.00±0.25 aD	9.80±0.350 aE
	5	14.00±0.36 bD	24.00±0.45 aC	11.76±0.45 bE
	10	32.00±0.78 aB	36.00±0.85 aB	27.45±0.75 bC
	20	40.00±0.55 bB	52.00±0.65 aA	39.22±1.45 bB
48	1	8.00±1.15 bE	14.00±0.23 aD	10.00±1.75 bE
	5	18.00±1.85 bD	28.00±1.15 aC	14.00±0.25 bE
	10	36.00±0.55 aB	40.00±0.85 aB	30.00±0.36 bC
	20	48.00±0.36 bA	56.00±0.45 aA	44.00±0.46 bB
96	1	12.00±0.74 aD	14.58±0.78 aD	14.00±0.75 aE
	5	26.00±0.89 aC	29.17±0.89 aC	20.00±0.15 bD
	10	38.00±1.85 bB	45.83±0.25 aB	34.00±0.25 bC
	20	50.00±0.48 bA	56.25±0.15 aA	58.00±0.45 aA

*: Different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to essential oils and application doses, respectively ($p<0.05$).

Contact toxicity on egg

The contact toxicity on egg stage in the susceptible and resistant populations of essential oils are given in Table 5. The contact toxicity on egg stage of pine, laurel and juniper essential oils in susceptible population were found to be higher than the resistant population. At 20 mL/l, the mortality was 48.9, 48.9 and 55.3% for juniper oil, pine oil and laurel oil, respectively, in the eggs of susceptible population, and 14.9, 23.3 and 41.1% for juniper oil, pine oil and laurel oil, respectively, in the eggs of resistant population.

Table 5. Contact toxicity of essential oils on egg stage of susceptible and resistant populations of *Tetranychus urticae*

Concentration (ml/l)	Mortality (%)		
	Pine	Laurel	Juniper
	Susceptible		
1	19.15±0.35 aC*	0.00±0.25 bD	21.28±0.75 aD
5	23.46±0.78 aB	11.11±0.85 bC	29.79±0.15 aC
10	42.55±1.12 aA	37.78±0.43 bB	46.81±0.45 aB
20	48.94±1.63 bA	48.89±0.45 bA	55.32±0.33 aA
	Resistant		
1	4.26±1.45 bC	2.33±0.75 cC	14.89±1.25 aC
5	10.64±0.45 cB	16.28±0.45 bB	28.30±1.85 aB
10	10.64±0.36 cB	19.95±0.15 bB	31.06±0.25 aB
20	14.89±0.85 cA	23.25±1.15 bA	41.06±0.55 aA

*: Different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to essential oils and application doses, respectively ($p<0.05$).

Discussion

Plant essential oil-containing pesticides or components can affect some pathogenic fungi that cause pre- and post-harvest diseases, agricultural pests, pests of stored products and urban pests (Koul et al., 2008). Essential oils consist of a mixture of hydrocarbons, terpenes, aldehydes, ketones, alcohols and phenol-like compounds. Each of these components, or their mixtures, can cause toxicity, repellent, behavioral, antifeeding and reproductive effects on some arthropod species (Lawless, 2002). Essential oils and their components are particularly suitable for use in IPM applications, as they cause many effects on pest species but are not deleterious to non-target organisms.

Studies showing that essential oils obtained from different plants are effective on phytophagous mite species and can be used as an alternative to synthetic acaricides. Lee et al. (1997) reported some of the monoterpenoids were lethal to the *T. urticae* at high concentrations; specially carvomenthenol and terpinen-4-ol. Rasikari et al. (2005) investigated contact toxicity of extracts from 67 species from six subfamilies of Australian Lamiaceae to *T. urticae* and determined some of the extracts had acaricidal effects. However, the number of studies on the effects of essential oils on acaricide-resistant mite populations has been limited. Han et al. (2010) reported essential oils from citronella Java, clover leaf, lemon eucalyptus, pennyroyal, peppermint and thyme showed acaricidal activity in the abamectin-resistant *T. urticae* population. In a study on eucalyptus oil components, menthol, β-citronellol and citral components were found to be effective in the chlorfenapyr-resistant CRT-53 population, geranyl acetate, citronellal and α-terpinene components in the fenpropathrin-resistant FRT-53 population, and citronellyl acetate, citral, eugenol and geraniol components in the pyridaben-resistant PRT-53 population (Han et al., 2011).

Rauch and Nauen (2003) and Van Pottelberge et al. (2009) determined the resistance against spirodiclofen developed as a result of selection pressure in *T. urticae*. A highly spirodiclofen-resistant (296-fold) *T. urticae* population used this study. The findings showed that the acaricidal activity of pine, laurel and juniper oils in the resistant population was consistent with the literature. Therefore, it is considered that the spraying method leads to the toxic effect in susceptible and resistant populations of *T. urticae,* and that this method is easily applicable, especially in greenhouse production areas.

In order for plant-based products, such as essential oil, to be used for insecticide resistance management in *T. urticae*, the resistance mechanisms and the mode of action of insecticide resistance should be known. The resistance mechanisms against acaricides in *T. urticae* are usually the formation of target-site insensitivity, such as decreased sodium channel sensitivity caused by pyrethroids, or decreased acetylcholinesterase sensitivity caused by organic phosphates (Knowles,1997). In the literature it has been reported that the effects of essential oils are on the octopaminergic system and GABA receptors (Kostyukovsky et al., 2002; Priestley et al., 2003). However, more studies on the the effects of essential oils to these target regions are needed, and the possibility of resistance development must be considered.

In conclusion, pine, laurel and juniper oils had a greater effect on the susceptible population than on the spirodiclofen-resistant population of *T. urticae*, but the effect on the resistant population was significant. Provided no side effects to natural enemies and phytotoxicity are found, essential oils could prove useful for control of spirodiclofen-resistant populations of *T. urticae*, especially in greenhouses.

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Original article (Orijinal araştırma)

Anomognathus ispartaensis sp. n. (Coleoptera: Staphylinidae: Aleocharinae) from Turkey[1]

Türkiye'den yeni bir tür: *Anomognathus ispartaensis* sp. n. (Coleoptera: Staphylinidae: Aleocharinae)

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Abstract

The genus *Anomognathus* Solier, 1849 (Coleoptera: Staphylinidae: Aleocharinae) comprises 10 species in the Palearctic Region. In the present study, a new species of the genus *Anomognathus* is described from Isparta Province of southwestern Anatolia and illustrated: *Anomognathus ispartaensis* sp. n. The new species is distinguished from all its congeners by the different body proportions, differently shaped tergite VIII of male, and by the completely different aedeagus. Three species of this genus are now known from Turkey. Specimens of this unknown species were collected from damaged cones of *Pinus brutia* Tenore by larvae of the Turkish red pine cone moth *Dioryctria mendacella* (Staudinger, 1859) (Lepidoptera: Pyralidae: Phycitinae) on 10 May 2016. The adult specimens emerged between 17 and 25 May 2016. This new species is probably a predator of *D. mendacella*.

Keywords: Aleocharinae, *Anomognathus*, cones of *Pinus brutia*, new species, Staphylinidae, Turkey

Öz

Anomognathus Solier, 1849 (Coleoptera: Staphylinidae: Aleocharinae) cinsi Palearktik Bölgede 10 tür ile temsil edilmektedir. Bu çalışmada, Güneybatı Anadolu'daki Isparta ilinden *Anomognathus* cinsine bağlı yeni bir tür tanımlanmış ve şekillendirilmiştir: *Anomognathus ispartaensis* sp. n. Bu yeni tür, bütün yakın türlerden farklı vücut oranları, erkek 7. tergitinin değişik biçimi ve tamamen farklı aedeagus yapısı ile ayrılmaktadır. Böylece, bu cinse bağlı olarak Türkiye'de bilinen tür sayısı üç olmuştur. Bu bilinmeyen türün örnekleri kızılçam kozalak kelebeği *Dioryctria mendacella* (Staudinger, 1859) (Lepidoptera: Pyralidae: Phycitinae) larvaları tarafından zarar verilmiş olan *Pinus brutia* Tenore ağaçlarının kozaklarından 10 Mayıs 2016 tarihinde toplanmıştır. Ergin bireyler 17-25 Mayıs 2016 tarihleri arasında çıkmıştır. Bu yeni tür muhtemelen, *D. mendacella* türünün bir predatörüdür.

Anahtar sözcükler: Aleocharinae, *Anomognathus*, *Pinus brutia* kozalağı, yeni tür, Staphylinidae, Türkiye

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Introduction

Anomognathus Solier, 1849 (Coleoptera: Staphylinidae: Aleocharinae) is a genus of the tribe Homalotini Heer, 1839 belonging to the subfamily Aleocharinae Fleming, 1821. The genus contains 10 species in the Palearctic Region (Schülke & Smetana, 2015). *Anomognathus* species live mostly under tree bark, but the biology and ecology of the species are unknown. According to Anlaş (2009) and Assing (2009), *Anomognathus cuspidatus* (Erichson, 1839) has been found in Bitlis, Karabük and Konya Provinces and *Anomognathus tricuspis* (Eppelsheim, 1884) in Adana and Mersin Provinces (Assing, 2006, 2009). In the Palearctic Region, *A. cuspidatus* is widespread in Europe and is known from China, also this species is distributed in the Nearctic Region. The known distribution of *A. tricuspis* is confined to Greece, Italy, Ukraine and Turkey.

Dioryctria mendacella (Staudinger, 1859) (Lepidoptera: Pyralidae: Phycitinae) is a moth and one of the several important harmful species on *Pinus* spp. The Turkish red pine cone moth, *D. mendacella* attacks the cones of several pine species, such as *Pinus pinea* L., *Pinus halepensis* Miller, *Pinus brutia* Tenore and *Pinus pinaster* Aiton, around the Mediterranean Region. The larvae feed within the cones, causing the cones to disintegrate before reaching maturity and thereby preventing seed production. It is commonly found in the pine forests of the Mediterranean, Aegean and Marmara Regions of Turkey. The pest reduces tree reproductive success, impacting on the quality of seed supply for regeneration and reforestation, and affecting abundance, distribution and dynamics of tree populations (Özek & Avcı, 2017). Can & Özçankaya (2006) and Özçankaya et al. (2013) observed specimens of *Carpelimus* spp. (Staphylinidae: Oxytelinae) abundantly in cones of *P. pinea* as a natural enemy of *D. mendacella*. Specimens of the new species were collected from *P. brutia* cones damaged by *D. mendacella* larvae. Thus, this is the second staphylinid natural enemy species of this pest found in Turkey.

The aim of this paper is to present a description of a new species of *Anomognathus* from southwestern Anatolia.

Material and Methods

The specimens of the new species were obtained from *P. brutia* cones, which were collected on 10 May 2016, with the Turkish red pine cone moth *D. mendacella*. The adult specimens emerged between 17 and 25 May 2016. Emerged specimens were individually preserved in 70% ethanol. Terminology of the primary and secondary sexual characters of the species described here follows Assing (2009). The morphological studies were conducted using a Stemi 2000-C microscope (Zeiss, Carl Zeiss AG, Oberkochen, Germany). For the photographs a digital camera (Zeiss Axiocam ERC5s) was used. Corel Draw X5 and Corel Photo-Paint X5 were used for editing the photos.

The following abbreviations are used for the measurements, which are given in mm: AL, length of antenna; AW, maximal width of abdomen; EL, length of elytra from apex of scutellum to posterior margin; EW, combined width of elytra; HL, head length from anterior margin of clypeus to posterior margin of head; HW, head width (including eyes); ML, length of aedeagus from apex of ventral process to base; PL, length of pronotum along median line; PW, maximal width of pronotum; and TL, total body length.

The material referred to in this study is preserved in the following collections: AZMM, Alaşehir Zoological Museum, Manisa, Turkey; and cAvc, private collection of the second author.

Results

Anomognathus ispartaensis sp. n. (Figure 1a-i)

Type material: Holotype: TURKEY: ♂ "TR – Isparta Province, Sütçüler, Yeşilyurt Village, Karadağ Forest Enterprise Depots, 890 m, 37°32'48" N, 30°53'24" E, 10-25.V.2016, leg. Avcı / Holotype ♂ *Anomognathus ispartaensis* sp. n. det. S. Örgel, M. Avcı & T. Özek 2018" (AZMM). Paratypes: TURKEY: 3♂♂, 2♀♀, same data as holotype (AZMM, cAvc).

Measurements (in mm) and ratios (range, n = 6): AL 0.56-0.59; HL 0.18-0.25; HW 0.29-0.36; PW 0.36-0.42; PL 0.25-0.31; EL 0.33-0.39; EW; 0.57-0.62; AW 0.42-0.48; ML 0.52 (n = 4); TL 1.78-2.40; HL/HW 0.62-0.69; PW/HW 1.17-1.24; PW/PL 1.35-1.44; EL/PL 1.26-1.32; EW/PW 1.48-1.58; AW/EW 0.75-0.77.

Type locality: Turkey, Isparta Province, Sütçüler District, Yeşilyurt Village.

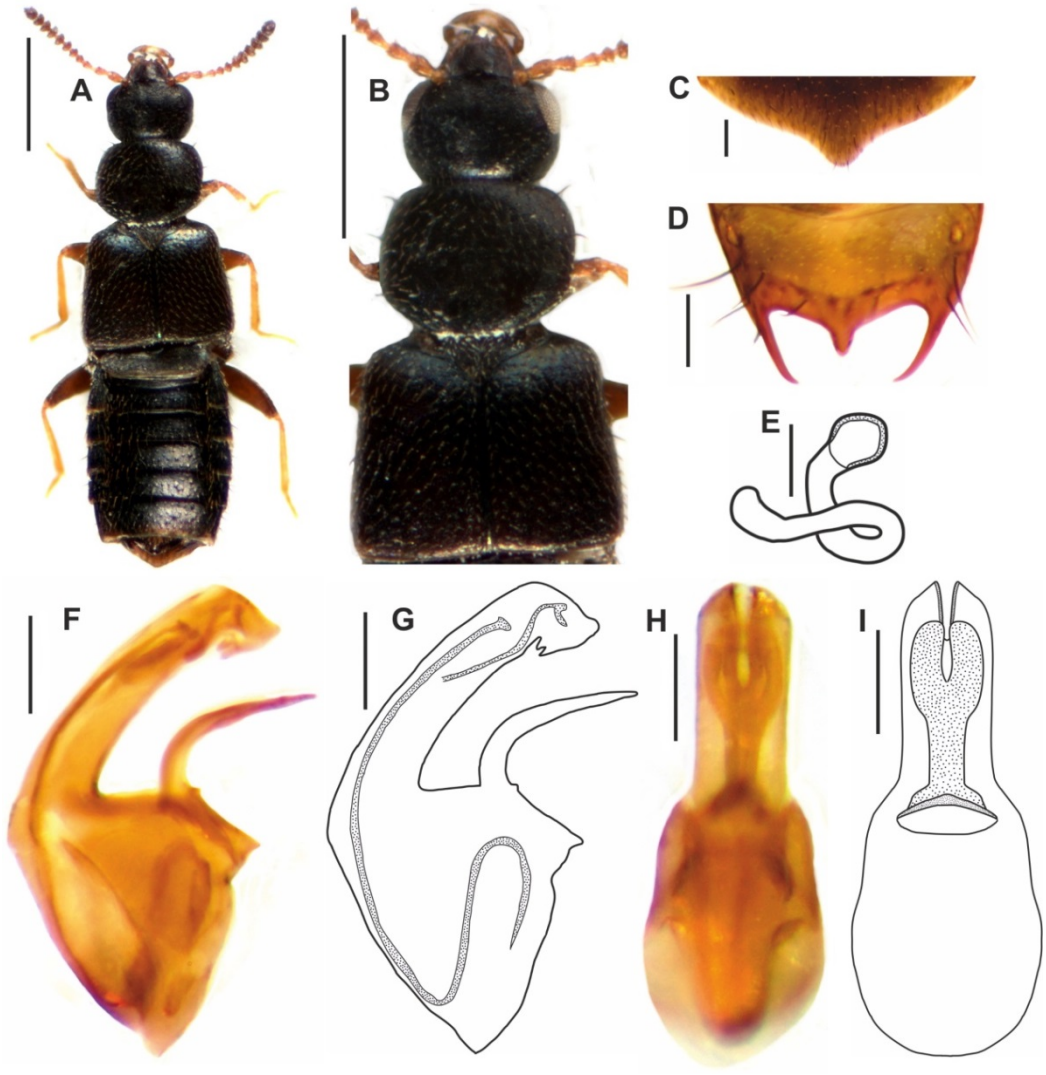

Figure 1. Details of *Anomognathus ispartaensis* sp. n. A) habitus; B) forebody; C) male sternite VIII; D) male tergite VIII; E) spermatheca; F & G) aedeagus in lateral view; H & I) aedeagus in ventral view. Scale bars: 0.5 mm (A-B) and 0.1 mm (C-I).

Description: Body length 1.8-2.4 mm. Coloration as Figure 1a: uniformly black with lighter posterior part of elytra; legs pale brown; antennae reddish brown with antennomeres I-IV yellowish.

Head 0.62-0.69 times as long as broad; narrower and shorter than pronotum (Figure 1A, B), with large eyes longer than postocular area in dorsal view; postocular area strongly converging basally and rounded posteriorly; antennae with articles I-III elongate and IV-X subquadrate (Figure 1A); punctation sparse and barely visible, microreticulation explicit. Pronotum approximately 1.4 times as broad as long and 1.2 times as wide as head (Figure 1A, B), lateral margins parallel, posterior margin convex, punctation and microreticulation similar to head, obviously noticeable in the microsculpture. Elytra longer and wider than pronotum (Figure 1A, B); microsculpture obviously noticeable and regular, punctation and

microreticulation similar to pronotum. Abdomen subparallel (Figure 1A); slightly narrower than elytra, microsculpture distinct, shallower than that of elytra, microreticulation obviously noticeable.

♂: posterior margin of tergite VIII with three processes; two long and apically acute lateral teeth and one shorter and apically rotate median teeth (Figure 1D); posterior margin sternite VIII convex with median process (Figure 1C). Aedeagus 0.52 mm long, ventral process of distinctive shape in both lateral and ventral view (Figure 1F-I).

♀: tergite VIII with two long and apically acute lateral teeth, median part of tergite as long as lateral teeth and strongly converging basally; posterior margin of sternite VIII convex. Bulbus distalis of spermatheca wide, introflexio apicalis convex; manica interiecta strongly curved proximally, bulbus proxsimalis slightly curved as in Figure 1E.

Distribution and bionomics. The new species was collected in only one locality in the Yeşilyurt Village, Sütçüler District, Ispartaü Province in southwestern Anatolia, where it was collected from *P. brutia* cones damaged by larva of *D. mendacella* at an elevation of 890 m. According to Özek & Avcı (2017), this new species is a predator of *D. mendacella*.

Etymology. The name is derived from the Isparta Province, where the type locality is situated.

Comparative notes. The species is distinguished from its congeners (*A. cuspidatus* and *A. tricuspis*) by the different body proportions, by the differently shaped tergite VIII of male, with two large, acute and hooked apically lateral teeth, and also by the completely different aedeagus.

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