PRINT ISSN 1302-0250
ONLINE ISSN 2651-3579

Journal of the Entomological Research Society

Volume: 22 Part: 1 2020

JOURNAL OF THE ENTOMOLOGICAL RESEARCH SOCIETY

Published by the Gazi Entomological Research Society

Editor (in Chief)
Abdullah Hasbenli

Managing Editor
Zekiye Suludere

Associate Editor
Selami Candan

Review Editors
Doğan Erhan Ersoy
Damla Amutkan Mutlu
Nurcan Özyurt Koçakoğlu

Language Editor
Nilay Aygüney

Subscription information

Published by GERS in single volumes three times (March, July, November) per year. The Journal is distributed to members only. Non-members are able to obtain the journal upon giving a donation to GERS.

Papers in *J. Entomol. Res. Soc.* are indexed and abstracted in Biological Abstract, Zoological Record, Entomology Abstracts, CAB Abstracts, Field Crop Abstracts, Organic Research Database, Wheat, Barley and Triticale Abstracts, Review of Medical and Veterinary Entomology, Veterinary Bulletin, Review of Agricultural Entomology, Forestry Abstracts, Agroforestry Abstracts, EBSCO Databases, Scopus and in the Science Citation Index Expanded.

Publication date: March 15, 2020

© 2020 by Gazi Entomological Research Society

Printed by Hassoy Ofset
Tel:+90 3123415994   www.hassoy.com.tr

J. Entomol. Res. Soc., 22(1): 01-12, 2020
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

The Potential of *Sympherobius pygmaeus* (Rambur, 1842) as a Biological Agent Against *Planococcus citri* (Risso, 1813) in Citrus Orchards

Miraç YAYLA[1]*   Adnan TUSUN[2]   Serdar SATAR[2]

[1]Biological Control Research Institute, Kışla Avenue 01321, Yüreğir, Adana, TURKEY
[2]Department of Plant Protection, Faculty of Agriculture,
Çukurova University, 01330, Adana, TURKEY
e-mails: *miracyayla01@gmail.com, adnantusun@gmail.com, hserhat@cu.edu.tr
ORCID IDs: [1]0000-0002-4896-5325, [2]0000-0003-4492-120X, [3]0000-0003-0449-205X

ABSTRACT

Planococcus citri (Risso) (Hemiptera: Pseudococcidae) is one of the most significant pests for especially citrus, crop plants and ornamental plants. Besides its worldwide importance, chemical control is a significant, major method to suppress the population of *P. citri* in Turkey. Moreover, biological control has become more important in recent years because of the increase in consumer consciousness about pesticides. For this reason, the population dynamic of *P. citri* and its predator, *Sympherobius pygmaeus* (Rambur) (Neuroptera: Hemerobiidae) in three different grapefruit orchards were studied. For the study, 10 trees were randomly chosen from each orchard and the individuals on the plants, 4 branches, and stem of each tree were counted to determine the population level. When *P. citri* population reached the peak level, approximately 500 *S. pygmaues* individuals were released to each orchard. Population levels of prey and predators were monitored for two years, with weekly intervals between May-October and one time for two weeks between December and April of the following year. As the result of the treatment process, it is observed that the predator *S. pygmaeus* succeeded in decreasing the population of *P. citri* under economic damage threshold level. As a result, it is demonstrated that the predator *S. pygmaeus* can be used as a biological agent against *P. citri* in integrated pest management programs as an environmentally friendly method.

Key words: Hemerobiidae, Neuroptera, mealybug, biological control, grapefruit, population.

Yayla, M. Tusun, A., & Satar, S. (2020). The potential of *Sympherobius pygmaeus* (Rambur, 1842) as a biological agent against *Planococcus citri* (Risso, 1813) in Citrus Orchards. *Journal of the Entomological Research Society,* 22(1), 01-12.

INTRODUCTION

Citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)is an important pest for many fruit trees and ornamental plants, especially for citrus in Turkey (Kansu & Uygun, 1980; Polat, Ulgenturk, & Kaydan, 2008; Uygun & Satar, 2008; Karacaoğlu & Satar, 2017). It feeds on fruits and twigs of citrus by sucking the sap and causes dark-colored sooty mold and fugacity (Karacaoğlu & Satar, 2017). It also opens the way to secondary pests like *Cryptoblabes gnidiella* Millière (Lepidoptera: Pyralidae). *P. citri* prefers Washington navel and Star ruby rather than Interdonata lemon and Satsuma mandarin. On the other hand, it cannot complete a generation on Okitsu Wase mandarin variety (Canbolat, 2016). The grapefruit fruits are in the tree as cluster, so it is a great harbor for mealybug as it is protected from unfavorable weather conditions such as extreme heat or low humidity and from various big predators such as *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) adult. Through this protection, insecticide cannot reach this fruit, and mealybug control can be unsuccessful on the grapefruit. Chemical and biological control are widely used methods for suppressing pests both in our country and around the world. There are various negative effects of chemical control on human health and environment such as residue on fruit, resistance development and having effect on non-target organisms; in order to be able to prevent and control such effects, biological control provides an alternative for farmers. Thirty native predators and parasitoids on *P. citri* in the citrus orchards of East Mediterranean Region, Turkey have been recorded until today. Moreover mealybug control in citrus orchards has been done both with natural enemies such as *C. montrouzieri* and *Leptomastix dactylopii* How. (Hymenoptra: Encyrtidae) which are commercially available for a long time (Soylu & Ürel, 1977; Kansu & Uygun, 1980; Türkyılmaz, 1986; Uygun & Satar, 2008; Yayla & Satar 2012; Karacaoğlu & Satar, 2017). Change in vegetation pattern and pesticide use pattern, especially in broad spectrum pesticide with Chloranicotinyl Insecticide (CNI) group instead of summer oil and cultural practices, have resulted in insufficient biological control tactics in grapefruit orchards because of the biology of mealybug on grapefruit, which is in abetter position for *P. citri* when compared to other citrus varieties (Canbolat & Satar, 2016). At this point, importance of alternative biological control agents increases to support current biological control tactics; in addition, the process may sometimes become a solution. *S. pygmaeus* is a promising natural enemy that controls mealybug. Easy production in laboratory, low labor requirement, and rapid growth puts the predator forward for this project (Yayla & Satar, 2012; 2013). Therefore, the biological control of *P. citri* determined by using *S. pygmaeus* under field conditions. For this aim, *S. pygmaeus* was released to citrus plantations to detect its efficiency on mealybug in field condition; population dynamics of both prey and the predator were carefully observed in the study.

MATERIALS AND METHODS

Production of sprouted potatoes

Potatoes used for the experiment were stored in a dark room at +4 ˚C. Then the potato tubers were washed with tap water and cleaned. The cleaned potato tubers were placed in the climatics room with 20 ± 4 ˚C temperature and at 60 ± 10% relative humidity condition. It was observed that, sprouts began to appear on the tubers in 30 to 45 days. Sprouts reaching about 4 to 5 cm in length were used for mass production of *P. citri*. Each time the process were started from new potatoes which were kept in the +4 ˚C This process continued during the 2014 and 2015.

Planococcus citri rearing

Planococcus citri were reared on sprouted potatoes in cages in a climate controlled room with 25 ± 2 ˚C temperature, at 60 ± 10% relative humidity and were waited for 16: 8 hours (light: dark) daily light period (Yayla & Satar, 2012). *P. citri* were obtained from the citrus orchards of Çukurova University, Faculty of Agriculture, Research and Implementation Farm and transformed into the pure culture by subculturing at the egg stage of P. citri several times on the sprouted potatoes (Fisher, 1963).

Sympherobius pygmaeus rearing

The Neuropteran adults were collected from *P. citri* infected shoots and fruit clusters through a Steiner funnel before rearing from 20 years old grapefruits orchards at Çukurova University, Faculty of Agriculture, Research and Implementation Farm. These individuals were classified according to their morphological characteristics under Leica S8APO binocular microscope in the laboratory, and *S. pygmaeus* individuals belonging to Hemerobiidae family were distinguished by following character combination; anterior radial tarce with at least 2-12 radial sectoral branches and nygmata absent (Oswald, 1993). Moreover as it is name their color commonly brownish or greyish compare to other Neuroptera family (New, 2001). The adults collect from were released on mealybugs which were reared on the sprouted potatoes, in a five liter plastics jars in the insect production rooms with 25 ± 2 ˚C and 16 h day light conditions. Each of the production unit have 5-10 sprouted potatoes and released *S. pygmaeus* adults in the unit finding enough place to continue its life. Before starting rearing process, sponges or fiber-like materials were placed in jars for individuals egg laying. After first generations the obtained adult were sent to Prof. Dr. Norm Penny at California University for identifications. After identification and several generation later the adult individuals were start to use in the experiment.

Population dynamic

Population studies were carried out in three different regions to investigate the activity of *S. pygmaeus* and its prey *P. citri* in the citrus orchards. Alata Horticultural Research Institute in Erdemli district of Mersin province, Subtropical Fruits Research and Application Center in Çukurova University and a farmer orchard in Erzin district of Hatay were selected for this aim and population dynamic of the predator and its prey were observed on randomly selected 10 trees within experimental area. The trees were selected from *S. pygmaeus released area* which have generally 40-50 citrus trees during the 2014-2015. *P. citri* population on four main branches at different directions and 10 fruits on each one of 10 trees for every week in each orchard was observed. Totally, 10 trunks, 40 branches and 100 fruits were examined in each orchard. At each sampling point, egg packs, nymph1+nymph2, nymph3 stages and adults of *P. citri* individuals were counted separately and population fluctuations were determined accordingly. When *P. citri* population reached a peak level, approximately 10 *S. pygmaeus* adults per tree were released into the trees. The released individuals did not caged at all. Larvae stages and *S. pygmaeus* adults were taken into account while determining the population by using Steiner funnel (Steiner, 1962) and/or through naked eyes. Population of prey and predator were recorded once a week from April 2014 to December 2015.

RESULTS AND DISCUSSION

Sympherobius pygmaeus suppressed *P. citri* populations in three grapefruit orchards successfully and showed that it is an effective predator on its prey. *P. citri* individuals that overwinter as adult or at 3rd Nymph stage started to lay egg package actively at end of April 2014 in the grapefruit orchard of Çukurova University Subtropical Fruits Research and Application Centre (Fig. 1). Mealybug reached its first peak on June 19th, 2014, with mean 0.30 egg packs/branches. However, when it reached the first peak, summer oil was applied to control for other citrus pests. As a result of this spraying, a decrease in *P. citri* population was observed. The pest reached to second and highest peak level on August 8th, 2014 as mean 5.18 Nymph 1st + 2nd/fruit and 1.23 Nymph 3rd/fruit. After *S. pygmaeus* individuals were released on the trees following population increase of *P. citri* on July 24th, 2014, mealybug population began to decrease in the next 15 to 20 days. It was observed that the larvae of the predator first fed on the egg packages. Then, seven adult individuals were caught in the field with Steiner funnel on September 11st, 2014. When the data were examined, it was determined that *P. citri* population was suppressed within two months after the releasing of the predator. *P. citri* population data in the first year was in line with the data of the second year.

In the second year, the pest reached the first peak on June 5th 2015 and mean 0.50 egg packages/trunk were counted. Mean 0.38 Nymph 1st + 2nd/fruit were counted on August 14th, 2015 as the second peak. *S. pygmaeus* were released on the 24th of July 2015 due to the increasing mealybug population. Five adults were caught with Steiner funnel on September 18th, 2015. *S. pygmaeus* adults were found in all samples taken from the field surveys for a whole year. For instance, two larvae were found on January and February in 2015. *P. citri* population was suppressed by *S. pygmaeus* individuals within 45 days after the release of *S. pygmaeus* in the second year (Fig. 1). When two years were compared, it was observed that the second year had lower population density except adult on trunk. A turbo atomizer without fan is used for spraying the insecticide in this farm. It couldn't be effective on 40-60 cm above ground, as there is no fan. So the insecticide couldn't reach the trunk and *P. citri* adults used the trunk as reservoir; crawlers could spread out from trunk to the upper parts of trees and keep the population active.

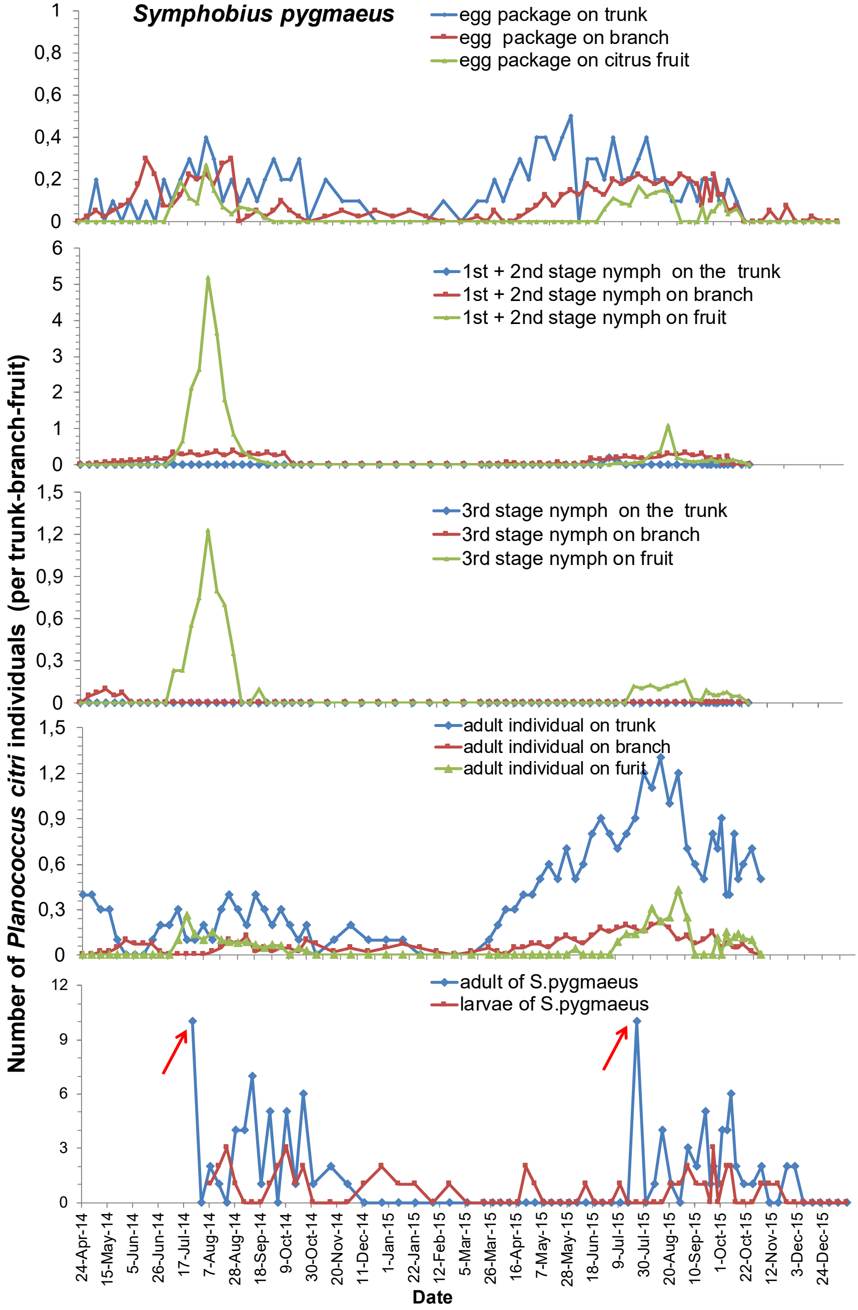


Fig. 1. Population fluctuation of *Sympherobius pygmaeus* and *Planococcus citri* grapefruit orchard in Çukurova University Subtropical Fruit Research and Application Center from April 2014 to December 2015 (arrow shows *S. pygmeous* releasing date).

Planococcus citri activity was observed as egg package, third nymph stage and adult individuals in the first week of May in 2014 at Alata Horticultural Research Institute in the Erdemli district of Mersin province. The mealybug reached its first and highest peak on May 17th, 2014, and were counted as mean 5.9 eggs packages/trunk; 29.7 Nymph 1st + 2nd/trunk, 7.4 Nymph 1st + 2nd/brunch; 6.20 Nymph 3rd/trunk, 1.48 Nymph 3rd/brunch. Second pick is on June 21st, 2014 as 24.5 Nymph 1st + 2nd/fruit, 9.2 Nymph 3rd/fruit, and 4.96 adult/fruit. *S. pygmaeus* individuals were released on June 21st, 2014 after the rapid increase in *P. citri* population and suppressed the pest. As a result of Steiner funnel sampling after the releasing on *P. citri* infected fruits, 54 larvae and 37 adult *S. pygmaeus* individuals were collected (Fig. 2).

Planococcus citri population in the second year was lower than the previous year. As can be seen in Figure 2, the pest reached its first peak on May 23rd, 2015 and mean 1.2 eggs were counted on the trunk. Nymph 1st + 2nd and Nymph 3rd were counted as approximately 1.12 and 0.75 individuals on fruit in June 2015, respectively. Adult individuals on fruits reached the highest level on May 30, 2015 and 1.7 adult individuals were observed on trunk. *P. citri* population began to decrease in 25-30 days following the releasing of *S. pygmaeus* on June 6th, 2015. Eight *S. pygmaeus* individuals were obtained from infected fruit samples with mealybug by using Steiner funnel in July 18th, five individuals were obtained in August 22nd, and eight individuals were obtained in September 10th. It was observed that *S. pygmaeus* individuals were active throughout the year except winter (Fig. 2). Although significantly high population density of the mealybug is observed in the first year, it was suppressed in the short term by *S. pygmaeus. S*econd year *P. citri* population didn't reach a high level as the predator settled in that area

When Figure 3 is examined, it can be seen that the pest reached its first peak in June 15th, 2014 and 3rd nymph stage on the trunk were observed in grapefruit orchard in Erzin. Hatay province. Nymph 1st + 2nd stages were determined to be 5.9 average on the trunk on the 6th of July 2014. 1.2 adult/trunk were counted on June 27th, 2014. Declines began in *P. citri* population in 20-35 days after releasing *S. pygmaues* on the 20th of July, 2014. *S. pygmaues* obtained with Steiner funnel was highest with seven individuals on October 5th, 2014. *P. citri* population was lower than the previous year according to the data in 2015. The first peak on *P. citri* population was 0.3 adult/trunk on May 17th, 2015 and 1.4 Nymph 3rd/trunk on July 5th, 2015. Maximum population level was 9.1 Nymph 1st + 2nd stages/trunk on July 19th, 2015. After releasing of *S. pygmaeus* on the 19th of July, 2015, *P. citri* population began to decrease in 20-30 days. *S. pygmaeus* individuals reached the peak on August 30th, 2015 with 5 individuals. *S. pygmaeus* population was low in both years, as there was an uncontrolled spraying against the disease and pests by farmers and untargeted natural enemies like *S. pygmaeus* were destroyed. Six peaks were detected in each grove in the months of May on main trunk, June, July, August and September on fruit and finally in October-November on fruit trunk during 2014 and 2015. Karacaoğlu & Satar (2017) reported that, there can be 4 generations of the same fruit in the same geographic area, but the number of generations changes according to climatic factors;

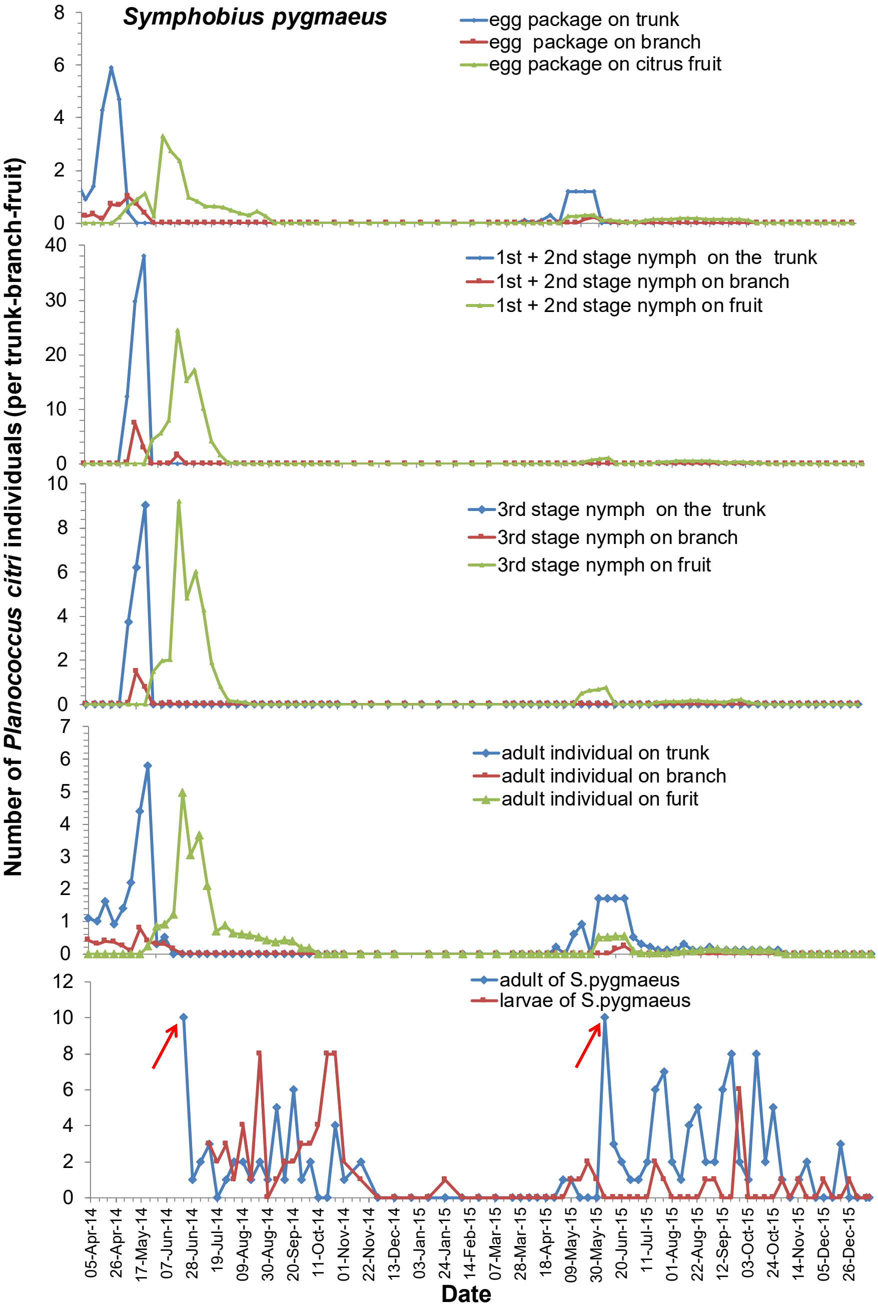


Fig. 2. Population fluctuation of *Sympherobius pygmaeus* and *Planococcus citri* in grapefruit orchard in Alata Horticultural Research Institute in Erdemli district of Mersin province in 2014 and 2015 (arrow shows *S. pygmeous* releasing date).

it is also reported that *P. citri* can give two to three generations in California (Ebeling, 1959); three generations in Texas (Harlan, Hart, Ingle, & Mayerdirk, 1977); eight generations in Palestine (Bodenheimer, 1951); 4-5 generations in Greece (Santorini, 1977); 5 generations in Spain (Martínez-Ferrer, Garcia-Marí, & Moles, 2003). Avidov & Harpaz (1969) reported that citrus mealybug can give six generations per year in coastal plain, 4-5 generations in hill region and up to 7 generation in upper Jordan Valley based on 8.2˚C developmental threshold and 698 days-degree for development of one generation. Karacaoğlu & Satar (2017) calculated 8.53 ˚C developmental threshold and 666.67 days-degree for one generation (from egg to egg stage). *P. citri* can generally hibernate the trunk of citrus tree till the end of April and at this point they give first generation on the trunk, hatched crawler feeding branch and leaf of the tree and moving up and settle under the calix when fruits reach 2-3 cm Ø (Figs. 1, 2 and 3).

In the project, not only *S. pygmaeus* but also other predators, Coleoptera and Neuroptera orders were collected. Seven species and one genus level in Coccinellidae family (Coleoptera), one species from Hemerebiidae, three species from Chrysopidae and Coniopterygidae family (Neuroptera) were identified (Table 1). *Chrysoperla carnea,* which is common predator species, was obtained at highest number followed by *S. pygmaeus.* All *C. carnea* individuals were adults and possibly came to feed on honeydew excreted by mealybug. Only a few individuals could be collected from *Nephus includens* which is a native and a mealybug specific predator. Erzin has the poorest species richness and lower predators than Adana and Alata (Erdemli).

Sympherobius pygmaeus is worldwide available but it is rather neglected by researchers, as the related literature is generally limited with taxonomic studies (Pröse, 1995; Bayram, 2008; Duelli, Bolt, & Henry, 2015). In our country, *S. pygmaues* was firstly detected by (Şengoca, 1979) in Kahramanmaraş province. In addition, Türkyılmaz (1984) reported that it feeds on mealybugs in citrus fruits in Antalya region.

According to the study of Türkyılmaz (1986) in citrus orchards in Antalya province, *S. pygmaues* population’s feed on *P*. citri increased in parallel with the increase in mealybug population and reached the highest level in July, which is similar to the findings of this study. Moreover, The researcher revealed that *S. pygmaues* is the most important predator of *P. citri* and also he indicated the population development between prey and predator. However, Bozdoğan, Özcan, Satar, & Tusun (2016) surveyed pasture, wooded, swamp, and heath land areas between 2011 and 2013 in Osmaniye and found that *S. pygmaeous* reached the peak level on May. It can resulted from the fact that the chosen areas were non-cultured.

Martínez-Ferrer et al. (2003) emphasized that *P. citri* population started to increase at end of June through mid of July, but the population decreased when predator and parasitoid occurred. Karacaoğlu (2016) also studied the relationship between *P. citri* population and the predator population in citrus fields in Aegean and Mediterranean regions. The study was carried out in 2013-2015 by using Steiner (Steiner, 1962) and visual control methods in citrus orchards. As a result of this study, 70 adult from *S. pygmaues* species belonging to Hemerobiidae family and 239 adult individuals from *S. fallax* species were identified. Study of Karacaoğlu (2016) revealed a direct relationship

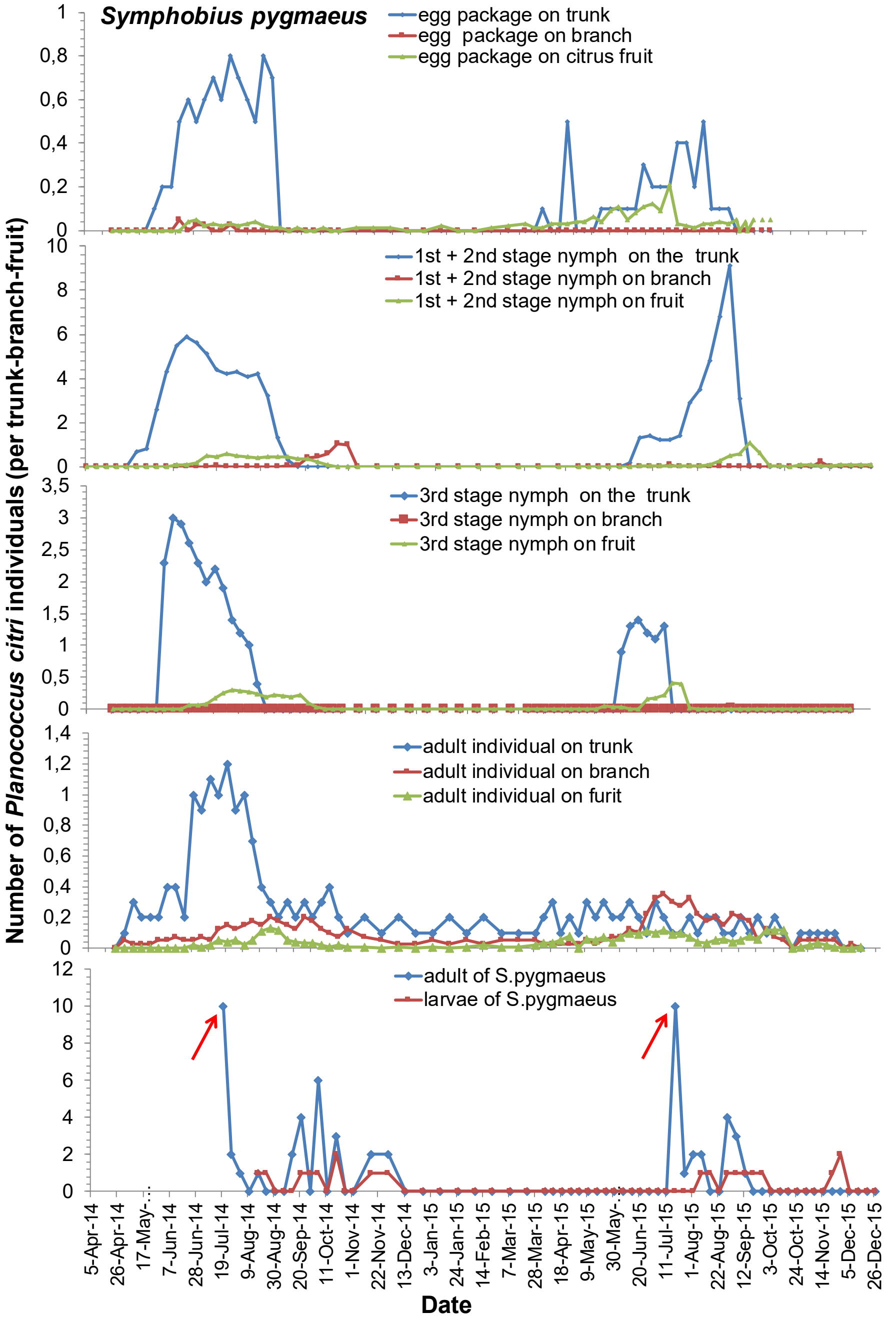


Fig. 3. Population fluctuation of *Sympherobius pygmaeus* and *Planococcus citri* in grapefruit orchard in Erzin district of Hatay province in 2014 and 2015 (arrow shows *S. pygmeous* releasing date).

between *S. pygmaues* and *S. fallax* with *P. citri* and showed the similarity between them in his study. *S. pygmaeus* has some advantages and disadvantages as a biological control. Its detection is very tricky as it is a good flyer. During the project, it was observed that it suppressed the pest successfully in another citrus orchard which is five kilometers away from project area. Commercial *Cryptolaemus montrouzieri* and parasitoid *Leptomastix dactylopii* are released to *P. citri* infected orchards by farmers every year as it cannot overwinter in our region. However, *S. pygmaeus* activity occurs in all seasons and is able to overwinter in our region. On the other hand Şimşek & Uygun (2016) determined that *S. pygmaeus* is very sensitive to insecticides. Increasing pesticide use affects it negatively and its population significantly decreases in the field condition. It proved that the predator is insufficient to control the pest at citrus orchards in regular pesticide usage process. In conclusion, it has high feeding capacity, short developmental time, high progeny, and overwintering ability in subtropical areas like Mediterranean; these features make *S. pygmaeus* an important candidate for biological solution of citrus mealybug but it should be produced in laboratory condition and should be released like other commercial natural enemies in order to obtain successful control. At that point, results of this research show that it functions as a solution in farm condition and can be used as an alternative in *P. citri* control.

Table 1. Coleoptera and Neuroptera predator species and numbers obtained by Steiner and visual control in three grapefruit orchards in Adana-Çukurova, Mersin-Alata and Hatay-Erzin from April 2014 to December 2015.

Order-Family	**Scientific name**	**Adana**	**Mersin**	**Hatay**
Coleoptera				
Coccinellidae	*Adalia bipunctata*	23	42	2
	Coccinella septempuntata	45	50	10
	Chilocorus bipustulatus	58	75	15
	Rodolia cardinalis	25	10	5
	Stethorus punctillum	21	15	5
	Stethorus gilvifron	15	12	0
	Nephus includens	10	12	0
	Scymnus spp.	36	62	15
Neuroptera				
Hemerobiidae	***Sympherobius pygmaeus***	**94**	**114**	**56**
Chrysopidae	*Chrysoperla carnea*	150	125	32
	Dichochrysa flavifrons	5	11	1
	Dichochrysa prasina	2	0	0
Coniopterygidae	*Conwentzia hageni*	32	16	0
	Semidalis aleyrodiformis	26	34	0
	Coniopteryx parthenia	15	5	0

ACKNOWLEDGEMENTS

This study is a part of first authors PhD thesis, carried out in Çukurova University. The authors thank to TAGEM for providing the funding of this project (TAGEM 2013 / AR-GE40). The manuscript was presented at IOBC-WPRS WG Congress “Integrated Control in Citrus Fruit Crops” Valencia (Spain) 25-27 Sept. 2017.

REFERENCES

Avidov, Z. & Harpaz, I. (1969). Plant Pest of Israel. Israel Universities Press, Jerusalem, 549p.

Bayram, Ş. (2008). Ankara’da Karaağaç (Ulmus glabraMill.)’da gal yapan yaprak bitlerinde avcı Coccinellidae (Coleoptera), Chrysopidae ve Hemerobiidae (Neuroptera) familyasına bağlı türler. *Ankara Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi*, 14(4), 386-393.

Bodenheimer, F.S. (1951). Citrus entomology in the Middle East. *W. Junk. The Hague, The Netherlands*.

Bozdoğan, H., Özcan, İ., Satar, A., & Tusun, S. (2016). Notes on the seasonal and ecological dynamics of some Hemerobiidae family Latreille, 1803 (Neuroptera: Hemerobiidae) species in Osmaniye province, East Mediterranenan Anatolia. *Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, 16(3), 484-494.

Canbolat, U. (2016). Farklı Turunçgil Türleri *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)’nin Bazı Biyolojik Özellikleri ile Parazitoidi *Leptomastix dactylopii* Howard (Hym.: Encyrtidae) Arasındaki İlişkiler. Yüksek Lisans Tezi (Master’s Thesis), Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana, 55s.

Duelli, P., Bolt, D., & Henry, C.S. (2015). Neuroptera of the Caucasian Republic of Georgia. *Entomological News*, 124(4), 229-244.

Ebeling, W. (1959). Mealybugs. Subtropik Fruit Pest. *University of California, Division of Agricultural Sciences,* 436p.

Fisher, T.W. (1963). Mass culture of Cryptolaemus and Zeptomastic-Natural enemies of citrus mealybug. *California Agriculture Experiment Station Bulletin,* 797, 38p.

Harlan, D.P., Hart, W.G., Ingle, S.J., & Mayerdirk, D.E. (1977). Citrus Mealybug: Population in the lower rio grande valley of Texas. *Journal of the Rio Grande Valley Horticultural Society*, 31, 33-36.

Kansu, İ., & Uygun, N. (1980). Doğu Akdeniz bölgesinde turunçgil zararlıları ile tüm savaş olanakları araştırılması. *Çukurova Üniversitesi Ziraat Fakültesi Yayınları 141 Bilimsel Araştırma ve İnceleme* 33, 69s.

Karacaoğlu, M. (2016) Akdeniz ve Ege bölgesi Turunçgil Bahçelerinde Unlubit Türlerinin Belirlenmesi ile Turunçgil Unlubiti *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)’nin Bazı Biyo-ekolojik Özellikleri Üzerine Araştırmalar. Doktora Tezi (PhD Dissertation), Çukurova Üniversitesi Fen Bilimleri Enstitüsü, 148s.

Karacaoğlu, M., & Satar, S. (2017). Bioecological characteristics of *Planococcus citri* Risso, 1813 (Hemiptera: Pseudococcidae) under constant and alternating temperatures 1. *Turkiye Entomoloji Dergisi-Turkish Journal of Entomology*, 41(2), 147-157.

Martínez-Ferrer, M.T., Garcia-Marí, F., & Moles, J.L.R. (2003). Population dynamics of *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) in citrus groves in Spain. *Integrated Control in Citrus Fruit Crops IOBC WPRS Bulletin*, 26(6), 149-162.

New, T. (2001). Introduction to the systematics and distribution of Coniopterygidae, Hemerobiidae, and Chrysopidae used in pest management. In P.K. McEwen, T.R New, A.E Whittington (Eds.). *Lacewings in the crop environment* (1st ed., pp. 6-28). Cambridge University Press, New York.

Oswald, J.D. (1993). Revision and cladistic analysis of the world genera of the family Hemerobiidae (Insecta: Neuroptera). *Journal of the New York Entomological Society,* 143-299.

Polat, F., Ulgenturk, S., & Kaydan, M.B. (2008). Developmental biology of citrus mealybug, *Planococcus citr*i (Risso) (Hemiptera: Pseudococcidae), on ornamental plants. In *Proceedings of the International Symposium on Scale Insect Studies*, 11, 177-184.

Pröse, H. (1995). Kommentierte Artenliste der Netzflügler Bayerns (Insecta: Neuropteroidea). *Beiträge zur bayerischen Entomofaunistik,* 1: 151-158.

Santorini, A.P. (1977). Etude de quelques caracteres de *Planococcus citri* (Risso) en Grece (Homoptera, Coccoidea, Pseudococcidae). *Fruits*, 10(32), 611-612.

Soylu, O.Z. & Ürel, N. (1977). Güney Anadolu bölgesi turunçgillerinde zararlı böceklerin parazitoit ve predatörlerinnin tesbiti üzerine araştırtmalar. *Bitki Koruma Bülteni*,17(2-4), 77-112.

Steiner, H. (1962). Methoden Zur Untersuchung Des Populationdinamik in Obstanlagen. *Entomophaga*, 7, 207-214.

Şengonca, Ç. (1979). Beitrag zur Neuropterenfauna der Türkei. *Nachrichtenblatt der Bayerischen Entomologen,* 28, 10-15.

Şimşek, V.M. & Uygun, N. (2016). Bazı tarımsal savaş ilaçlarının turunçgil bahçelerindeki önemli parazitoit ve predatörlere etkilerinin laboratuvar koşullarında araştırılması. *Türkiye Biyolojik Mücadele Dergisi,* 4(2), 141-154.

Türkyılmaz, N. (1984). Antalya ve yöresi turunçgil plantasyonlarında bulunan Neuroptera türleri, Tanımları, konukçuları ve etkinlik durumları üzerinde araştırmalar. *T.C. Tarım Orman ve Köyişleri Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü, Antalya Biyolojik Mücadele Araştırma Enstitüsü Müdürlüğü Araştırma Eserleri Serisi*, 2, 42.

Türkyılmaz, N. (1986). Antalya ve yöresi turunçgil plantasyonlarında ilk defa bulunan *Sympherobius pygmaeus* (Rambur) (Neuroptera: Hemerobiidae)'un konukçuları ve popülasyon dalgalanmaları üzerinde araştırmalar. *Türkiye 1. Biyolojik Mücadele Kongresi* 12-14 Şubat 1986 Adana, 363-370, 476s.

Uygun, N. & Satar, S. (2008). The current situation of citrus pests and their control methods in Turkey. *IOBC-WPRS Bulletin*, 38, 2-9.

Yayla, M. & Satar, S. (2012). Temperatura influence on development of *Sympherobius pygmaeus* (Rambur) (Neuroptera: Hemerobiidae) reared on *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae). *Türkiye Entomoloji Dergisi*, 36(2), 11-22.

Yayla, M. & Satar, S. (2013). Effect of different food on adult productivity of *Sympherobius pygmaeus* (Rambur) (Neuroptera: Hemerobiidae. *Integrated Control in Citrus Fruit Crops IOBC-WPRS Bulletin*, 95, 153-160.

Received: February 15, 2019 *Accepted: December 03, 2019*

J. Entomol. Res. Soc., 22(1): 13-40, 2020 *Research Article*
Print ISSN:1302-0250 *Online ISSN:2651-3579*

New Data on Little-known Beetle Families and a Summary of the Project: Coleoptera of the Eastern Beskid Mts (Western Carpathians, Poland)

Artur TASZAKOWSKI[1] Natalia KASZYCA-TASZAKOWSKA[2]
Wojciech T. SZCZEPAŃSKI[3] Lech KARPIŃSKI[4*]

[1,2]Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, POLAND
[3]Kościelna 34B/19, 41-103 Siemianowice Śląskie, POLAND
[4]Museum and Institute of Zoology, Polish Academy
of Sciences, Wilcza 64, 00-679 Warsaw, POLAND
e-mails: [1]artur.taszakowski@us.edu.pl, [2]nkaszyca@us.edu.pl,
[3]szczepanski.w@interia.pl, [4*]lkarpinski@miiz.waw.pl
ORCID IDs: [1]0000-0002-0885-353X, [2]0000-0001-5031-6167,[3]0000-0003-0858-519X,
[4*]0000-0002-5475-3732

ABSTRACT

New data on the distribution of the beetles from the Eastern Beskid Mountains are given. A list of 100 species from 37 families is presented. New localities of some species rare in Poland such as *Elodes johni* Klausnitzer, 1980, *Lyctus pubescens* Panzer, 1792, *Malachius scutellaris* Erichson, 1840, *Osphya bipunctata* (Fabricius, 1775), *Peltis grossa* (Linnaeus, 1758), *Pyrochroa serraticornis* (Scopoli, 1763) and *Soronia punctatissima* (Illiger, 1794) are also given. Eight taxa were recorded in the Eastern Beskid Mts for the first time: *Agabus congener* (Thunberg, 1794), *Amphotis marginata* (Fabricius, 1781), *Dolichosoma lineare* (Rossi, 1794), *Dytiscus dimidiatus* Bergsträsser, 1778, *Glischrochilus quadrisignatus* (Say, 1835), *Ips cembrae* (Heer, 1836), *Orthoperus nigrescens* Stephens, 1829 and *Stephostethus alternans* (Mannerheim, 1844). A comprehensive summary of our entire research, which was conducted in the Eastern Beskid Mts between 1999 and 2017, is provided with an emphasis on species protected by Polish law or included in various lists of endangered species.

Key words: Biodiversity, endangered species, faunistics, primeval forest relics.

Taszakowski, A., Kaszyca-Taszakowska, N., Szczepański, W.T. & Karpiński, L. (2020). New data on little-known beetle families and a summary of the project: Coleoptera of the Eastern Beskid Mts (Western Carpathians, Poland). *Journal of the Entomological Research Society,* 22(1), 13-40.

INTRODUCTION

Beetles (Coleoptera) are the largest and the most diverse group of insects and they inhabit nearly all habitats. In Poland, they are among the richest groups in respect to the number of species. To date, more than 6000 species have been recorded in the country and new species are constantly being discovered (Biodiversity Map, 2018). Although mountainous areas only cover approx. 8% of the area of Poland, including about 6% of strictly mountain areas, they are very attractive in terms of species richness due to their huge diversity of environments, vegetation zones and climatic conditions as well as a high level of preservation (Knutelski & Tykarski, 2010). Extensive literature data show that beetles have been of considerable interest in these areas since the beginning of the 19th century. Nevertheless, the knowledge of beetles in particular mountain regions is both insufficient and very unequal. For example, the Bieszczady, Pieniny and Beskidy Mountains have been relatively well investigated; however, many of the remaining areas have not been comprehensively studied. Therefore, the currently published data only concern some groups of Coleoptera from specific regions (Knutelski & Tykarski, 2010).

An analysis of the data contained in the Catalogue of Fauna of Poland, which was conducted by Knutelski & Tykarski (2010), showed that 5120 species of beetles (approx. 85.5% of the Polish fauna) are found in the mountainous areas. Among the three main mountain ranges of Poland, the largest number of beetles (4570 species) is found in the Carpathian Mountains due to the greatest diversity of habitats. In the subregion of the Eastern Beskid Mts, approx. 3500 species have been found to date (Knutelski & Tykarski, 2010). Therefore, while the state of the knowledge on the Coleoptera fauna in this area can be considered to be relatively good, it is still quite unequal. The high number of recorded species is to a large degree the result of the intensive research conducted by Trella (e.g. 1923a; 1923b; 1925) in the environs of Przemyśl in the early 20th century. However, recently collected information on distribution of beetles in this zoogeographical region has been limited mainly to the data that were published by a group of the researchers from the Department of Zoology of the University of Silesia (e.g. Karpiński, Taszakowski, & Szczepański, 2015; Szczepański, Taszakowski, & Karpiński, 2015a; Szczepański, Taszakowski, Karpiński, & Kubusiak, 2015c; Szczepański, Taszakowski, Karpiński, & Tomecka, 2015d; Kaszyca & Taszakowski, 2017; Taszakowski, Kaszyca, & Mazur, 2017a; Taszakowski, Morawski, Szołtys, & Szczepański, 2017b; Taszakowski, Kaszyca, & Szołtys, 2018; Taszakowski, Masłowski, Karpiński, Szczepański, & Szołtys, 2019), which was gathered into a Biodiversity Map (2018). As a consequence, the area of the Low Beskid Mts can still be considered to be a “blank spot” on the map of Poland (Gil & Melke, 2017; Taszakowski et al, 2017b).

This paper is part of a series presenting the results of the faunistic research on the beetles in the Eastern Beskid Mts, which were conducted intensively in 2010-2015. Therefore, the present study aims to supplement the faunistic data on many species not included in the above-mentioned works as well as to summarise the results of the entire field research project conducted in 1999-2017.

MATERIAL AND METHODS

According to the division of Poland that is used in the Catalogue of Fauna of Poland (Burakowski, Mroczkowski, & Stefańska, 1973), the research area is located in the Eastern Beskid Mountains (Beskid Wschodni). It is a large zoogeographical region (about 8670 km^2) in south-eastern Poland (Fig. 1). In terms of the physical-geographical division of Poland (Kondracki, 2013), this region is situated in two provinces in the Carpathian megaregion - the Western Carpathians, which includes Western and Northern Subcarpathia and the Eastern Carpathians, which includes Eastern Subcarpathia. The study area did not cover the entire area of the Eastern Beskidy Mts, only a part that is located in three mesoregions - the Ciężkowickie Foothills (Pogórze Ciężkowickie), the Jasielskie Foothills (Pogórze Jasielskie), and the Low Beskids (Beskid Niski). In this paper, we use the names of the regions that are used in the Catalogue of Fauna of Poland when we refer to our data in the Eastern Beskid Mountains. However, we treated separately the Low Beskids, which is a mountain range that forms a transition zone between the Eastern and Western Beskids (Kondracki, 2013). In this way, we wanted to highlight the specificity of the fauna of the region, which is at a relatively low altitude and has many mountain passes. Recent studies have shown that they constitute a very important Transcarpathian route (Ondavian migratory route) for the migration of small fauna from the south to the north, between the Pannonian Basin, which includes the Ondava Upland and the drainage basin of the San and Wisłoka Rivers (Taszakowski & Gorczyca, 2018).

Research plots (Fig. 1) were selected in the following locations (in alphabetical order): Bednarka [EA20], Dobrynia [EV39], Libusza [EA10], Lipinki [EA20], Lisów [EA21], Małastów [EV18], Nowy Żmigród [EV39], Rozdziele [EV19], Wójtowa [EA20], Załęże [EV39] and in the Low Beskids: Bartne [EV28, EV29], Bednarka [EV29], Blechnarka [EV17], Folusz [EV29], Jaśliska [EV57], Kąty [EV39], Krempna [EV38], Mrukowa [EV39], Nieznajowa [EV28], and Wysowa-Zdrój [EV17].

The material was primarily collected during the growing seasons. Due to the mild winters, it was also possible to sample overwintering insects. The most effective standard methods for collecting beetles such as attracting them to artificial light sources (with yellow and ultraviolet light), shaking them down from trees and shrubs into an entomological umbrella, sweep netting (grasslands and ecotone zones), sifting plant litter using an entomological sieve and Winkler-Moczarski eclector, and analyses of the immature stages and feeding galleries found in inhabited wood material were used during the field research. The beetles were collected in different plant communities [the nomenclature after Matuszkiewicz (2013)] (Fig. 2), e.g. Carpathian alder forest (*Alnetum incanae*), Carpathian beech forest (*Dentario glandulosae-Fagetum*) and its bordering shrub vegetation (*Rhamno-Prunetea*) (mainly in the Low Beskids), linden-oak-hornbeam forest (*Tilio-Carpinetum*) (Bednarka env.), subxerothermic plant communities (Dobrynia env.) and from meadows, pastures or shrubs that were slowly overgrown.

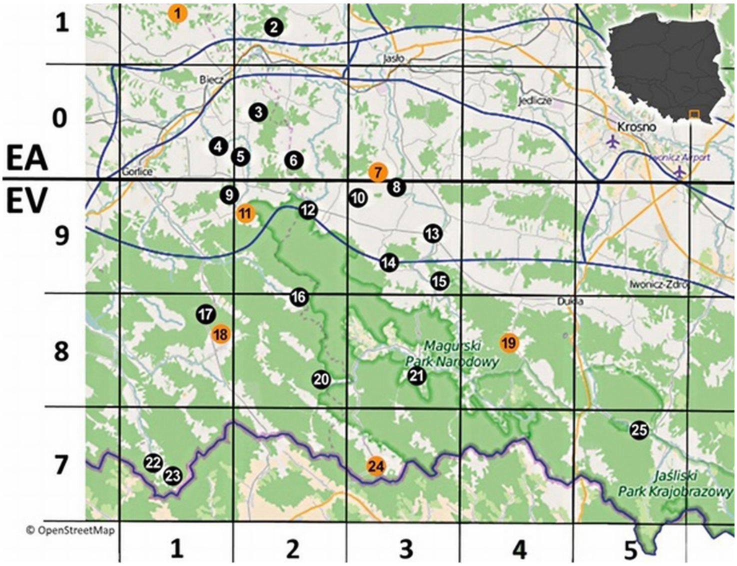


Fig. 1. Research sites: 1-Ołpiny, 2-Lisów, 3-Wójtowa, 4-Libusza, 5-Lipinki, 6-Bednarka (a), 7-Wola Dębowiecka, 8-Załęże, 9-Rozdziele, 10-Dobrynia, 11-Wapienne, 12-Bednarka (b) and Folusz, 13-Nowy Żmigród, 14-Mrukowa, 15-Kąty, 16-Bartne, 17-Małastów, 18-Gładyszów, 19-Chyrowa, 20-Nieznajowa, 21-Krempna, 22-Wysowa-Zdrój, 23-Blechnarka, 24-Ożenna, and 25-Jaśliska. Black circles - the sites on which the material used in this article was collected, orange circles - the sites on which the material was presented only in previous publications from the series. Lines and numbers of black color constitute the UTM grid, blue lines represent the boundaries of the mesoregions; © authors OpenStreetMap.

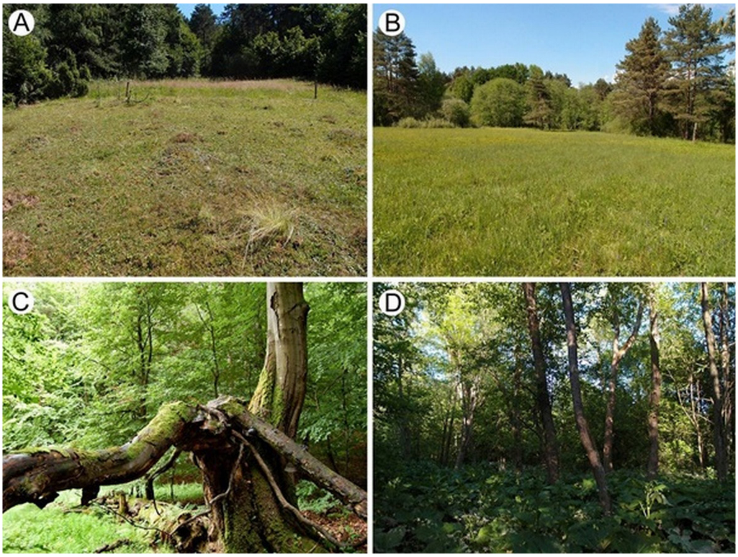


Fig. 2. Characteristic habitats; A-subxerothermic plant communities in Bartne; B-wet meadow in Wysowa-Zdrój; C-Carpathian beech forest in Bartne; D-Carpathian alder forest in Jaśliska.

The nomenclature for the beetles was adopted from the Catalogue of Palearctic Coleoptera (Löbl & Smetana, 2003; 2004; 2006; 2007; 2008; 2010; 2011; 2013). The specimens are preserved in the collections of the Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice (IoBUS), the Upper Silesian Museum in Bytom (USMB) and in the collections of the authors.

The specimens were imaged using a Leica M205C stereo microscope with a Leica DFC495 digital camera and the Leica application suite 4.9.0 software.

The potential number of species was estimated using the Chao1 formula (Chao & Bunge, 2002; Gotelli & Colwell, 2011).

$$S_{Chao1} = S_{obs} + (f_1^2 / 2f_2)$$

Where:

S_{Chao1} - potential number of species,

S_{obs} - empirical number of species,

f_1 - number of species represented by one specimen in the collection,

f_2 - number of species represented by two specimens in the collection.

RESULTS

The following list contains 100 species that belong to 37 beetle families. The species that were recorded in the Eastern Beskid Mountains for the first time are indicated by an asterisk (*).

Adephaga Schellenberg, 1806

Dytiscidae Leach, 1815

***Acilius canaliculatus* (Nicolai, 1822)**

Wysowa-Zdrój [EV17], 2007, 1 ex.

***Agabus congener* (Thunberg, 1794) ***

Libusza [EA10], 22.07.2006, 1 ex.

The species is widely distributed in the northern part of the Holarctic. In Poland, it occurs throughout the country in both the lowlands and mountains. Although *Agabus congener* primarily inhabits small water reservoirs that are overgrown with grasses and sedges, it is sometimes also found in the coastal zone of lakes and ponds (Burakowski, Mroczkowski, & Stefańskal, 1976).

***Agabus melanarius* Aubé, 1837**

Libusza [EA10], 22.07.2006, 1 ex.; Wysowa-Zdrój [EV17] 01.05.2016, 1 ex.

***Dytiscus marginalis* Linnaeus, 1758**

Lipinki [EA20], 2007, 2 exx.

***Dytiscus dimidiatus* Bergsträsser, 1778 ***

Lipinki [EA20], 2009, 2 exx.

This is a common species that is distributed over almost whole Europe, Asia Minor, Transcaucasia, Iran, Syria and Central Asia. In Poland, it is known from numerous scattered localities. It occurs in various types of waters, both standing and flowing, that are abundantly overgrown with plants (Burakowski et al, 1976).

***Ilybius fuliginosus* (Fabricius, 1792)**

Załęże [EV39], 07.08.2009, 1 ex.

***Platambus maculatus* (Linnaeus, 1758)**

Libusza [EA10], 10.08.2009, 1 ex.

***Rhantus suturalis* (Macleay, 1825)**

Załęże [EV39], 07.08.2009, 2 exx.

Polyphaga Emery, 1886

Elateriformia Crowson, 1960

Scirtoidea Fleming, 1821

Scirtidae Fleming, 1821

***Contacyphon palustris* C.G. Thomson, 1855**

Libusza [EA10], scrubs (*Rhamno-Prunetea*), 15.05.2013, 4 exx.; Nieznajowa [EV28], at light (UV), 26.07.2015, 1 ex.

***Contacyphon coarctatus* Paykull, 1799**

Bartne [EV28], scrubs, 11.07.2013, 2 exx.

***Elodes minuta* (Linnaeus, 1767)**

Libusza [EA10], scrubs, 15.05.2013, 1 ex.

***Elodes johni* Klausnitzer, 1975**

Bartne [EV29], scrubs, 11.07.2013, 2 exx.

Elodes johni was relatively recently described from Germany and to date, it is only known from a few countries, primarily in Central Europe (Burakowski, Mroczkowski & Stefańska, 2000). In Poland, it was recorded for the first time from the Bieszczady Mts by Borowiec and Kania (1996). In the past, it was probably misidentified with the common *Elodes minuta* (Linnaeus, 1767). Currently, after the revision of specimens, it is recorded from four zoogeographical regions. In the Eastern Beskidy Mts, it is known from several localities (Biodiversity Map, 2018). The species is found on banks of streams and at forest edges (Burakowski et al, 2000).

***Microcara testacea* (Linnaeus, 1767)**

Blechnarka [EV17], olszyna, 21.05.2014, 1 ex.

***Scirtes hemisphaericus* (Linnaeus, 1767)**

Libusza [EA10], at light, 08.08.2013, 1 ex.

Byrrhoidea Latreille, 1804

Byrrhidae Latreille, 1804

***Byrrhus pilula* (Linnaeus, 1758)**

Dobrynia [EV39], subxerothermic plant community, 12.05.2015, 1 ex.

***Cytilus sericeus* (Forster, 1771)**

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*) 12.05.2015, 2 exx.

Elateroidea Leach, 1815

Omalisidae Lacordaire, 1857

***Omalisus fontisbellaquaei* Geoffroy, 1785**

Nieznajowa [EV28], scrubs, 26.07.2015, 1 ex.

The species was previously recorded also in two other localities (Szczepański, Karpiński & Taszakowski, 2013).

Lampyridae Latreille, 1817

***Lamprohiza splendidula* (Linnaeus, 1767)**

Nieznajowa [EV28], at light (UV), 26.07.2015, 1 ex.

***Phosphaenus hemipterus* (Goeze, 1777)**

Jaśliska [EV57], scrubs (*Rhamno-Prunetea*), 06.06.2015, 1 ex.

Both firefly species were previously recorded in Szczepański & Karpiński (2013) and Szczepański et al (2015d).

Bostrichiformia Forbes, 1927

Bostrichoidea Latreille, 1802

Dermestidae Latreille, 1804

***Anthrenus scrophulariae* (Linnaeus, 1758)**

Libusza [EA10], scrubs, 15.05.2013, 2 exx.

***Attagenus pellio* (Linnaeus, 1758)**

Libusza [EA10], 2008, 1 ex.

Dermestes laniarius **Illiger, 1801**

Libusza [EA10], 03.06.2010, 1 ex.; 20.07.2013, 1 ex.

Dermestes lardarius **Linnaeus, 1758**

Libusza [EA10], 2008, 1 ex.; riverbank, 29.04.2013, 1 ex.

Dermestes murinus **Linnaeus, 1758**

Lipinki [EA20], subxerothermic plant community, 21.04.2014, 1 ex.

Bostrichidae Latreille, 1802

Lyctus pubescens **Panzer, 1792**

Libusza [EA10] 2006, 1 ex.

A rare species, which is distributed in southern and central Europe, the Caucasus and Asia Minor (Burakowski, Mroczkowski, & Stefańska, 1986a). In Poland, it is known from nine zoogeographical regions (Biodiversity Map, 2018). In the Eastern Beskidy Mts, it is known only from the Przemyśl env. (Trella, 1925). It inhabits trees and especially prefers oaks, which mainly grow in wet and shady places. Adults lead a secretive way of life by hiding in wood crevices during the day. However, specimens have sometimes been found on freshly barked or injured trees (Burakowski et al, 1986a).

Ptinidae Latreille, 1802

Ptilinus pectinicornis **(Linnaeus, 1758)**

Libusza [EA10], woodshed, 2006, 3 exx.

Cucujiformia Lameere, 1938

Lymexyloidea Fleming, 1821

Lymexylidae Fleming, 1821

Elateroides dermestoides **(Linnaeus, 1761)**

Libusza [EA10], 2007, 1 ex.; Wysowa-Zdrój [EV17], scrubs (*Rhamno-Prunetea*), 18 May 2013, 2 exx.; scrubs (*Rhamno-Prunetea*), shaken down, 01.05.2014, 2 exx.; meadow, 01.05.2014, 2 exx.

Cleroidea Latreille, 1802

Trogossitidae Latreille, 1802

Peltis grossa **(Linnaeus, 1758) (Fig. 3A)**

Jaśliska [EV57], Carpathian alder forest (*Alnetum incanae*), 06.06.2015, 1 ex.

The species is widespread in the forested areas of Fennoscandia, Karelia and Siberia and in the primeval forests of central and southern Europe. In Poland, it is rarely recorded and is primarily known from the southern part of the country (Burakowski et al, 1986a). Recent records come from the Białowieża Primeval Forest (Kubisz & Szwałko, 1991), the Świętokrzyskie Mts (Byk, 2007), Roztocze (Papis & Mokrzycki, 2015) and the Bieszczady Mts (Holly, 2016). In the Eastern Beskidy Mts, it is only known from the Przemyśl env. (Trella, 1923b).

Peltis grossa is a relatively rare saproxylic beetle. Its larvae develop in the soft, decaying wood of standing or fallen trees (both coniferous and deciduous) that are infested with fungi (Kolibáč, 2013; Procházka, Kment, Németh, & Kolibáč, 2017). The species is considered to be an indicator species of natural forest habitats (Müller et al, 2005).

Cleridae Latreille, 1802

Thanasimus formicarius (Linnaeus, 1758)

Libusza [EA10], 20.06.2005, 1 ex.; scrubs, 04.06.2015, 1 ex.; dead wood, 15.05.2013, 2 exx.; Wysowa-Zdrój [EV17], wood pile, 02.05.2014, 2 exx.

Tillus elongatus (Linnaeus, 1758)

Libusza [EA10], 20.06.2005, 1 ex.

Trichodes apiarius (Linnaeus, 1758)

Bartne [EV29], meadow, 09.07.2013, 1 ex.; Carpathian beech forest (*Dentario glandulosae-Fagetum*), 10.07.2013, 1 ex.; Bednarka [EV29], Carpathian beech forest (*Dentario glandulosae-Fagetum*), 29 .07.2014, 2 exx.; Libusza [EA10], scrubs, 17.08.2012, 1 ex.; 20.06.2005, 1 ex.; 04.08.2009, 1 ex.; Wysowa-Zdrój [EV17], scrubs, 03.08.2011, 1 ex.; Carpathian beech forest (*Dentario glandulosae-Fagetum*), 11.07.2011, 1 ex.

Dasytidae de Castelnau, 1840

Dasytes caeruleus (De Geer, 1774)

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 16.05.2013, 1 ex.; Dobrynia [EV39], subxerothermic plant community, 22.07.2013, 1 ex.

Dasytes fusculus (Illiger, 1801)

Dobrynia [EV39], subxerothermic plant community, 16.05.2013, 1 ex.; Libusza [EA10] meadow, 20.05.2014, 1 ex.; dead wood, 30.04.2014, scrubs, 20.04.2014., 1 ex.; Lipinki [EA20], subxerothermic plant community, 21.04.2014, 1 ex.; Wysowa-Zdrój [EV17] scrubs, 17.05.2013, 1 ex., 21.05.2014 1 ex.

Dasytes niger (Linnaeus, 1761)

Dobrynia [EV39], subxerothermic plant community, 16.05.2013, 1 ex. Libusza [EA10], scrubs, 10.06.2013, 1 ex.; Mrukowa [EV39], meadow, 13.06.2013, 1 ex.; Wysowa-Zdrój [EV17], wet meadow, 27.07.010, 1 ex.

Dolichosoma lineare **(Rossi P., 1794) ***

Bartne [EV29], meadow, 11.07.2013, 1 ex.; scrubs, 10.07.2013, 1 ex.; Libusza [EA10], scrubs, 12.06.2013, 1 ex.; subxerothermic plant community, 15.06.2013, 1 ex.; Nowy Żmigród [EV39], meadow, 19.05.2013, 3 exx.

The species is widespread in Europe; it reaches as far as Transbaikal and Siberia to the east. In Poland, it is probably distributed throughout the entire country, but it still has not been recorded from several regions (Burakowski et al, 1986a). Adults usually occur in open areas such as grasslands or wastelands where they are frequently observed.

Malachiidae Fleming, 1821

Anthocomus fasciatus **(Linnaeus, 1758)**

Libusza [EA10], herbs community, 16.06.2013, 1 ex.

Axinotarsus marginalis **(Laporte, 1840)**

Libusza [EA10], meadow, 12.08.2014, 1 ex.

Axinotarsus ruficollis **(A.G. Olivier, 1790)**

Lipinki [EA20], subxerothermic plant community,12.06.2013, 1 ex.

Charopus flavipes **(Paykull, 1798)**

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 13.06.2014, 1 ex.; Dobrynia [EV39], subxerothermic plant community, 16.06.2013, 1 ex.

Malachius bipustulatus **(Linnaeus, 1758)**

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 13.06.2013, 1 ex.; Libusza [EA10], meadow, 10.06.2013, 1 ex.; meadow, 15.05.2013, 1 ex.; Mrukowa [EV39], scrubs, 13.06.2013, 1 ex.; Wójtowa [EA20], scrubs (*Rhamno-Prunetea*), 16.05.2013, 1 ex.; Wysowa-Zdrój [EV17], wet meadow, 01.05.2014, 1 ex.; scrubs, 23.05.2011, 1 ex.

Malachius scutellaris **Erichson, 1840 (Fig. 3B)**

Libusza [EA10]; scrubs, 15.05.2013, 2 exx.; Wysowa-Zdrój [EV17], scrubs, 17.05.2013, 1 ex.

This in one of the rarest representatives of the genus *Malachius* Fabricius, 1775 in Poland. Until 1986, it was only known from six regions: the Baltic Coast, Lower Silesia, the Kraków-Wieluń Upland, the Sandomierska Lowland, the Western Beskidy Mts and the Pieniny Mts (Burakowski et al, 1986a). In the Eastern Beskidy Mts, it was recorded by H. Szołtys for the first time in 1984 (Biodiversity Map, 2018). In the neighbouring region - the Western Beskidy Mts - its presence was recently confirmed by Kubisz (1990) and Szarfaniec, Szarfaniec, & Mazur (2010), and later, it was collected in the Białowieża Primeval Forest (Borowiec, Kania, & Wanat, 1992) and the Mazovian Lowland (Marczak, Borowski, & Jędryczkowski, 2016) for the first time.

Malachius scutellaris is mainly distributed in the central and eastern part of Europe. In Asia, it was only recorded in Syria (Mayor, 2007). The species is considered to be rare in its entire range (Szarfaniec et al, 2010). Its biology is still poorly known. The larvae are predators and prey on immature stages of other insects. Adults are palynivores and they prefer warm slopes that are overgrown by *Barbarea* spp. and *Isatis* spp. (Kolibáč, Majer, & Švihla, 2005; Szarfaniec et al, 2010).

Cucujoidea Latreille, 1802

Kateretidae Erichson, 1846

***Brachypterus urticae* (Fabricius, 1792)**

Bednarka [EV29], scrubs, 11.07.2013, 1 ex.

***Kateretes pedicularius* (Linnaeus, 1758)**

Rozdziele [EV19], scrubs, 01.05.2013, 1 ex.

Nitidulidae Latreille, 1802

***Amphotis marginata* (Fabricius, 1781) ***

Libusza [EA10], sieve analysis, 22.03.2014, 1 ex.

The species is widespread in Europe. In Poland, it has rarely been collected and is known from 14 zoogeographical regions (Lasoń & Bidas, 2010; Biodiversity Map, 2018).

Amphotis marginata is a myrmecophilous species. Adults lead a cryptic life in their relationship with the ant species - *Lasius fuliginosus* (Latreille, 1798). The beetles use tactile cues to mimic the begging behaviour of ants and, if an ant is fooled, this will solicit the regurgitation of a liquid food drop that is offered to the beetle (Hölldobler & Wilson, 1990).

***Cychramus luteus* (Fabricius, 1787)**

Wysowa-Zdrój [EV17], meadow, 23.07.2010, 1 ex.

***Cyllodes ater* (Herbst, 1792)**

Wysowa-Zdrój [EV17], 27.08.2009, 1 ex.

***Glischrochilus quadrisignatus* (Say, 1835) ***

Bednarka [EV29], Carpathian beech forest (*Dentario glandulosae-Fagetum*), 23.07.2013, 1 ex.

The species is widespread throughout North America, where it is listed as a pest on fruit plantations and corn crops. It was brought to Europe with imported vegetables and fruits in the middle of the 20th century. Since then, its range is gradually increasing and it is currently distributed in most European countries. In Poland, the species was recorded in 1990 (Lasoń, 1999b) for the first time, and currently, it is known from 12 zoogeographical regions (Biodiversity Map, 2018).

In natural conditions, it mainly feeds on the fermenting sap of deciduous tree trunks and on decaying mushrooms. The species has become adapted to the environments near human settlements (Lasoń, 1999a).

Omosita depressa **(Linnaeus, 1758)**

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 16.05.2013, 2 exx.

Pocadius adustus **Reitter, 1888**

Lipinki [EA20], subxerothermic plant community, 24.05.2014, 1 ex.

Pocadius ferrugineus **(Fabricius, 1775)**

Dobrynia [EV39], subxerothermic plant community,12.05.2015, 1 ex.

Soronia grisea **(Linnaeus, 1758)**

Libusza [EA10], dead wood, 22.10.2013, 1 ex.

Soronia punctatissima **(Illiger, 1794) (Fig. 3C)**

Libusza [EA10], 2006, 1 ex.

Although the species is widespread in Europe, it has rarely been collected in Poland. It is known from single plots in 13 zoogeographical regions (Burakowski, Mroczkowski & Stefańska, 1986b; Lasoń, 1997; 1999a; Lasoń & Bidas, 2010; Lasoń & Miłkowski, 2011), which are mainly located in the southern and western parts of the country. The species is usually found under wet and rotten bark soaked by leaking tree sap or in similar habitats such as composts (Burakowski et al, 1986b).

Silvanidae Kirby, 1837

Uleiota planatus **(Linnaeus, 1761)**

Blechnarka [EV17], wood pile, 20.08.2010, 1 ex.; Libusza [EA10], dead wood, 30.04.2013, 1 ex.; dead wood, 15.05.2013, 2 exx.

Silvanus bidentatus **(Fabricius, 1792)**

Libusza [EA10], dead wood, 30.04.2013, 1 ex.; scrubs, 15.05.2013, 1 ex.

Cucujidae Latreille, 1802

Cucujus cinnaberinus **(Scopoli, 1763)**

Bednarka [EV29], Carpathian beech forest (*Dentario glandulosae-Fagetum*), under bark of *Acer pseudoplatanus*, 01.03.2014, 1 ex.

Phalacridae Leach, 1815

Olibrus bicolor **(Fabricius, 1792)**

Libusza [EA10], scrubs, 15.05.2013, 1 ex.

Erotylidae Latreille, 1802

Tritoma bipustulata Fabricius, 1775

Wysowa-Zdrój [EV17], 30.04.2014, 1 ex.

Byturidae Jacquelin du Val, 1858

Byturus ochraceus (Scriba, 1790)

Bednarka [EV29], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 16.05.2013, 1 ex.; Libusza [EA10], meadow, 16.06.2013, 1 ex.; riverbank, 29.04.2013, 1 ex., scrubs, 12.06.2014, 1 ex.

Byturus tomentosus (De Geer, 1774)

Bartne [EV29], Carpathian beech forest (*Dentario glandulosae-Fagetum*), 10.07.2013, 1 ex.; Bednarka [EV29], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 16.05.2013, 1 ex.; Libusza [EA10], riverbank, 29.04.2013, 1 ex.; scrubs, 15.05.2013, 2 exx.; scrubs, 10.06.2013, 1 ex.; 20.07.2013, 1 ex.; Rozdziele [EV19], scrubs, 01.05.2013, 1 ex.; Wysowa-Zdrój [EV17], meadow, 02.05.2014, 1 ex.

Endomychidae Leach, 1815

Endomychus coccineus (Linnaeus, 1758)

Bartne [EV29], Carpathian beech forest (*Dentario glandulosae-Fagetum*), 9.07.2013, 2 exx.; scrubs, 11.07.2013, 1 ex.; Libusza [EA10], 08.08.2008, 1 ex.; 14.08.2009, 3 exx.; woodshed, 13.08.2014, 2 exx.

Mycetaea subterranea (Fabricius, 1801)

Libusza [EA10], woodshed, 26.12.2013, 7 exx.

Corylophidae LeConte, 1852

Orthoperus nigrescens Stephens, 1829 *

Lisów [EA21], 15.08.2014, 1 ex.

This rare species is mainly distributed in northern Europe, where it reaches the Asian part of Turkey to the East (Bowestead, 2007). In Poland, it is only known based on several plots that are located in six zoogeographical regions: the Masurian Lake Region, the Wielkopolska-Kujawy Lowland, the Białowieża Primeval Forest (Burakowski et al, 2000), the Małopolska Upland, Lower Silesia and the Świętokrzyskie Mts (Ruta, Gawroński, Jałoszyński, & Miłkowski, 2010). *Orthoperus nigrescens* has sporadically been observed in the all of its range. It inhabits deciduous forests in which the adults can be found under bark that is overgrown with mycelium, on mouldy piles of brushwood or in forest litter at the base of tree trunks (Burakowski et al, 2000).

Sericoderus lateralis **(Gyllenhal, 1827)**

Libusza [EA10], 30.07.2014, at light, 2 exx.

Latridiidae Erichson, 1842

Stephostethus alternans **(Mannerheim, 1844) ***

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 12 .05.2015, 1 ex.

Stephostethus alternans is widespread in Europe (Johnson, 2007). In Poland, the species has been recorded only from four regions: the Wielkopolska-Kujawy Lowland, Lower Silesia, the West Sudetes and the Białowieża Primeval Forest (Biodiversity Map, 2018). It mainly inhabits rotten wood of various deciduous trees (especially *Quercus* and *Fagus*) (Burakowski, Mroczkowski & Stefańska, 1986c). This is the first record from the Eastern Beskidy Mts.

Tenebrionoidea Latreille, 1802

Mycetophagidae Leach, 1815

Litargus connexus **(Geoffroy in Fourcroy, 1785)**

Libusza [EA10], scrubs, 15.05.2013, 1 ex.

Ciidae Leach, 1819

Cis boleti **(Scopoli, 1763)**

Bartne [EV28], Carpathian beech forest (*Dentario glandulosae-Fagetum*), 11.07.2013, 1 ex.

Melandryidae Leach, 1815

Osphya bipunctata **(Fabricius, 1775)**

Blechnarka [EV17], scrubs, shaken down, 21.05.2014, 1 ex.

The species has rarely been recorded in Poland, and therefore, it is only known from dozen or so dispersed localities in eight zoogeographical regions (Kubisz, Iwan, & Tykarski, 2014): the Pomeranian Lake District, the Wielkopolska-Kujawy Lowland, Lower Silesia, the Małopolska Upland, the Świętokrzyskie Mts, the Lublin Upland, the Roztocze Upland and the Eastern Beskid Mts. In the last mentioned region, it was previously found in Hołubla (Kubisz, Ruta, Jałoszyński, Konwerski, & Królik, 2010) and Prałkowce (Trella, 1923a). Adults are found on blooming shrubs (Kubisz et al, 2014). *Osphya bipunctata* is classified as a DD category species in the Polish Red List (Pawłowski, Kubisz & Mazur, 2002).

Hypulus bifasciatus **(Fabricius, 1792)**

Libusza [EA10], 2008, 1 ex.

Zopheridae Solier, 1834

Bitoma crenata (Fabricius, 1775)

Libusza [EA10], scrubs, 15.05.2013, 1 ex.; scrubs, shaken down, 20.05.2014, 1 ex.

Mordellidae Latreille, 1802

Mordella holomelaena Apfelbeck, 1914

Bartne [EV28], meadow, 09.07.2013, 1 ex.; Libusza [EA10], scrubs, 15.05.2013, 1 ex.; Wysowa-Zdrój [EV17], meadow, 20.08.2010, 1 ex.

Mordellistena humeralis (Fabricius, 1758)

Libusza [EA10], meadow, 27.07.2015, 1 ex.

Variimorda villosa (Schrank von Paula, 1781)

Libusza [EA10], 22.07.2009, 1 ex.; 20.06.2005, 1 ex. Murkowa [EV39], scrubs, 13.06.2013, 1 ex.; Wysowa-Zdrój [EV17], meadow, 07.07.2010, 1 ex.

Tenebrionidae Latreille, 1802

Allecula morio (Fabricius, 1787)

Libusza [EA10], scrubs, 20.07.2013, 1 ex.

Allecula rhenana (Bach, 1856)

Libusza [EA10], synanthropic habitat, 12.07.2011, 1 ex.

Bolitophagus reticulatus (Linnaeus, 1767)

Jaśliska [EV57], scrubs (*Rhamno-Prunetea*), 06.06.2015, 1 ex.; Wysowa-Zdrój [EV17], 27.08.2009, 2 exx.; 04.05.2014, 1 ex.

Corticeus unicolor Piller et Mitterpacher, 1783

Wysowa-Zdrój [EV17], 27.08.2009, 1 ex.

Isomira murina (Linnaeus, 1758)

Lipinki [EA10], shrubs, 15.06.2013, 1 ex.; subxerothermic plant community, 4.06.2015, 2 exx.

Lagria hirta (Linnaeus, 1758)

Bartne [EV29], subxerothermic plant community, 09.07.2013, 4 exx.; scrubs, 11.07.2013, 3 exx.; Bednarka [EV29], 29.07.2014, 2 exx.; Krempna [EV38], scrubs, 11.08.2014, 1 ex.; Libusza [EA10], scrubs, 20.07.2013, 1 ex.; Lisów [EA21], mixed coniferous forest, 15.08.2014, 1 ex.; Wysowa-Zdrój [EV17], meadow, 23.07.2010, 1 ex.; wet meadow, 13.06.2011, 1 ex.

Mycetochara flavipes **(Fabricius, 1792)**

Libusza [EA10], scrubs, shaken down, 20.05.2014, 1 ex.

Opatrum riparium **Scriba W., 1865**

Dobrynia [EV39], subxerothermic plant community, 12.05.2015, 1 ex.

Stenomax aeneus **(Scopoli, 1763)**

Bartne [EV29], pitfall trap, 11.07.2013, 1 ex.; Libusza [EA20] scrubs, 30.04.2013, 1 ex.

Oedemeridae Latreille, 1810

Chrysanthia geniculata **Schmidt W.L.E., 1846**

Bartne [EV28], scrubs, 10.07.2013, 1 ex.; Kąty [EV39], scrubs (*Rhamno-Prunetea*), 22.07.2013, 1 ex.

Oedemera femorata **(Scopoli, 1763)**

Dobrynia [EV39], subxerothermic plant community, 13.06.2013, 1 ex.; Libusza [EA10], 12.08.2009, 1 ex.; scrubs, 8.08.2013, 1 ex., 10.06.2013, 1 ex.; meadow, 16.06.2013, 1 ex.; Wysowa-Zdrój [EV17], meadow, 07.07.2010, 1 ex.; 23.07.2010, 2 exx., scrubs, 13.06.2011, 1 ex.

Oedemera virescens **(Linnaeus, 1767)**

Libusza [EA10], 2003, 1 ex.; meadow, 15.05.2013, 1 ex.; scrubs, 30.04.2013, 1 ex., 10.06.2013, 1 ex. 15.05.2013, 4 exx.; Lipinki [EA20], scrubs, 30.04.2013, 1 ex.; Murkowa [EV39], scrubs, 13.06.2013, 1 ex.; Wójtowa [EA20], scrubs (*Rhamno-Prunetea*), 16.05.2013, 1 ex.; Wysowa-Zdrój [EV17], meadow, 23.07.2010, 1 ex.; scrubs, 17.05.2013, 1 ex.

Meloidae Gyllenhal, 1810

Meloe proscarabaeus **Linnaeus, 1758**

Blechnarka [EV17], scrubs (*Rhamno-Prunetea*), 13.06.2011, 3 exx.

Pyrochroidae Latreille, 1807

Pyrochroa coccinea **(Linnaeus, 1760)**

Libusza [EA10], 8.08.2008, 1 ex.

Pyrochroa serraticornis **(Scopoli, 1763) (Fig. 3D)**

Libusza [EA10], 03.06.2010, 1 ex.; scrubs (*Rhamno-Prunetea*), 15.05.2013, 4 exx.; meadow, 10.06.2013, 2 exx.

This species is known in Poland from scattered sites, and it has usually been captured in small numbers. However, *P. serraticornis* is probably far more widespread than the scant literature data suggest. The species prefers riparian forests, which was confirmed by our and Holly's (2010) observations. In the Eastern Beskidy Mts, it has been recorded four times (Kubisz, Iwan, & Tykarski, 2015).

Schizotus pectinicornis **(Linnaeus, 1758)**

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 16.05.2013, 2 exx.; 12.05.2015, 2 exx.; Libusza [EA10], 08.08.2008, 3 exx.; Wysowa-Zdrój [EV17], Carpathian beech forest (*Dentario glandulosae-Fagetum*), 17.05.2013, 4 exx.

Salpingidae Leach, 1815

Salpingus planirostris **(Fabricius, 1787)**

Libusza [EA10], scrubs, 15.05.2013, 1 ex.; sieve analysis, 11.11.2014, 1 ex.

Anthicidae Latreille, 1819

Anthicus antherinus **(Linnaeus, 1760)**

Wysowa-Zdrój [EV17], scrubs (*Rhamno-Prunetea*), shaken down, 30.04.2014, 1 ex.

Scraptiidae Mulsant, 1856

Anaspis frontalis **(Linnaeus, 1758)**

Bednarka [EA20], linden-oak-hornbeam forest (*Tilio-Carpinetum*), 13.06.2013, 1 ex.; Dobrynia [EV39], subxerothermic plant community, 16.05.2013, 1 ex. Libusza [EA10], meadow, 10.06.2013, 1 ex.; Wysowa-Zdrój [EV17], scrubs, 17.05.2013, 1 ex.

Chrysomeloidea Latreille, 1802

Megalopodidae Latreille, 1802

Zeugophora subspinosa **(Fabricius, 1781)**

Blechnarka [EV17], scrubs (*Rhamno-Prunetea*), 13.06.2011, 1 ex.; Dobrynia [EV39], subxerothermic plant community, 16.05.2013, 1 ex.; Wysowa-Zdrój [EV17], scrubs, 23.05.2011, 1 ex.

Curculionoidea Latreille, 1802

Curculionidae Latreille, 1802

Dryocoetes autographus **(Ratzeburg, 1837)**

Małastów [EV18], wood pile, 14.06.2013, 1 ex.

Hylastes ater **(Paykull, 1800)**

Libusza [EA10], 21.04.2014, 1 ex.

Hylesinus varius **(Fabricius, 1775)**

Libusza [EA10], 24.07.2010, 2 exx.

***Ips cembrae* (Heer, 1836) ***

Wysowa-Zdrój [EV17], wood pile, pine, 02.05.2014, 1 ex.

Although this species has been recorded from eleven zoogeographical regions in Poland (Mokrzycki et al, 2011), it has not been recorded from the Eastern Beskidy Mts to date. Its host plants are *Larix* spp.

***Ips sexdentatus* (Börner I.K.H., 1766)**

Bednarka [EV29], 07.2004, 1 ex.

***Ips typographus* (Linnaeus, 1758)**

Blechnarka [EV17], wood pile, 20.08.2010, 2 exx.; Wysowa-Zdrój [EV17], 23.06.2010, 1 ex.; 23.07.2010, 1 ex.; wood pile, pine, 02.05.2014, 1 ex.

***Pityogenes bidentatus* (Herbst, 1784)**

Wysowa-Zdrój [EV17], wet meadow, 30.04.2014, 1 ex.

***Tomicus piniperda* (Linnaeus, 1758)**

Wysowa-Zdrój [EV17], wood pile, pine, 02.05.2014, 1 ex.

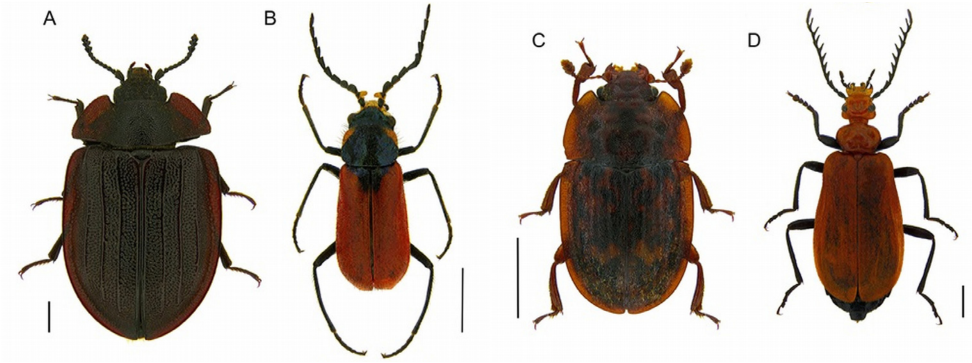


Fig. 3. A - *Peltis grossa* (Trogossitidae), B - *Malachius scutellaris* (Malachiidae), C - *Soronia punctatissima* (Nitidulidae), D - *Pyrochroa serraticornis* (Pyrochroidae); a linear scale - 2 mm.

DISCUSSION

Summary of the data presented in this paper

One hundred species belonging to 37 beetle families were found during our research studies. As many as 49 species were only represented by a single specimen and the next ten were only represented by two specimens. The estimated number of species that was calculated on this basis (Chao1 formula) was 220, which means that there are twice as many as have been recorded. Within the particular mesoregions, the following species were found: the Lower Beskids - 50, the Jasielsko-Krośnieńska Basin - 70 and the Ciężkowickie Foothills - 2 species.

Among the collected material, eight species were found in the Eastern Beskids for the first time: *Agabus congener*, *Dytiscus dimidiatus*, *Dolichosoma lineare*, *Amphotis marginata*, *Glischrochilus quadrisignatus*, *Orthoperus nigrescens*, *Stephostethus alternans* and *Ips cembrae*. Moreover, species that are rarely found in Poland, such as *Elodes johni*, *Lyctus pubescens*, *Peltis grossa*, *Malachius scutellaris*, *Soronia punctatissima*, *Osphya bipunctata* and *Pyrochroa serraticornis* deserve special attention.

Summary of the entire project: Coleoptera of the Eastern Beskid Mountains

Considering the entire research, which was conducted in the Eastern Beskid Mts, a total of 744 species from 63 beetle families have been found (Szczepański et al, 2013; 2015a; 2015c; 2015d; Szczepański, Taszakowski & Karpiński, 2014; Szczepański, Taszakowski & Karpiński, 2015b; Szczepański, Taszakowski, Karpiński & Kaszyca, 2016; Taszakowski, Szczepański, Baran & Morawski, 2014; Taszakowski, Baran, Kaszyca & Depa, 2015a; Taszakowski et al, 2017a; 2017b; 2018; Karpiński et al, 2015; Kaszyca & Taszakowski, 2017; Szołtys & Taszakowski, 2017). This constitutes approx. 12% of the Polish beetle fauna. The number of recorded taxa can be considered high, especially since representatives of the largest family Staphylinidae, which according to Szujecki (2017) comprises more than 1400 species in the country, constitute only a small part of the collected material. Only 49 species of this family were recorded during the sporadic samplings (Taszakowski et al, 2018). The estimated number of species that was calculated using the Chao1 formula for the entire project was 983 species, which is 233 more species than the number in our results.

Within the particular mesoregions, the following species were found: the Lower Beskids - 448, the Jasielsko-Krośnieńska Basin - 544 and the Ciężkowickie Foothills - 14 species. Forty-four species (approx. 6% of a total number) were recorded in the Eastern Beskids for the first time; among them as many as eight species (of the 49 collected in this family) belong to the family Staphylinidae. This proves an insufficient recognition of the staphylinid fauna of this region. The buprestids are even more poorly studied; of the 18 species that were collected as many as seven (approx. 39%) are new to the Eastern Beskids (Szczepański et al, 2015a). Moreover, one species - *Isorhipis nigriceps* (Mannerheim, 1823) (Eucnemidae) was recorded in Poland for the first time (Szołtys & Taszakowski, 2017). On the other hand, the state of knowledge on the beetles in this area is relatively high when this data is compared with the results of recent research on true bugs (Hemiptera). Among the 478 collected species of Hemiptera, as many as 175 (approx. 37%) were listed in this zoogeographic region for the first time and seven species were recorded as being new to the fauna of the country (Depa & Mróz, 2013; Taszakowski, Walczak & Baran, 2015b; Taszakowski, Walczak, Morawski & Baran, 2015c; Walczak, Taszakowski, Skrynetska & Kaszyca, 2016; Walczak, Kaszyca & Taszakowski, 2018; Depa, Mróz, Bugaj-Nawrocka & Orczewska, 2017; Kanturski, Mruk, Morawski, Wojciechowski & Depa, 2017; Kaszyca, Morawski, Taszakowski & Depa, 2018a; Kaszyca et al, 2018b; Taszakowski & Gorczyca, 2018).

Fifty-two species are protected by Polish law or are included in various lists of endangered species as follows: 1-strict protection, 2-partial protection, 3-Habitats

Directive (Annexes II and III) (EEC 1992), 4-Polish Red Data Book of Animals. Invertebrates (Głowaciński & Nowacki, 2004), 5-Red List Of Threatened Animals in Poland (Pawłowski et al, 2002), 6-The IUCN Red List of Threatened Species (IUCN 2003), 7-Carpathian List of Endangered Species (Pawłowski, 2003), 8-European Red List of Saproxylic Beetles (Nieto & Alexander, 2010). The red list category abbreviations are EN-endangered, VU-vulnerable, NT-near threatened, LC-least concern and DD-data deficient.

Allecula morio (Fabricius, 1787) ^{6} (LC)

Ampedus erythrogonus (P.W.J. Müller, 1821) ^{8} (LC)

Ampedus elongatulus (Fabricius, 1787) ^{8} (NT)

Ampedus melanurus Mulsant & Guillebeau, 1855 ^{5} (VU), $^{6, 8}$ (DD)

Ampedus pomorum (Herbst, 1784) ^{8} (LC)

Ampedus sanguineus (Linnaeus, 1758) ^{8} (LC)

Ampedus sanguinolentus (Schrank, 1776) ^{8} (LC)

Anaglyptus mysticus (Linnaeus, 1758) ^{8} (LC)

Aphodius scrutator (Herbst, 1789) ^{5} (LC)

Aromia moschata (Linnaeus, 1758) ^{7} (VU), ^{8} (LC)

Betarmon bisbimaculatus (Fabricius, 1803) ^{5} (EN), ^{7} (CR)

Callidium violaceum (Linnaeus, 1758) ^{8} (LC)

Carabus auronitens Fabricius, 1792 ^{2}

Carabus coriaceus Linnaeus, 1758 ^{2}

Carabus intricatus Linnaeus, 1761 $^{2, 5}$ (LC), ^{6} (NT)

Carabus irregularis Fabricius, 1792 $^{2, 5}$ (NT)

Carabus ulrichii Germar, 1824 ^{2}

Carabus variolosus Fabricius, 1787 $^{1, 3}$

Ceruchus chrysomelinus (Hochenwarth, 1785) $^{2, 5}$ (VU), ^{6} (NT), ^{7} (EN/VU), ^{8} (NT)

Chlorophorus herbsti (Brahm, 1790) ^{8} (LC)

Claviger longicornis Müller, 1818 ^{5} (VU)

Clytus arietis (Linnaeus, 1758) ^{8} (LC)

Clytus lama Mulsant, 1847 $^{6, 8}$ (LC)

Cucujus cinnaberinus (Scopoli, 1763) $^{1, 3, 5}$ (LC), $^{6, 8}$ (NT)

Denticollis borealis (Paykull, 1800) ^{5} (DD)

Diachromus germanus (Linnaeus, 1758) ^{5} (NT)

Drypta dentata (Rossi, 1790) ^{5} (LC)

Elaphrus ullrichii Redtenbacher G., 1842 ^{5} (EN)

Emus hirtus (Linnaeus, 1758) ^{5} (NT), ^{7} (VU)

Eurythyrea austriaca (Linnaeus, 1767) $^{2, 5}$ (VU), ^{7} (EN)

Evodinus clathratus (Fabricius, 1792) [6] (LC)
Gnorimus nobilis (Linnaeus, 1758) [8] (LC)
Hylotrupes bajulus (Linnaeus, 1758) [8] (LC)
Isoriphis nigriceps (Mannerheim, 1823) [6] (DD)
Larinus sturnus (Schaller, 1783) [5] (VU)
Litargus connexus (Faucroy, 1785) [8] (LC)
Lixus punctiventris Boheman, 1842 [5] (VU)
Molorchus minor (Linnaeus, 1758) [8] (LC)
Monochamus sartor (Fabricius, 1787) [6, 8] (LC)
Monochamus sutor (Linnaeus, 1758) [8] (LC)
Obrium brunneum (Fabricius, 1793) [8] (LC)
Onthophagus illyricus (Scopoli, 1763) [5] (DD)
Oodes helopioides (Fabricius, 1792) [5] (VU)
Oryctes nasicornis (Linnaeus, 1758) [5] (NT)
Osphya bipunctata (Fabricius, 1775) [5] (DD)
Peltis grossa (Linnaeus, 1758) [5] (VU), [7] (EN)
Pidonia lurida (Fabricius, 1792b) [6] (LC)
Platycerus caraboides Linnaeus, 1758 [8] (LC)
Prionus coriarius (Linnaeus, 1758) [8] (LC)
Protaetia lugubris (Herbst, 1786) [8] (LC)
Pyrrhidium sanquineum (Linnaeus, 1758) [8] (LC)
Ropalopus macropus (Germar, 1824) [8] (LC)
Rosalia alpina (Linnaeus, 1758) [1, 3, 4] (EN), [5] (EN), [6] (VU), [7] (EN), [8] (LC)
Saperda perforata Pallas, 1773 [8] (LC)
Sinodendron cylindricum Linnaeus, 1758 [8] (LC)
Trichius fasciatus (Linnaeus, 1758) [8] (LC)
Tritoma bipustulata Fabricius, 1775 [6, 8] (LC)
Xylotrechus rusticus (Linnaeus, 1758) [8] (LC)

Many of the recorded species, primarily in the families Bostrichidae, Buprestidae, Cerambycidae, Cucujidae, Elateridae, Eucnemidae, Lucanidae, Lymexylidae, Nitidulidae, Ptinidae, Scarabaeidae, Silvanidae and Trogossitidae, belong to the group of saproxylic beetles. Some of them, the so-called *saproxylobionts*, are obligatorily associated with dead wood (e.g. *Ampedus*, *Agrilus*, *Anthaxia*, *Buprestis*, *Ceruchus*, *Chrysobothris*, *Cucujus*, *Denticollis*, *Monochamus*, *Nivellia*, *Oxymirus*, *Peltis*, *Platycerus*, *Rosalia*, *Sinodendron*, *Soronia*, *Stenocorus* and *Stictoleptura*), while others only facultatively associated - *saproxylophiles* (e.g. *Carabus*, *Chilocorus*, *Thanasimus* and *Tillus*). In this interesting and increasingly threatened group of insects, numerous species are extraordinarily valuable from ecological point of view, and these are often referred to as *relict species* or *primeval forest species*. These include the rarest taxa, many of which are extremely vulnerable, especially in Europe. Although the definition of the *primeval forest species* is not yet unambiguous, among the collected material, the following taxa can certainly be included in this category: *Carabus intricatus*, *C. irregularis*, *Ceruchus chrysomelinus*, *Eurythyrea austriaca*, *Peltis grossa* and *Rosalia alpina*.

Among the studied habitats, the ecotone environments were characterised by the highest species diversity of beetles. These are habitats that are created at the junction of forests and meadows and in similar shrub environments, which are at various stages of succession. Although these plant communities are difficult to classify because of their considerable variation and mutual penetration, they can be placed in the class *Rhamno-Prunetea*, order *Prunetalia spinosae*, alliance *Pruno-Rubion fruticosi* and in the class *Trifolio-Geranietea sanguinei*, order *Origanetalia* and alliance *Trifolion medii* (Matuszkiewicz, 2013). Because of declining agricultural activities, such habitats are very common in the study area. Among the beetles that were recorded in the above-mentioned habitats, some species rarely collected in Poland were found, *inter alia*, *Omalisus fontisbellaquaei, Phosphaenus hemipterus, Osphya bipunctata, Malachius scutellaris* (these species are recorded herein)*, Scymnus suffrianioides apetzoides* Capra et Fürsch*,* 1967 (Szczepański et al, 2015c), *Agrilus sinuatus* (Olivier, 1790) (Szczepański et al, 2015b), *Phytoecia nigricornis* (Fabricius, 1781) (Karpiński et al, 2015), *Tachyerges rufitarsis* (Germar, 1821) (Taszakowski et al, 2017a) and *Cassida panzeri* Weise, 1907 (Szczepański et al, 2015b). Some species were collected only in the scrub biotopes and ecotone areas. This is probably connected with both the presence of a large number of suitable host plants and the specific environmental conditions, which permit the existence of helio- and thermophilic species. While such habitats play a big role as local biodiversity centres, unfortunately, they are often overlooked in faunistic studies.

Many species that have the southern type of range such as *Benibotarus taygetanus* (Pic, 1905), *Betarmon bisbimaculatus* (Fabricius, 1803), *Isorhipis nigriceps* (Mannerheim, 1823) and *Rhagonycha nigriceps* (Waltl, 1838) were found during the research (Szczepański et al, 2015d, 2016). Most probably, they came to Poland *via* passes in the Low Beskids. This path (the Ondavian migratory route) is a very important Transcarpathian route of migration for small fauna from the south to the north between the Pannonian Basin with the Ondava Upland and the drainage basin of the San and Wisłoka Rivers. For species that are characterised by a southern type of range, this route makes it possible to migrate to Poland and the effect of this migration is clearly visible in both the immediate vicinity of the passes in the Low Beskids and on the foothills of this mountain range (e.g. Szczepański et al, 2016; Taszakowski & Gorczyca, 2018). This issue is especially important nowadays in the era of the warming climate when this may be one of the most important routes for numerous species to cross the Carpathian Mountains and to extend their range to the north. Faunistic studies are necessary to follow the ongoing processes and transformations that are related to climate change, both dynamic (e.g. anthropogenic environmental transformations) and long-term (Banaszak, 2008; Gurung et al, 2009). Unfortunately, there is still not enough research of this type, and it has become less and less supported by the government and scientific units. The state of knowledge, even in cases that involve the most valuable and best-preserved fragments of nature in Poland, is far from satisfactory (Banaszak, 2008). The invertebrate fauna of the Carpathians still requires a fundamental inventory in order to draw some solid conclusions on the species lists they belong on, their distribution and threats. The lack of knowledge of Carpathian invertebrate fauna is not an exception; this problem can be found all over the world (Pawłowski, 2003; Gurung et al, 2009). Unfortunately, the scientific policies that prevail in many countries not only do not support broadly understood ecological (especially faunistic) research, but on the contrary, they render them less profitable (Banaszak, 2008; Neff, 2018).

ACKNOWLEDGEMENTS

We would like to express our special thanks to Dr. Czesław Greń, Dr. Rafał Ruta (Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, Wrocław, Poland) and Henryk Szołtys for their help in identifying some of the specimens. We are also grateful to all of the people who participated in the project: Prof. Dr. Lech Borowiec (Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, Wrocław, Poland), Dr. Łukasz Depa (Department of Zoology, University of Silesia in Katowice, Katowice, Poland), Roman Królik, Dr. Miłosz A. Mazur (Department of Biosystematics, Opole University, Opole, Poland), Andrzej Melke, Dr. Mieczysław Stachowiak (Department of Environmental Development and Protection, University of Technology and Life Sciences in Bydgoszcz, Bydgoszcz, Poland), Prof. Dr. Dariusz Tarnawski (Department of Invertebrate Biology, Evolution and Conservation, University of Wrocław, Wrocław, Poland) and members of the Students' Scientific Association of Zoologists "Faunatycy" (Department of Zoology, University of Silesia in Katowice, Katowice, Poland): Bartosz Baran, Magdalena Domagała, Arkadiusz Imiela, Karolina Krzyżowska, Angelina Kubusiak, Adrian Masłowski, Miłosz Morawski, Miłosz Obrzut and Małgorzata Tomecka.

REFERENCES

Banaszak, J. (2008). Najważniejsze trendy oraz wyzwania entomologii XXI wieku w Polsce. *Wiadomości Entomologiczne*, 27(supl.), 59-76. (Major trends and challenges of entomology in the 21st century in Poland) (in Polish)

Biodiversity Map. (2018, August 11). Polish Biodiversity Information Network. http://baza.biomap.pl

Borowiec, L., Kania, J., & Wanat, M. (1992). Chrząszcze (Coleoptera) nowe dla Puszczy Białowieskiej. *Wiadomości Entomologiczne*, 11(3), 133-141. (Coleoptera new for the Białowieża Primeval Forest) (in Polish)

Borowiec, L. & Kania, J. (1996). Chrząszcze (Coleoptera) nowe i rzadkie w faunie Bieszczadów. *Wiadomości Entomologiczne*, 14(3), 153-157. (Beetles (Coleoptera) new and rare to the fauna of the Bieszczady Mts) (in Polish)

Bowestead, S. (2007). Corylophidae. In I. Löbl, A. Smetana (Eds.). *Catalogue of Palaearctic Coleoptera* (4, 935 pp). Stenstrup, Apollo Books.

Burakowski, B., Mroczkowski, M., & Stefańska J. (1973). Chrząszcze Coleoptera. Biegaczowate-Carabidae, cz. 1. *Katalog Fauny Polski*, 23(2), 1-232. (Beetles (Coleoptera). Carabidae) (in Polish)

Burakowski, B., Mroczkowski, M., & Stefańska, J. (1976). Chrząszcze-Coleoptera. Adephaga prócz Carabidae, Myxophaga, Polyphaga: Hydrophiloidea. *Katalog Fauny Polski*, XXIII, 4, Warszawa. (Beetles (Coleoptera). Adephaga excl. Carabidae, Myxophaga, Polyphaga: Hydrophiloidea) (in Polish)

Burakowski, B., Mroczkowski, M. & Stefańska, J. (1986a). Chrząszcze Coleoptera. Dermestoidea, Bostrichoidea, Cleroidea i Lymexyloidea. *Katalog Fauny Polski*, 23(11), 1-244. (Beetles (Coleoptera). Dermestoidea, Bostrichoidea, Cleroidea and Lymexyloidea) (in Polish)

Burakowski, B., Mroczkowski, M. & Stefańska, J. (1986b). Chrząszcze-Coleoptera. Cucujoidea, część 1. *Katalog Fauny Polski*, 23(12), 1-266. (Beetles (Coleoptera). Cucujoidea, part 1) (in Polish)

Burakowski, B., Mroczkowski, M. & Stefańska, J. (1986c). Chrząszcze-Coleoptera. Cucujoidea, część 2. *Katalog Fauny Polski*, 23(13), 1-277. (Beetles (Coleoptera). Cucujoidea, part 2) (in Polish)

Burakowski, B., Mroczkowski, M., Stefańska, J. (2000). Chrząszcze Coleoptera. Uzupełnienia tomów 2-21. *Katalog Fauny Polski*, Warszawa, 23(22), 1-252. (Beetles (Coleoptera). Supplement of volumes 2-21) (in Polish)

Byk, A. (2007). Waloryzacja lasów Gór Świętokrzyskich na podstawie struktury zgrupowań chrząszczy saproksylicznych. In J. Borowski, S. Mazur (Eds.). *Waloryzacja ekosystemów leśnych Gór Świętokrzyskich metodą zooindykacyjną* (pp. 57-118). Wydawnictwo SGGW, Warszawa. (Valorization of the forests of the Świętokrzyskie Mts. based on the structure of saproxylic beetle communities. In: Valorization of Forest Ecosystems bythe Zooindication Method) (in Polish)

Chao, A. & Bunge, J. (2002). Estimating the number of species in a stochastic abundance model. *Biometrics*, 58, 531-539.

Depa, Ł. & Mróz, E. (2013). Central European Acer-and Salicaceae-feeding aphids of the genus *Stomaphis* (Insecta: Aphidoidea: Lachnidae)-separate species or populations? *Zoological Science*, 30(6), 509-518.

Depa, Ł., Mróz, E., Bugaj-Nawrocka, A., & Orczewska, A. (2017). Do ants drive speciation in aphids? A possible case of ant-driven speciation in the aphid genus *Stomaphis* Walker (Aphidoidea, Lachninae). *Zoological Journal of the Linnean Society*, 179(1), 41-61. doi: 10.1111/zoj.12437

European Economic Community (EEC). (1992). Council directive 92/43EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal*, L206, 7-50.

Gil, R. & Melke, A. (2017). Owady z rzędu Coleoptera na terenie projektowanego Parku Krajobrazowego Beskidu Niskiego. [In:] Park Krajobrazowy „Beskidu Niskiego" Dokumentacja projektowa. *Fundacja Dziedzictwo Przyrodnicze*, Przemyśl: 168-191. (Coleoptera of the area of the designed Low Beskids Landscape Park) (in Polish)

Gotelli N.J. & Colwell R.K. (2011). Estimating species richness. In A.E. Magurran, B.J. McGill (Eds.). *Frontiers in measuring biodiversity* (pp. 39-54). New York: Oxford University Press.

Głowaciński, Z., Nowacki, J. (Eds). (2004). Polish Red Data Book of Animals. Invertebrates. Instytut Ochrony Przyrody PAN, Akademia Rolnicza im. A. Cieszkowskiego, Kraków-Poznań, 447 pp.

Gurung, A.B., Bokwa, A., Chełmicki, W., Elbakidze, M., Hirschmugl, M., Hostert, P., Ibisch, P., Kozak, J., Kuemmerle, T., Matei, E., Ostapowicz, K., Pociask-Karteczka, J., Schmidt, L., van der Linden, S., Zebisch, M. (2009). Global change research in the Carpathian Mountain region. *Mountain Research and Development,* 29, 282-288. doi: 10.1659/mrd.1105

Hölldobler, B. & Wilson, E.O. (1990). The Ants. Springer Verlag, Berlin. 733 pp.

Holly, M. (2010). Nowe dla Bieszczadzkiego Parku Narodowego oraz rzadko spotykane gatunki chrząszczy Coleoptera stwierdzone w 2009 roku. Roczniki Bieszczadzkie 18, 432-435. (The new and rare species of beetles Coleoptera recorded in the Bieszczady National Park in 2009) (in Polish with English abstract)

Holly, M. (2016). Rzadko spotykane oraz nowe dla fauny Bieszczadów gatunki chrząszczy (Coleoptera) stwierdzone w Bieszczadzkim Parku Narodowym i otulinie w latach 2012-2015. Roczniki Bieszczadzkie 24, 199-213. (Rare and new for the fauna of the Bieszczady Mts species of beetles (Coleoptera) recorded from the Bieszczady National Park and its buffer zone in the years 2012-2015) (in Polish with English abstract)

IUCN. (2003). IUCN Red List of Threatened Species. International Union for Conservation of Nature and Natural Resources-The World Conservation Union, Species Survival Commission (http://www.redlist.org).

Johnson, C. (2007). Latridiidae. In I. Löbl, A. Smetana (Eds.). *Catalogue of Palaearctic Coleoptera* (4, 935 pp). Apollo Books, Stenstrup.

Kanturski, M., Mruk, K., Morawski, M., Wojciechowski, W., & Depa, Ł. (2017). *Nearctaphis bakeri* (Cowen, 1895) and *Illinoia liriodendri* (Monell, 1879)-two aphid species (Hemiptera: Aphididae) of alien origin new to the Polish fauna. Annals of The Upper Silesian Museum in Bytom. *Entomology* 26 (online 002), 1-6.

Karpiński, L., Taszakowski, A. & Szczepański, W.T. (2015). New data on the occurrence of longhorn beetles (Coleoptera: Cerambycidae) in the Eastern Beskid Mountains. *Fragmenta Faunistica*, 58(1), 7-16.

Kaszyca, N., Morawski, M., Taszakowski, A., & Depa, Ł. (2018a.) Aphid species (Hemiptera: Aphididae) new in fauna of the Eastern Beskid Mountains (south Poland). *Fragmenta Faunistica*, 61(1), 35-38. doi: 10.3161/00159301FF2018.61.1.035

Kaszyca, N., Mruk, K., Kuna, E., Taszakowski, A., Depa, Ł., & Wojciechowski W. (2018b). Nowe dane o występowaniu mszyc (Hemiptera: Aphidomorpha) w Beskidzie Wschodnim. *Acta entomologica silesiana*, 26(online 032), 1-8. doi: 10.5281/zenodo.1419960 (New data on the occurrence of aphids (Hemiptera: Aphidomorpha) in the Eastern Beskidy Mountains) (in Polish with English abstract)

Kaszyca, N. & Taszakowski, A. (2017). Materiały do znajomości chrząszczy z nadrodziny Scarabaeoidea Beskidu Wschodniego. *Acta entomologica silesiana*, 25(online 013), 1-7. (Materials to the knowledge of Scarabaeoidea of the Eastern Beskid Mountains) (in Polish with English abstract)

Knutelski, S. & Tykarski, P. (2010). Chrząszcze obszarów górskich Polski (Insecta: Coleoptera). *Wiadomości Entomologiczne*, 29, 39-51. (Beetles of mountain areas of Poland (Insecta: Coleoptera) (in Polish with English abstract)

Kolibáč, J. (2013). Trogossitidae: A review of the beetle family, with a catalogue and keys. *ZooKeys*, 366, 1-194.

Kolibáč, J., Majer, K. & Švihla, V. (2005). Beetles of the superfamily Cleroidea in the Czech and Slovak Republics and neighbouring areas. Clarion Production, Praha, 186 pp.

Kondracki, J. (2013). Geografia regionalna Polski. Wydawnictwo Naukowe PWN. Warszawa: 440 pp. (Regional geography of Poland) (in Polish)

Kubisz, D. (1990). Nowe stanowiska niektórych rzadkich Malachiidae (Coleoptera) w Polsce. *Wiadomości Entomologiczne*, 9(3-4), 90. (New stands of some rare Malachiidae (Coleoptera) in Poland) (in Polish)

Kubisz, D., Iwan, D., & Tykarski, P. (2014). Tenebrionoidea: Tetratomidae, Melandryidae, Ripiphoridae, Prostomidae, Oedemeridae, Mycteridae, Pythidae, Aderidae, Scraptiidae. Critical checklist, distribution in Poland and meta-analysis. Coleoptera Poloniae 2, University of Warsaw-Faculty of Biology, Natura optima dux Foundation, Warszawa, 470 pp.

Kubisz, D., Iwan, D., & Tykarski, P. (2015). Tenebrionoidea: Mycetophagidae, Ciidae, Mordellidae, Zopheridae, Meloidae, Pyrochroidae, Salpingidae, Anthicidae. Critical checklist, distribution in Poland and meta-analysis. Coleoptera Poloniae 3, University of Warsaw-Faculty of Biology, Natura optima dux Foundation, Warszawa, 744 pp.

Kubisz, D., Ruta, R., Jałoszyński, P., Konwerski, S. & Królik, R. (2010). A faunistic review of beetle families Tetratomidae and Melandryidae (Coleoptera: Tenebrionoidea) of Poland. *Polskie Pismo Entomologiczne*, 79(2), 107-138.

Kubisz, D. & Szwałko P. (1991). Nowe dla Podlasia i Puszczy Białowieskiej gatunki chrząszczy (Coleoptera). *Wiadomości Entomologiczne*, 10(1), 5-14. (Coleoptera-species new to the fauna of Podlasie and the Białowieża Primeval Forest) (in Polish with English abstract)

Lasoń, A. (1997). Nowe stanowiska chrząszczy z rodzin Kateretidae i Nitidulidae (Coleoptera) na Podlasiu. *Wiadomości Entomologiczne*, 16(1), 7-10. (New records of some Kateretidae and Nitidulidae (Coleoptera) species fromthe Podlasie region) (in Polish with English abstract)

Lasoń, A. (1999a). Nowe stanowiska polskich Kateretidae i Nitidulidae (Coleoptera). *Wiadomości Entomologiczne*, 17(3-4), 157-163. (New records of Polish Kateretidae and Nitidulidae (Coleoptera) (in Polish with English abstract)

Lasoń, A. (1999b). Nowe dane o występowaniu w Polsce przedstawicieli rodzaju *Glischrochilus* Reitter, 1873 (Coleoptera: Nitidulidae: Cryptarchinae). *Wiadomości Entomologiczne*, 17, 169-173. (New data on the occurrence of species of the genus *Glischrochilus* Reitter, 1873 (Coleoptera: Nitidulidae: Cryptarchinae) in Poland) (in Polish with English abstract)

Lasoń, A. & Bidas, M. (2010). Stan poznania fauny łyszczynek (Coleoptera: Kateretidae, Nitidulidae) Gór Świętokrzyskich. *Wiadomości Entomologiczne*, 29(4), 263-272. (The state of knowledge about sap beatles (Coleoptera: Kateretidae, Nitidulidae) of the Świętokrzyskie Mts.) (in Polish with English abstract)

Lasoń, A. & Miłkowski, M. (2011). Kateretidae i Nitidulidae (Coleoptera) okolic Radomia. *Wiadomości Entomologiczne*, 30(3), 149-158. (Short-winged flowers beetles and sap beetles (Coleoptera: Kateretidae, Nitidulidae) of Radom environs) (in Polish with English abstract)

Löbl, I. & Smetana, A. (Ed). (2003). *Catalogue of Palaearctic Coleoptera. Vol. 1. Archostemata-Myxophaga-Adephaga.* Apollo Books, Stenstrup, 819 pp.

Löbl, I. & Smetana, A. (Ed). (2004). *Catalogue of Palaearctic Coleoptera. Vol. 2. Hydrophyloidea-Histeroidea-Staphylinoidea.* Apollo Books, Stenstrup, 942 pp.

Löbl, I. & Smetana, A. (Ed). (2006). *Catalogue of Palaearctic Coleoptera. Vol. 3. Scarabaeoidea, Scirtoidea, Dascilloidea, Buprestoidea, Byrrhoidea.* Apollo Books, Stenstrup, 690 pp.

Löbl, I. & Smetana, A. (Ed). (2007). *Catalogue of Palaearctic Coleoptera. Vol. 4. Elateroidea, Derodontoidea, Bostrichoidea, Lymexyloidea, Cleroidea and Cucujoidea.* Apollo Books, Stenstrup, 935 pp.

Löbl, I. & Smetana, A. (Ed). (2008). *Catalogue of Palaearctic Coleoptera. Vol. 5. Tenebrionoidea.* Apollo Books, Stenstrup, 670 pp.

Löbl, I. & Smetana, A. (Ed). (2010). *Catalogue of Palaearctic Coleoptera. Vol. 6. Chrysomeloidea.* Apollo Books, Stenstrup, 924 pp.

Löbl, I. & Smetana, A. (Ed). (2011). *Catalogue of Palaearctic Coleoptera. Vol. 7. Curculionoidea I.* Apollo Books, Stenstrup, 373 pp.

Löbl, I. & Smetana, A. (Ed). (2013). *Catalogue of Palaearctic Coleoptera. Vol. 8. Curculionoidea II.* Apollo Books, Stenstrup, 707 pp.

Marczak, D., Borowski, J., & Jędryczkowski, W. (2016). Materiały do poznania fauny Kampinoskiego Parku Narodowego: Dasytidae, Malachiidae (Coleoptera: Cleroidea). *Wiadomości Entomologiczne*, 35(2), 72-81. (A contribution to the knowledge of the fauna of the Kampinos National Park: Dasytidae, Malachiidae (Coleoptera: Cleroidea) (in Polish with English abstract)

Matuszkiewicz, W. (2013). Przewodnik do oznaczania zbiorowisk roślinnych Polski. PWN, Warszawa, 537 pp. (Guide for the determination of Polish plant communities) (in Polish)

Mayor, A. (2007). Malachiidae. In I. Löbl, A. Smetana (Eds.). *Catalogue of Palaearctic Coleoptera* (4, 935 pp). Apollo Books, Stenstrup.

Mokrzycki, T., Hilszczański, J., Borowski, J., Cieślak, R., Mazur, A., Miłkowski, M., & Szołtys, H. (2011). Faunistic review of Polish Platypodinae and Scolytinae (Coleoptera: Curculionidae). *Polish Journal of Entomology*, 80, 343-364.

Müller, J., Bussler, H., Bense, U., Brustel, H., Flechtner, G., Fowles, A., Kahlen, M., Möller, G., Mühle, H., Schmidl, J., & Zábranský, P. (2005). Urwald relict species Saproxylic beetles indicating structural qualities and habitat tradition. *Waldoekologie Online*, 2, 106-113.

Neff, M. (2018). Publication incentives undermine the utility of science: Ecological research in Mexico. *Science and Public Policy*, 45(2), 191-201.

Nieto, A. & Alexander, K.N.A. (2010). *European Red List of Saproxylic Beetles*. Luxembourg, Publications Office of the European Union, 45 pp.

Papis, M. & Mokrzycki, T. (2015). Chrząszcze saproksyliczne (Coleoptera) obszaru ochrony ścisłej Bukowa Góra w Roztoczańskim Parku Narodowym. *Leśne Prace Badawcze*, 76(3), 229-239. doi: 10.1515/frp-2015-0022 (Saproxylic beetles (Coleoptera) of the strictly protected area Bukowa Góra in the Roztoczański National Park) (in Polish with English abstract)

Pawłowski, J. (2003). Invertebrates. In Z.J. Witkowski, W. Król, W. Solarz (Eds.). *Carpathian list of endangered species* (pp. 64). WWF and Institute of Nature Conservation, Polish Academy of Sciences. Vienna-Krakow.

Pawłowski, J., Kubisz, D., & Mazur, M. (2002). Coleoptera chrząszcze. In Z. Głowaciński (Ed.). *Red list of threatened animals in Poland* (pp. 88-110). Instytut Ochrony Przyrody PAN, Kraków. (Coleoptera. The Beetles) (in Polish)

Procházka, J., Kment, P., Németh, T., & Kolibáč, J. (2017). New data on the distribution of *Peltis grossa* and *P. gigantea* (Coleoptera: Trogossitidae). *Acta Musei Moraviae*, *Scientiae Biologicae*, 102, 25-33.

Ruta, R., Gawroński, R., Jałoszyński, P., & Miłkowski, M. (2010). Contribution to the knowledge of Corylophidae (Coleoptera: Cucujoidea) of Poland. *Polish Journal of Entomology*, 79, 223-234.

Szarfaniec, S., Szarfaniec, P., & Mazur, M.A. (2010). *Malachius scutellaris* Erichson, 1840 and some other interesting species of Melyridae (Coleoptera: Cleroidea) from the Western Beskidy Mts. *Nature Journal*, 43, 95-100.

Szczepański, W.T. & Karpiński, L. (2013). Nowe stanowiska świetlikowatych (Coleoptera: Lampyridae) w Polsce południowo-wschodniej. *Acta Entomologica Silesiana*, 21, 72. (New localities of fireflies (Coleoptera: Lampyridae) in south-east Poland) (in Polish)

Szczepański, W.T., Karpiński, L., & Taszakowski, A. (2013). Nowe stanowiska *Omalisus (Omalisus) fontisbellaquaei* Geoffroy, 1785 (Coleoptera: Omalisidae) w południowej Polsce. *Acta entomologica silesiana*, 21, 73. (New localities of *Omalisus* (*Omalisus*) *fontisbellaquaei* Geoffroy, 1785 (Coleoptera: Omalisidae) in Southern Poland) (in Polish)

Szczepański, W.T., Taszakowski, A., & Karpiński, L. (2014). Nowe stanowisko *Hemicoelus costatus* (Aragona, 1830) (Coleoptera: Ptinidae) w Polsce*. Acta Entomologica Silesiana*, 22, 55. (New locality of *Hemicoelus costatus* (Aragona, 1830) (Coleoptera: Ptinidae) in Poland) (in Polish)

Szczepański, W.T., Taszakowski, A., & Karpiński, L. (2015a). Nowe stanowiska bogatkowatych (Buprestidae) w Beskidzie Wschodnim. *Acta Entomologica Silesiana*, 23(online 030), 1-4. (New localities of jewel beetles (Coleoptera: Buprestidae) in the Eastern Beskid Mts) (in Polish with English abstract)

Szczepański, W.T., Taszakowski, A. & Karpiński, L. (2015b). Nowe stanowiska *Cassida panzeri* Weise, 1907 (Coleoptera: Chrysomelidae) w południowej Polsce. *Acta Entomologica Silesiana*, 23(online 023), 1. (New locality of *Cassida panzeri* Weise, 1907 (Coleoptera: Chrysomelidae) in south Poland) (in Polish)

Szczepański, W.T., Taszakowski, A., Karpiński, L. & Kubusiak, A. (2015c). New data on the distribution of ladybird beetles (Coleoptera: Coccinellidae) of the Eastern Beskid Mts. *Nature Journal* (Opole Scientific Society), 48, 24-33.

Szczepański, W.T., Taszakowski, A., Karpiński, L., & Tomecka, M. (2015d). Materiały do znajomości omomiłkowatych, karmazynkowatych i świetlikowatych (Coleoptera: Elateroidea: Cantharidae, Lycidae, Lampyridae) Beskidu Wschodniego. *Acta Entomologica Silesiana*, 23(online 003), 1-7. (Materials to the knowledge of soldier beetles, net-winged beetles and fireflies (Coleoptera: Elateroidea: Cantharidae, Lycidae, Lampyridae) of the Eastern Beskid Mountains) (in Polish with English abstract)

Szczepański, W.T., Taszakowski, A., Karpiński, L., & Kaszyca, N. (2016). Nowe stanowiska sprężykowatych (Coleoptera: Elateridae) w Beskidzie Wschodnim. *Acta entomologica silesiana*, 24(online 001), 1-9. (New localities of click beetles (Coleoptera: Elateridae) in the Eastern Beskidy Mountains) (in Polish with English abstract)

Szołtys, H. & Taszakowski, A. (2017). *Isorhipis nigriceps* (Mannerheim, 1823)-nowy dla fauny Polski gatunek chrząszcza (Coleoptera: Eucnemidae). *Acta Entomologica Silesiana*, 25(online 031), 1-5. (*Isorhipis nigriceps* (Mannerheim, 1823)-a species of beetle (Coleoptera: Eucnemidae) new to the Polish fauna) (in Polish with English abstract)

Szujecki, A.J. (2017). Kusakowate (Staphylinidae) lasów Polski. Aspekt różnorodności i monitoringu zooindykacyjnego. Centrum Informacyjne Lasów Państwowych, Warszawa. 147 pp. (Rove-beetles (Staphylinidae) of Polish forests. Diversity and biomonitoring) (in Polish)

Taszakowski, A., Baran, B., Kaszyca, N., & Depa, Ł. (2015a). Genus *Claviger* Preyssler, 1790 (Coleoptera: Staphylinidae: Pselaphinae) in the Low Beskid Mts. (Poland)-new sites and host affiliation. *Nature Journal* (Opole Scientific Society), 48, 114-119.

Taszakowski, A. & Gorczyca, J. (2018). Lądowe pluskwiaki różnoskrzydłe (Hemiptera: Heteroptera) Beskidu Wschodniego-geneza fauny. *Monograph of the Upper Silesian Museum in Bytom*, 8, 1-159. (Terrestrial true-bugs (Hemiptera: Heteroptera) of the Eastern Beskidy Mountains-origin of fauna) (In Polish with English abstract and summary)

Taszakowski, A., Kaszyca, N., & Mazur, M.A. (2017a). Materiały do znajomości ryjkowców (Coleoptera: Curculionoidea) Beskidu Wschodniego. *Acta entomologica silesiana*, 25(online 006), 1-18. (New data on the weevils (Coleoptera: Curculionoidea) of the Eastern Beskidy Mountains) (in Polish with English abstract)

Taszakowski, A., Morawski, M., Szołtys, H., & Szczepański, W.T. (2017b). Materiały do znajomości stonkowatych (Coleoptera: Chrysomelidae) Beskidu Wschodniego. *Rocznik Muzeum Górnośląskiego w Bytomiu-Przyroda*, 23(online 004), 1-17. (Materials to the knowledge of leaf beetle (Coleoptera: Chrysomelidae) of the Eastern Beskid Mountains) (in Polish with English abstract)

Taszakowski, A., Kaszyca, N., & Szołtys, H. (2018). Materiały do znajomości Staphyliniformia (Coleoptera) Beskidu Wschodniego. *Acta entomologica silesiana*, 26(online 003), 1-10. doi: 10.5281/zenodo.1175615 (Materials to the knowledge of Staphyliniformia (Coleoptera) of the Eastern Beskidy Mountains) (in Polish with English abstract)

Taszakowski, A., Masłowski, A., Karpiński, L., Szczepański, W.T., & Szołtys, H. (2019). Materiały do znajomości biegaczowatych (Coleoptera: Carabidae) Beskidu Wschodniego. *Rocznik Muzeum Górnośląskiego w Bytomiu-Przyroda*, 25 (online 002), 1-18. doi: 10.5281/zenodo.3336072 (Materials to the knowledge of ground beetles (Coleoptera: Carabidae) of the Eastern Beskid Mountains) (in Polish with English abstract)

Taszakowski, A., Szczepański, W.T., Baran, B., & Morawski, M. (2014). Nowe stanowiska *Oxythyrea funesta* (Poda, 1761) (Coleoptera: Scarabaeidae) w Polsce. *Acta entomologica silesiana*, 22, 56-57. (New localities of *Oxythyrea funesta* (Poda, 1761) (Coleoptera: Scarabaeidae) in Poland) (in Polish)

Taszakowski, A., Walczak, M., & Baran, B. (2015b). *Reptalus quinquecostatus* (Dufour, 1833) (Hemiptera: Fulgoromorpha)-new species of cixiid in Poland. *Acta entomologica silesiana*, 23(online 031), 1-8.

Taszakowski, A., Walczak, M., Morawski, M., & Baran, B. (2015c). Piewiki (Hemiptera: Fulgoromorpha et Cicadomorpha) Beskidu Wschodniego. *Acta entomologica silesiana*, 23, 83-96. (Planthoppers and leafhoppers (Hemiptera: Fulgoromorpha et Cicadomorpha) of the Eastern Beskidy Mountains) (in Polish with English abstract)

Trella, T. (1923a). Wykaz chrząszczów okolic Przemyśla. Heteromera. *Polish Journal of Entomology*, 2, 12-19. (Checklist of beetles of Przemyśl environs. Heteromera) (in Polish)

Trella, T. (1923b). Wykaz chrząszczów okolic Przemyśla. Cliavicornia. *Polish Journal of Entomology*, 2, 110-123. (Checklist of beetles of Przemyśl environs. Cliavicornia) (in Polish)

Trella, T. (1925). Wykaz chrząszczów okolic Przemyśla. Wżerki-Teredilia, Miękopokrywe-Malacodermata. *Polish Journal of Entomology*, 3, 122-127. (Checklist of beetles of Przemyśl environs. Teredilia and Malacodermata) (in Polish)

Walczak, M., Kaszyca, N., & Taszakowski, A. (2018). Materiały do znajomości piewików (Hemiptera: Fulgoromorpha & Cicadomorpha) Beskidu Wschodniego. *Acta Entomologica Silesiana*, 26(online 034), 1-9. doi: 10.5281/zenodo.1420001 (Materials to the knowledge of planthoppers and leafhoppers (Hemiptera: Fulgoromorpha, Cicadomorpha) of the Eastern Beskids Mountains) (In Polish with English abstract)

Walczak, M., Taszakowski, A., Skrynetska, I., & Kaszyca, N. (2016). First record of *Criomorphus williamsi* China, 1939 (Hemiptera: Fulgoromorpha: Delphacidae) in Poland. *Acta Entomologica Silesiana*, 24(online 029), 1-8.

Received: March 21, 2019 *Accepted: October 22, 2019*

J. Entomol. Res. Soc., 22(1): 41-51, 2020
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

The First Identification of a Serine Protease Inhibitor (Serpin) Encoding Gene in the Mosquito Species *Aedes cretinus* (Edwards, 1921)

Meryem Şenay ŞENGÜL DEMİRAK

Tokat Gaziosmanpasa University, Department of Molecular Biology and Genetics, 60250
Tokat, TURKEY e-mail: senay.sengul@gop.edu.tr, ORCID ID: 0000-0003-4879-1908

ABSTRACT

Mosquitoes are vectors for many infectious diseases including malaria, yellow fewer, dengue, encephalitis, lymphatic filariasis and others. The mosquito's immune system is critical in fight with the pathogens before infecting a human host. Serine protease inhibitors (serpins, SRPNs) have been shown in many biological processes in insects, such as reproductive and developmental processes, hematophagy, cellular secretion and immunity. Mosquito genome annotations have allowed identification of serpins in various mosquito species but there is little information on their role in mosquito immunity. In this study, a 534 bp nucleotide sequence of a putative serpin gene from the mosquito species *Aedes cretinus* has been identified for the first time through conventional molecular techniques. The amplified gene product encoded a 159 amino acid peptide sequence. The cDNA and peptide sequence analysis have indicated that the identified serpin gene has the highest identity to *Aedes aegypti Srpn2 a*nd *Aedes albopictus Srpn2* genes. Thus, the gene was named as *AcSrpn2* in *Ae. cretinus* due to its possible orthology with other *Aedes* mosquito *Srpn2* genes. Conservation among other mosquito species indicates that SRPN2 may have an important and common role in the mosquito immunity and physiology. The identification of serpin genes in different mosquito species is an important step to determine their biological functions and understand the mosquito immune system.

Key words: Aedes cretinus, mosquito, mosquito immune system, pathogen, serpin, orthologous gene.

Şengül Demirak, M.Ş. (2020). The first identification of a serine protease inhibitor (Serpin) encoding gene in the Mosquito species *Aedes cretinus* (Edwards, 1921). *Journal of the Entomological Research Society,* 22(1), 41-51.

INTRODUCTION

The vectoral capacity of the mosquitoes is determined by the complex interplay between the pathogen and the effectiveness of the vector's innate immune system. In insects, innate immune reactions are developed in response to viral, bacterial, parasitic and fungal infections which include melanization, phagocytosis, autophagy, cellular encapsulation, nodulation, apoptosis, RNA-mediated virus destruction and lysis mechanisms that involve different effector genes (reviewed in Hillyer, 2016). The pathogen is recognized when pathogen-associated molecular patterns (PAMPs) bind to pattern recognition receptors (PRRs) of the host. Following recognition, some PRRs elicit immune reactions such as melanization and phagocytosis while others activate intracellular signaling pathways that include transcriptional activation of immunity genes (Levashina et al, 2001; Choe, Werner, Stöven, Hultmark, & Anderson, 2002; Nakhleh, El Moussawi, & Osta, 2017).

PRR initiates proteolytic cascades that involve serine proteases and serine protease inhibitors (serpins, SRPNs). Serine proteases play critical roles in insect immunity by processing the signal and triggerring the response while their inhibitors diminish the signal and participate in a suicide inhibition. Serpins are usually 350-400 amino acid residues long with a reactive center loop (RCL) that is located 30-40 residues from the C-terminal end that occupies the proteinase active site (Huber & Carrell, 1989). Once the specific bond (scissile bond) at the loop is cleaved, serpin undergoes a conformational change that traps the target proteinase and causes its inactivation (Huntington, Read, & Carrell, 2000; Whisstock & Bottomley, 2006; Dunstone & Whisstock, 2011).

Serpins are structurally conserved but functionally distinct proteins that are present in all higher eukaryotes. The first arthropod serpins were isolated from the hemolymph of the silkworm *Bomyx mori* (Sasaki & Kobayashi, 1984). Similar peptides were also cloned in *Manduca sexta* genome (Kanost, Prasad, & Wells, 1989). With the availability of insect genome sequences, serpin genes have been identified in many insects, including 34 serpin genes in *B. mori* (Zou, Picheng, Weng, Mita, & Jiang, 2009), 32 in *M. sexta* (Li et al, 2018), 7 in *Apis mellifera* (Evans et al, 2006), 31 in *Tribolium castaneum* (Zou et al, 2007) and 35 in *Drosophila melanogaster* (Reichhart, Gubb, & Leclerc, 2011). The genomes of the mosquito species *Anopheles gambiae*, *Aedes aegypti* and *Culex pipiens quinquefasciastus* are also sequenced and includes 18, 23 and 31 serpin genes, respectively (Christophides et al, 2002; Suwanchaichinda & Kanost, 2009; Gulley, Zhang, & Michel, 2013).

D. melanogaster has served as a model organism to understand invertebrate immunity. Through biochemical and genetic studies, some serpins are functionally characterized but some remain to be discovered. Serpins named Spn43Ac and Spn27A were reported to play roles in Toll and prophenolexidase pathways in innate immunity of *D. melanogaster* (Reichhart et al, 2011). Spn27A has been found to activate phenoloxidase (PO) in blood and stimulate melanization reactions (De Gregorio et al, 2002). Mutations in the Spn43Ac gene showed negative regulation of serine proteases as a result of constitutive activation of the Toll pathway which elicit activation of the antimicrobial peptide Drosomycin (Levashina et al, 1999). Other *D. melanogaster* serpins, such as Spn28, Spn38, Spn42, Spn55 and Spn88 have been demonstrated to have roles in development (Reichhart, 2005).

Mosquito are vectors for devastating diseases and the interaction between the pathogen and the mosquito immune system determines the impact of disease transmission. Some serpins have been functionally characterized in different mosquito species. In *Ae. aegypti* and *An.gambiae* genomes, three serpins (SRPN1, SRPN2, SRPN3) are clustered and could be possible orthologs of *D. melanogaster* Spn27A (Michel, Budd, Pinto, Gibson, & Kafatos, 2005). In *An. gambiae*, SRPN2 was shown to be involved in melanization response through inhibition of prophenoloxidase activating proteinase (PAP) (An, Lovell, Kanost, Battaile, & Michel, 2011). In *Ae. aegypti*, SRPN1 and SRPN2 were also shown to be key regulators in melanization response (Zou, Shin, Alvarez, Kokoza, & Raikhel, 2010). SRPN6 in *An. gambiae* was determined to be involved in immunity against malaria parasites since knockdown of *SRPN6* resulted in increased malaria parasite number in the mosquito midgut and salivary glands (Abraham et al, 2005; Pinto, Kafatos, & Michel, 2008). While these studies indicates important roles of serpins in mosquito immunity, biological functions of many mosquito serpins are still unknown.

Aedes cretinus (Edwards, 1921) is a closely related mosquito species to *Ae. albopictus* and *Ae. aegypti* and has been found in Greece and other Mediterranean countries, including Turkey (Patsoula et al, 2006). This species lacks a sequenced genome. Additionally, no immunity related study has been performed with this pecies. *Aedes* mosquitoes are vectors for yellow fever virus, dengue viruses, Zika virus, and other disease agents. Thus, identification and comparison of immunity related genes, such as serpins, will provide insights into the mechanisms involved in *Aedes* mosquito immunity. This study demonstrates the first report of molecular cloning and identification of a serpin encoding gene in the mosquito species, *Ae. cretinus*. About 534 bp nucleotide sequence of a putative serpin gene sequence has been determined, named AcSrpn2, which showed more than 90% peptide identity with possible mosquito ortholog SRPN2. Its existence in the genome and high conservation among other mosquito species are indications that mosquito serpin2 has an important and a common role in mosquito innate immune system. Serpins that are well conserved within the mosquito lineage could help our understanding of their function in relation with mosquito-borne disease transmission. Genome-wide identification of mosquito serpins and comparative genomics will unravel serpin genes in the mosquito genomes and will enrich our knowledge on serpin roles in immune pathways. Therefore, cloning and identification of serpins through molecular analysis is an important step in understanding their roles to prevent mosquito disease transmission.

MATERIALS AND METHODS

Mosquitoes

Aedes cretinus (Edwards 1921) mosquitoes were reared at 25±2 ºC and 80-90% relative humidty with a photoperiod of 16:8 L:D at the Department of Entomology and Agricultural Zoology at Benaki Phytopathological Institute, Athens, Greece.

DNA extraction

Total DNA from a pool of male and female adult mosquitoes were isolated using a protocol described by Barik, Hazra, Prusty, Rath, & Kar (2013). The quantity and purity of the isolated DNA were checked using Multiskan GO (Thermo Scientific) and 0.8% agarose gel electrophoresis.

Primer design

D. melanogaster Spn27A, *An. gambiae* AgSrpn2, *Ae. aegypti* AaSrpn2 and *Ae. albopictus* Srpn2 orthologs were obtained from National Center for Biotechnology (NCBI, https://www.ncbi.nlm.nih.gov/) and Vectorbase (https://www.vectorbase.org/) databases with accession numbers NP652024.1, AGAP006911, AAEL014078 and AALF012780, respectively. Peptide sequences for these serpin genes were aligned using Clustal Omega programme (Sievers et al, 2011). Conserved regions were selected to design degenerate primers: SrpnF1: 5'-GARATTGCCACCAAGTTCTTC (Tm: 57.4 ºC) and Srpn2R1: 5'-CACTTCRGTTTCATCCATGTA (Tm: 55.5 ºC) which were used in polymerase chain reaction (PCR) amplifications.

PCR amplification and sequencing

DNA amplification was performed in a 30 µl volume mixture containing 15 µl 2X Dream Taq Master mix (Thermo Scientific), 5 µl *Ae. cretinus* genomic DNA, 3 µl degenerate primer pair (final concentration of 1 µM each) and 4 µl sterile ddH$_2$O. A negative control reaction was also run in parallel in the absence of genomic DNA, including sterile ddH$_2$O instead. PCR amplification conditions included 3 min initial denaturation at 95 ºC followed by 40 cycles of denaturation at 95 ºC for 30 sec, annealing at 51 ºC for 30 sec and extension at 72 ºC for 30 sec and a final extension for 10 min. PCR products were run in a 1 % agarose gel electrophoresis and positive PCR products were gel purified by using Wizard SV Gel and PCR Clean-up System (Promega) and sent for sequencing (BM Labosis Inc., Ankara). PCR products were sequenced from both ends using forward and reverse PCR primers.

Sequence analysis

Genomic sequences generated from this study were examined using Basic Local Alignment Search Tool (BLAST) programme (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and amino acid sequence alignment was performed with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers et al, 2011) using default settings. Previously identified serpin peptide sequences were retrieved from GenBank or Vectorbase databases and used for phylogenetic analysis. An unrooted phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis software (MEGA 6.0; Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) using the Neighbor-Joining, Minimum Evolution and Maximum Parsimony methods with bootstrap support of 1000 replicates and default parameters. The bootstrap support values >50% were marked on the branches of the tree.

RESULTS AND DISCUSSION

Identification of a putative *Srpn2* gene ortholog in *Aedes cretinus*

Serpins have not been previously described from the mosquito species, *Ae. cretinus*. A putative serpin gene, *Srpn2*, which was previously identified in other *Aedes* mosquito species, was used to determine its possible ortholog in *Ae. cretinus*. Since the genome sequence is unavailable for this species, PCR amplifications using degenerate primers were conducted which resulted in a partial segment of SRPN2 in *Ae. cretinus*. BLAST results have confirmed that *An. gambiae* serpin 2 (*AgSrpn2*), *Ae. aegypti* serpin 2 (*AaSrpn2*) and *Ae. albopictus* serpin 2 (*AalbSrpn2*) genes are possible orthologs of the putative serpin gene identified in *Ae. cretinus*. As a result of high sequence identity among mosquito SRPN2 and orthology (see phylogenetic analysis below), this gene was named as *Ae. cretinus* serpin 2, *AcSrpn2* (GenBank Accession Number: MK592887), in order to be in accordance with the nomenclature of serpin genes in other mosquito species. As shown in Fig. 1, a partial genomic sequence corresponding to a 534 bp region has been determined which also included a 57 bp intronic sequence. In vectorbase database, *AaSrpn2* gene structure contains a long first intron region, thus the first coding exon and the intron regions were unable to be determined in *Ae. cretinus*. The identified *AcSrpn2* gene sequence was corresponding to the exons 4 and 5 of the *AaSrpn2*.

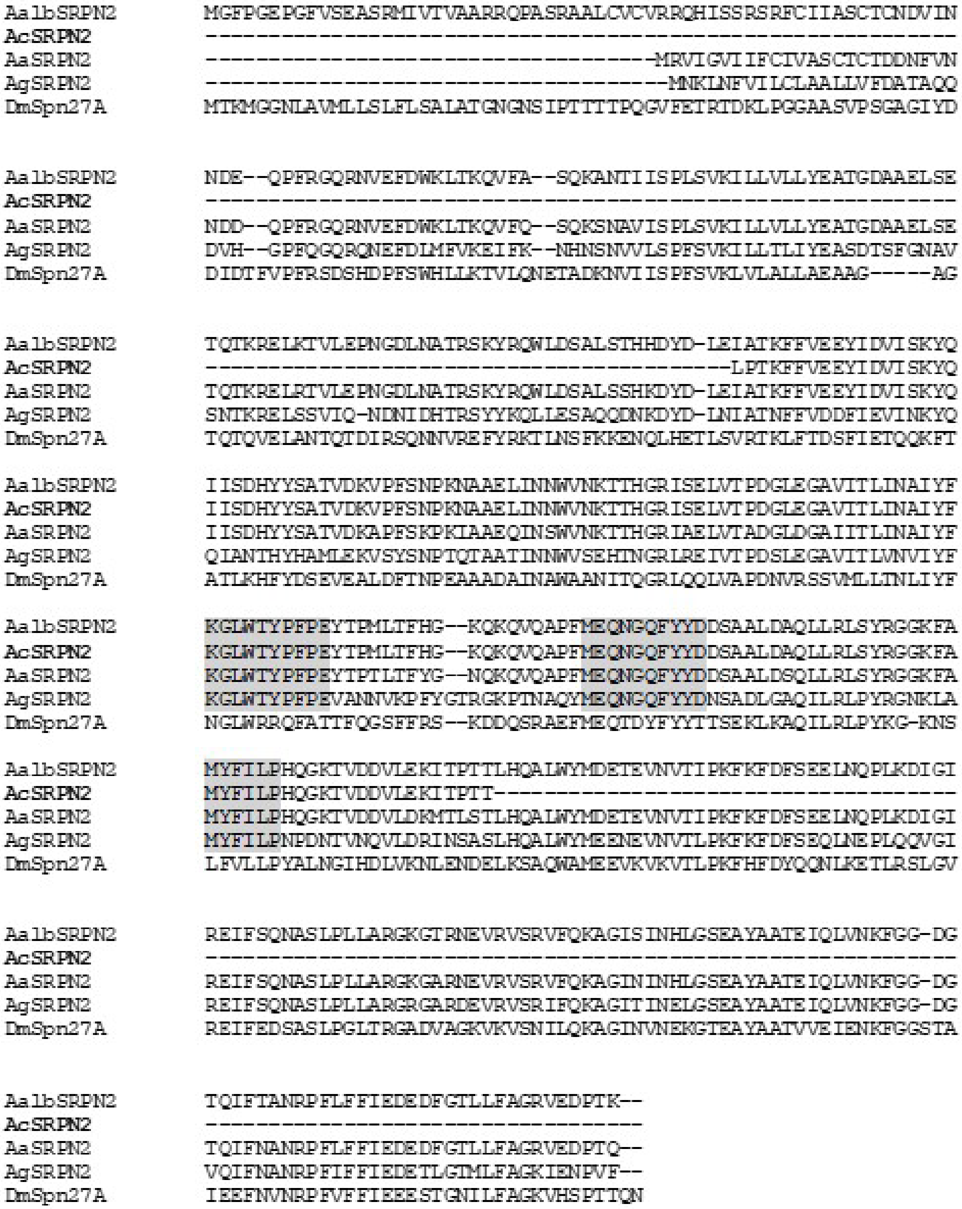
In order to examine the conserved regions of SRPN2 among different mosquito species, AcSRPN2 partial peptide sequence identified in *Ae. cretinus* was aligned with the previously annotated SRPN2 full length peptides from *An. gambiae*, *Ae. aegypti*, *Ae. albopictus* and its possible ortholog in *D. melanogaster* (Fig. 2). Amino acid residues at several positions were identical among *Aedes* species which may indicate that those regions could be structurally important domains of the protein. Additionally, there is a lack of peptide sequence identity when compared with that of *An. gambiae* and *D. melanogaster* SRPN2. While a high degree of conservation (98 % identity) was observed among *Aedes* mosquitoes, about 55 % identity was observed between *Aedes* and *Anopheles* mosquito SRPN2. The genomes of *Aedes* and *Anopheles* mosquitoes are estimated to have diverged about ~ 145-200 MYA (Krzywinski, Grushko, & Besansky, 2006), while *Anopheles* mosquitoes and *D. melanogaster* have diverged approximately 250 MYA (Zdobnov et al, 2002). *D. melanogaster* and mosquitoes have adapted different ecological niches and preferences for their survival. These adaptations and differences may have resulted in significant divergence of their innate immune systems.

```
1     TTGCCCACCAAGTTCTTCGTCGAGGAGTACATTGACGTTATCTCCAAGTATCAGATCATT
1      L  P  T  K  F  F  V  E  E  Y  I  D  V  I  S  K  Y  Q  I  I

61    TCGGATCACTACTACAGCGCCACGGTTGACAAAGTTCCTTTCTCCAATCCGAAAAATGCC
21     S  D  H  Y  Y  S  A  T  V  D  K  V  P  F  S  N  P  K  N  A

121   GCAGAACTGATCAATAACTGGGTGAACAAAACAACGCACGGACGCATTTCGGAACTCGTT
41     A  E  L  I  N  N  W  V  N  K  T  T  H  G  R  I  S  E  L  V

181   ACTCCTGGTAAAAATCCTTGTAATCTTACAAAAAGCATAATAAAAACAACCATTTCTTTT
61     T  P

241   ACAGATGGATTGGAAGGAGCGGTTATCACGTTGATCAACGCCATATACTTCAAGGGACTG
63        D  G  L  E  G  A  V  I  T  L  I  N  A  I  Y  F  K  G  L

301   TGGACTTACCCATTCCCAGAATACACGCCAATGTTGACCTTCCATGGCAAGCAGAAGCAA
82     W  T  Y  P  F  P  E  Y  T  P  M  L  T  F  H  G  K  Q  K  Q

361   GTGCAAGCTCCATTCATGGAACAAAATGGTCAGTTCTACTACGATGATTCAGCGGCATTG
102    V  Q  A  P  F  M  E  Q  N  G  Q  F  Y  Y  D  D  S  A  A  L

421   GACGCTCAGCTTCTGCGTTTGTCGTATCGTGGAGGAAAATTCGCCATGTATTTCATCCTA
122    D  A  Q  L  L  R  L  S  Y  R  G  G  K  F  A  M  Y  F  I  L

481   CCCCACCAAGGAAAGACTGTTGATGATGTTCTGGAGAAAATCACTCCTACCACC
142    P  H  Q  G  K  T  V  D  D  V  L  E  K  I  T  P  T  T
```

Fig. 1. Partial nucleotide sequence and the encoded amino acid sequence of the putative AcSRPN2. Bold sequences indicate the exon regions and deduced peptide sequences are shown benith the coding sequences. Underlined sequence is corresponding to the intronic region.

Mosquito *SRPN2* gene orthology

In order to understand the evolution of mosquito serpins, a phylogenetic tree was constructed using previously identified mosquito serpins from *Ae. aegypti*, *Ae. albopictus* and *An. gambiae* and their possible orthologs in *D. melanogaster*. Although many serpins were identified in these mosquito genomes, 6 different serpins that are more functionally related to SRPN2 were included in the phylogeny. Accordingly, the tree was constructed including mosquito serpins named SRPN1, 2, 3, 5, 8 and 9 and their *D. melanogaster* orthologs (Fig. 3). The tree showed five phylogenetic clusters which clearly indicates that orthologous serpin genes were grouped together. For example, AaSRPN1, AalbSRPN1 and AgSRPN1 serpins were clustered together while AcSRPN2 was clustered with AaSRPN2 and AalbSRPN2. Previously, it has been reported that mosquito serpins SRPN1, SRPN2 and SRPN3 were inhibitory serpins which regulate melanisation by inhibiting PPO-activating proteases (reviewed in Gulley et al, 2013). It appears that these serpins have retained common roles in the same immune pathways. Its ortholog in *D. melanogaster*, Spn27A also regulates the melanization pathway in the hemolymph. In addition to its function in immunity, Spn27A has been found to have a role in development during early embryogenesis. Although, mosquitoes and *D. melanogaster* have different adaptations for their innate immune systems, it is possible that mosquito SRPN2 and *D. melanogaster* Spn27A have retained conserved biological function which may indicate its role in insect-specific features. Additionally, our results also indicate that most mosquito serpins have one-to-one orthology which supports the idea that they had a common ancestral gene before the speciation event.



```
AalbSRPN2      MGFPGEPGFVSEASRMIVTVAARRQPASRAALCVCVRRQHISSRSRFCIIASCTCNDVIN
AcSRPN2        ------------------------------------------------------------
AaSRPN2        ------------------------------------MRVIGVIIFCIVASCTCTDDNFVN
AgSRPN2        -------------------------------------MNKLNFVILCLAALLVFDATAQQ
DmSpn27A       MTKMGGNLAVMLLSLFLSALATGNGNSIPTTTTPQGVFETRTDKLPGGAASVPSGAGIYD

AalbSRPN2      NDE--QPFRGQRNVEFDWKLTKQVFA--SQKANTIISPLSVKILLVLLYEATGDAAELSE
AcSRPN2        ------------------------------------------------------------
AaSRPN2        NDD--QPFRGQRNVEFDWKLTKQVFQ--SQKSNAVISPLSVKILLVLLYEATGDAAELSE
AgSRPN2        DVH--GPFQGQRQNEFDLMFVKEIFK--NHNSNVVLSPFSVKILLTLIYEASDTSFGNAV
DmSpn27A       DIDTFVPFRSDSHDPFSWHLLKTVLQNETADKNVIISPFSVKLVLALLAEAAG-----AG

AalbSRPN2      TQTKRELKTVLEPNGDLNATRSKYRQWLDSALSTHHDYD-LEIATKFFVEEYIDVISKYQ
AcSRPN2        ------------------------------------------LPTKFFVEEYIDVISKYQ
AaSRPN2        TQTKRELRTVLEPNGDLNATRSKYRQWLDSALSSHKDYD-LEIATKFFVEEYIDVISKYQ
AgSRPN2        SNTKRELSSVIQ-NDNIDHTRSYYKQLLESAQQDNKDYD-LNIATNFFVDDFIEVINKYQ
DmSpn27A       TQTQVELANTQTDIRSQNNVREFYRKTLNSFKKENQLHETLSVRTKLFTDSFIETQQKFT

AalbSRPN2      IISDHYYSATVDKVPFSNPKNAAELINNWVNKTTHGRISELVTPDGLEGAVITLINAIYF
AcSRPN2        IISDHYYSATVDKVPFSNPKNAAELINNWVNKTTHGRISELVTPDGLEGAVITLINAIYF
AaSRPN2        IISDHYYSATVDKAPFSKPKIAAEQINSWVNKTTHGRIAELVTADGLDGAIITLINAIYF
AgSRPN2        QIANTHYHAMLEKVSYSNPTQTAATINNWVSEHTNGRLREIVTPDSLEGAVITLVNVIYF
DmSpn27A       ATLKHFYDSEVEALDFTNPEAAADAINAWAANITQGRLQQLVAPDNVRSSVMLLINLIYF

AalbSRPN2      KGLWTYPFPEYTPMLTFHG--KQKQVQAPFMEQNGQFYYDDSAALDAQLLRLSYRGGKFA
AcSRPN2        KGLWTYPFPEYTPMLTFHG--KQKQVQAPFMEQNGQFYYDDSAALDAQLLRLSYRGGKFA
AaSRPN2        KGLWTYPFPEYTPTLTFYG--NQKQVQAPFMEQNGQFYYDDSAALDSQLLRLSYRGGKFA
AgSRPN2        KGLWTYPFPEVANNVKPFYGTRGKPTNAQYMEQNGQFYYDNSADLGAQILRLPYRGNKLA
DmSpn27A       NGLWRRQFATTFQGSFFRS--KDDQSRAEFMEQTDYFYYTTSEKLKAQILRLPYKG-KNS

AalbSRPN2      MYFILPHQGKTVDDVLEKITPTTLHQALWYMDETEVNVTIPKFKFDFSEELNQPLKDIGI
AcSRPN2        MYFILPHQGKTVDDVLEKITPTT-------------------------------------
AaSRPN2        MYFILPHQGKTVDDVLDKMTLSTLHQALWYMDETEVNVTIPKFKFDFSEELNQPLKDIGI
AgSRPN2        MYFILPNPDNTVNQVLDRINSASLHQALWYMEENEVNVTLPKFKFDFSEQLNEPLQQVGI
DmSpn27A       LFVLLPYALNGIHDLVKNLENDELKSAQWAMEEVKVKVTLPKFHFDYQQNLKETLRSLGV

AalbSRPN2      REIFSQNASLPLLARGKGTRNEVRVSRVFQKAGISINHLGSEAYAATEIQLVNKFGG-DG
AcSRPN2        ------------------------------------------------------------
AaSRPN2        REIFSQNASLPLLARGKGARNEVRVSRVFQKAGININHLGSEAYAATEIQLVNKFGG-DG
AgSRPN2        REIFSQNASLPLLARGRGARDEVRVSRIFQKAGITINELGSEAYAATEIQLVNKFGG-DG
DmSpn27A       REIFEDSASLPGLTRGADVAGKVKVSNILQKAGINVNEKGTEAYAATVVEIENKFGGSTA

AalbSRPN2      TQIFTANRPFLFFIEDEDFGTLLFAGRVEDPTK--
AcSRPN2        -----------------------------------
AaSRPN2        TQIFNANRPFLFFIEDEDFGTLLFAGRVEDPTQ--
AgSRPN2        VQIFNANRPFIFFIEDETLGTMLFAGKIENPVF--
DmSpn27A       IEEFNVNRPFVFFIEEESTGNILFAGKVHSPTTQN
```

Fig. 2. Alignment of mosquito SRPN2 peptides and its possible ortholog in *D. melanogaster*. AcSRPN2 was identified in this study and shown in bold. Other mosquito SRPN2 peptide sequences were obtained from Vectorbase and NCBI databases. A minimum of six consecutive amino acid residues that are conserved among all mosquito species are highlighted. Abbreviations: Aalb: *Aedes albopictus*, Ac: *Aedes cretinus*, Aa: *Aedes aegypti*, Ag: *Anopheles gambiae*, Dm: *Drosophila melanogaster*.

Due to different physiological challenges, the expression patterns of mosquito serpins could be highly variable but may provide insight into their biological roles. It is shown that AgSRPNs (including AgSRPN2) are expressed at similar levels in all developmental stages of the *An. gambiae* (Suwanchaichinda & Kanost, 2009).It is also reported that some *AgSrpn* genes show higher expression profile in female mosquitoes compared to males (Baker et al, 2011). Microarray analyses indicated that mRNA levels of serpins changed after a blood meal of the female mosquitoes (Marinotti, Nguyen, Calvo, James, & Ribeiro, 2005). It is indicated that a decrease in expression level of serpins may trigger stronger immune responses within midgut and hemolymph after a blood meal (Gulley et al, 2013). Mosquitoes challenge with different pathogens also elicit serpin expressions. In *Ae. aegypti*, it is reported that AaSRPN2 and other serpin transcript levels are increased after infection with the fungal pathogen *Beauveria bassiana* and regulated by REL1 transcription factors of the Toll pathways (Shin et al, 2005). These results clearly demonstrate that mosquito *Srpn2* has retained essential functions in mosquito innate immune responses in the Toll pathway.

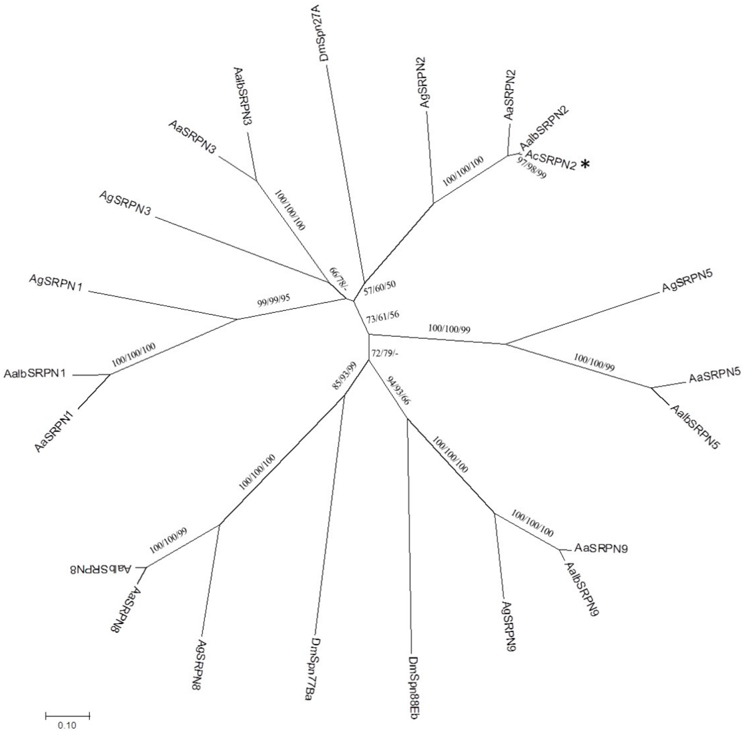


Fig. 3. Phylogenetic relationship between AcSRPN2 (indicated with asteriks) orthologs and representatives of other mosquito serpins. An unrooted tree was constructed using the neighbour-joining algorithm based on the alignment of serpin peptides with removed first exon region. Two additional methods, minimum evolution and maximum parsimony, were also used. Bootstrap values are based on 1000 replicates and represented as the first, second and the third numbers above the branches derived from neighbour-joining, minimum evolution and maximum parsimony analyses, respectively.

CONCLUSION

It is now clear that serpins are involved in a variety of functions in insect immunity. The mosquito genome projects have unravelled many serpin genes in different mosquito species. However, physiological functions of many serpins are still unknown. Studies conducted in *D. melanogaster* provide great wealth of information to understand their functions and thus, their orthologs found in other mosquito species will be helpful to better understand their biological roles in innate immunity.

This study shows the first molecular identification of a serpin encoding gene in the mosquito species, *Aedes cretinus*, in which the genome sequence is unavailable. The *AcSrpn2* gene sequence has been identified and the evolutionary relationship has been determined. The SRPN2 was well conserved among the different mosquito species. It is possible that SRPN2 could have conserved biological functions in the mosquito innate immune system. While bioinformatic analyses reveal existence and divergence of immunity genes in mosquito genomes, functional characterization of interspecies immune responses and immune competencies are still lacking. The biological functions of other mosquito serpins need to be determined in the future to undiscover potential roles in mosquitoes. Mosquito immune system can be manipulated through serpins. Among known serpins, SRPN2 is a good target to interfere with mosquito immune system and develop control strategies to cope with mosquito-borne diseases.

ACKNOWLEDGEMENTS

The author thanks to Dr. Antonios Michaelakis (Department of Entomology and Agricultural Zoology Benaki Phytopathological Institute, Athens, Greece) for providing *Ae. cretinus* adult mosquitoes and undergraduate student Ahmet Simsek for laboratory help. The author is grateful to Tokat Gaziosmanpasa University Research Fund (BAP Project No. 2018/63) for financial support.

REFERENCES

Abraham, E.G., Pinto, S.B., Ghosh, A., Vanlandingham, D.L., Budd, A., Higgs, S., Kafatos, F.C., Jacobs-Lorena, M., & Michel, K. (2005). An immune-responsive serpin, SRPN6, mediates mosquito defense against malaria parasites. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 16327-16332.

An, C., Lovell, S., Kanost, M.R., Battaile, K.P., & Michel, K. (2011). Crystal structure of native Anopheles gambiae serpin-2, a negative regulator of melanization in mosquitoes. *Proteins: Structure, Function, and Bioinformatics*, 79, 1999-2003.

Baker, D.A., Nolan, T., Fischer, B., Pinder, A., Crisanti, A., & Russell, S. (2011). A comprehensive gene expression atlas of sex-and tissue-specificity in the malaria vector, *Anopheles gambiae*. BMC Genomics, 12(1), 296.

Barik, S.K., Hazra, R.K., Prusty, M.R., Rath, A., & Kar, S.K. (2013). A simple, rapid and very efficient protocol for DNA isolation from mosquito species. *Protocol Exchange*.

Choe, K.M., Werner, T., Stöven, S., Hultmark, D., & Anderson, K.V. (2002). Requirement for a peptidoglycan recognition protein (PGRP) in Relish activation and antibacterial immune responses in *Drosophila*. *Science*, 296, 359-362.

Christophides, G.K., Zdobnov, E., Barillas-Mury, C., Birney, E., Blandin, S., Blass, C., Brey, P.T., Collins, F.H., Danielli, A., Dimopoulos, G., Hetru, C., Hoa, N.T., Hoffmann, J.A., Kanzok, S.M., Letunic, I., Levashina, E.A., Loukeris, T.G., Lycett, G., Meister, S., Michel, K., Moita, L.F., Muller, H.M., Osta, M.A., Paskewitz, S.M., Reichhart, J.M., Rzhetsky, A., Troxler, L., Vernick, K.D., Vlachou, D., Volz, J., von Mering, C., Xu, J., Zheng, L., Bork, P., & Kafatos, F.C. (2002). Immunity-related genes and gene families in *Anopheles gambiae*. *Science*, 298, 159-165.

De Gregorio, E., Han, S.J., Lee, W.J., Baek, M.J., Osaki, T., Kawabata, S., Lee, B.L., Iwanaga, S., Lemaitre, B., & Brey, P.T. (2002). An immune-responsive Serpin regulates the melanization cascade in *Drosophila*. *Developmental Cell,* 3, 581-592.

Dunstone, M.A. & Whisstock, J.C. (2011). Crystallography of serpins and serpin complexes. *Methods in Enzymology*, 501, 63-87.

Evans, J.D., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J.L., Jiang, H., Kanost, M., Thompson, G.J., Zou, Z., & Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology*, 15(5), 645-656.

Gulley, M.M., Zhang, X., & Michel, K. (2013). The roles of serpins in mosquito immunology and physiology. *Journal of Insect Physiology*, 59(2), 138-147.

Hillyer, J.F. (2016). Insect immunology and hematopoiesis. *Developmental and Comparative Immunology*, 58, 102-118.

Huber, R. & Carrell, R.W. (1989). Implications of the three-dimensional structure of alpha 1-antitrypsin for structure and function of serpins. Biochemistry, 28, 8951-8966.

Huntington, J.A., Read, R.J., & Carrell, R.W. (2000). Structure of a serpin-protease complex shows inhibition by deformation. *Nature*, 407, 923-926.

Kanost, M.R., Prasad, S.V., & Wells, M.A. (1989). Primary structure of a member of the serpin superfamily of proteinase inhibitors from an insect, *Manduca sexta*. *The Journal of Biological Chemistry*, 264, 965-972.

Krzywinski, J., Grushko, O.G., & Besansky, N.J. (2006). Analysis of the complete mitochondrial DNA from *Anopheles funestus*: an improved dipteran mitochondrial genome annotation and a temporal dimension of mosquito evolution. *Molecular Phylogenetics and Evolution*, *39*(2), 417-423.

Levashina, E.A., Langley, E., Green, C., Gubb, D., Ashburner, M., Hoffmann, J.A., & Reichhart, J.M. (1999). Constitutive activation of toll-mediated antifungal defense in serpin-deficient *Drosophila*. *Science*, 285, 1917-1919.

Levashina, E.A., Moita, L.F., Blandin, S., Vriend, G., Lagueux, M., & Kafatos, F.C. (2001). Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito, *Anopheles gambiae*. *Cell*, 104(5), 709-718.

Li, M., Christen, J.M., Dittmer, N.T., Cao, X., Zhang, X., Jiang, H., & Kanost, M.R. (2018). The *Manduca sexta* serpinome: Analysis of serpin genes and proteins in the tobacco hornworm. *Insect Biochemistry and Molecular Biology*, 102, 21-30.

Marinotti, O., Nguyen, Q.K., Calvo, E., James, A.A., & Ribeiro, J.M.C. (2005). Microarray analysis of genes showing variable expression following a blood meal in *Anopheles gambiae*. *Insect Molecular Biology*, 14(4), 365-373.

Michel, K., Budd, A., Pinto, S., Gibson, T.J., & Kafatos, F.C. (2005). *Anopheles gambiae* SRPN2 facilitates midgut invasion by the malaria parasite *Plasmodium berghei*. EMBO Reports 6, 891-897.

Nakhleh, J., El Moussawi, L., & Osta, M.A. (2017). Advances in Insect Physiology, Insect Immunity. In P., Ligoxygakis (Ed.) *The melanization response in insect immunity* (1st ed., pp. 83-109). United Kingdom, Academic Press.

Patsoula, E., Samanidou-Voyadjoglou, A., Spanakos, G., Kremastinou, J., Nasioulas, G., & Vakalis, N.C. (2006). Molecular and morphological characterization of *Aedes albopictus* in northwestern Greece and differentiation from *Aedes cretinus* and *Aedes aegypti*. *Journal of Medical Entomology*, 43, 40-54.

Pinto, S.B., Kafatos, F.C., & Michel, K. (2008). The parasite invasion marker SRPN6 reduces sporozoite numbers in salivary glands of *Anopheles gambiae*. *Cellular Microbiology*, 10, 891-898.

Reichhart, J.M. (2005). Tip of another iceberg: *Drosophila* serpins. *Trends in Cell Biology*, 15(12), 659-665.

Reichhart, J.M., Gubb, D., & Leclerc, V. (2011). The *Drosophila* serpins: multiple functions in immunity and morphogenesis. *Methods in Enzymology*, 499, 205-225.

Sasaki, T. & Kobayashi, K. (1984). Isolation of two novel proteinase inhibitors from hemolymph of silkworm larva, *Bombyx mori*. Comparison with human serum proteinase inhibitors. *Journal of Biochemistry,* 95, 1009-1017.

Shin, S.W., Kokoza, V., Bian, G., Cheon, H.M., Kim, Y.J., & Raikhel, A.S. (2005). REL1, a homologue of *Drosophila* dorsal, regulates toll antifungal immune pathway in the female mosquito *Aedes aegypti*. *Journal of Biological Chemistry*, 280(16), 16499-16507.

Sievers, F., Wilm A., Dineen D., Gibson T.J., Karplus K., Li W., Lopez R., McWilliam H., Remmert M., Söding J., Thompson J.D., & Higgins D.G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, 7, 539.

Suwanchaichinda, C. & Kanost, M.R. (2009). The serpin gene family in *Anopheles gambiae*. *Gene*, 442, 47-54.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725-2729.

Whisstock, J.C. & Bottomley, S.P. (2006). Molecular gymnastics: serpin structure, folding and misfolding. *Current Opinion in Structural Biology*, 16(6), 761-768.

Zdobnov, E.M., Von Mering, C., Letunic, I., Torrents, D., Suyama, M., Copley, R.R., Christophides, G.K., Thomasova, D., Holt, R.A., Subramanian, G.M., Mueller, H.M., Dimopoulos, G., Law, J.H., Wells, M.A., Birney, E., Charlab, R., Halpern, A.L., Kokoza, E., Kraft, C.L., Lai, Z., Lewis, S., Louis, C., Barillas-Mury, C., Nusskern, D., Rubin, G.M., Salzberg, S.L., Sutton, G.G., Topalis, P., Wides, R., Wincker, P., Yandell, M., Collins, F.H., Ribeiro, J., Gelbart, W.M., Kafatos, F.C., & Bork, P. (2002). Comparative genome and proteome analysis of *Anopheles gambiae* and *Drosophila melanogaster*. *Science*, *298*(5591), 149-159.

Zou, Z., Evans, J.D., Lu, Z., Zhao, P., Williams, M., Sumathipala, N., Hetru, C., Hultmark, D., & Jiang, H. (2007). Comparative genomic analysis of the *Tribolium* immune system. *Genome Biology*, 8(8), R177.

Zou, Z., Picheng, Z., Weng, H., Mita, K., & Jiang, H. (2009). A comparative analysis of serpin genes in the silkworm genome. *Genomics*, 93, 367-375.

Zou, Z., Shin, S.W., Alvarez, K.S., Kokoza, V., & Raikhel, A.S. (2010). Distinct melanization pathways in the mosquito *Aedes aegypti*. *Immunity*, 32, 41-53.

Received: April 19, 2019　　*Accepted: September 23, 2019*

J. Entomol. Res. Soc., 22(1): 53-73, 2020
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

A Comparative Study on Temperature and Relative Humidity Data of Three Caves in Different Climatic Regions of Turkey, with Notes on the Distribution of Anatolian Cave Crickets (Insecta, Orthoptera, Rhaphidophoridae)

Mehmet Sait TAYLAN[1] Nadim YILMAZER[2] Deniz ŞİRİN[3]*

[1]Department of Biology, Institute of Science, Hakkari University, Hakkari, TURKEY,
[2,3]Department of Biology, Faculty of Art and Science, Namık Kemal University, Tekirdağ, TURKEY
e-mails: msaittaylan@hakkari.edu.tr, nyilmazer@nku.edu.tr, denizsirin19@gmail.com
ORCID IDs: [1]0000-0002-2084-920X, [2]0000-0002-9935-9608, [3]0000-0001-5475-173X

ABSTRACT

The diversity of cave crickets (Orthoptera, Rhaphidophoridae) remarkably differs between geographic regions in Anatolia. While only 4 species are distributed in northern Anatolia where Black Sea climate prevails, 14 species are found throughout southern and western Anatolia where the Mediterranean climate is dominant. However, no cave crickets were reported from the middle and eastern Anatolia where the continental climate is present. There is no data-based study on the distribution pattern of cave cricket species in Anatolia. This study aimed to reveal any possible relation between climatic conditions and distribution of cave crickets in three caves selected as representatives for three climate types present in Anatolia. Temperature and relative humidity in the ecological zones of the given caves and the surface were periodically measured by using data loggers. Our data show within cave variations in temperature and relative humidity among all three caves: (i) variations in temperature and relative humidity decreased from the entrance zone to the dark zone, (ii) over the winter period, temperature increased from the entrance zone to the dark zone, (iii) over the summer period, temperature decreased from the entrance zone to the dark zone, (iv) the most distinct temperature variation was measured in continental climate, and (v) the major similarity of climatic conditions between cave and climate type was observed in the Black Sea region which cave crickets species have widened distribution and limited speciation.

Key words: Rhaphidophoridae, cave crickets, cave zones, climatic factors, distribution, Anatolia.

Taylan, M.S., Yılmazer, N., & Şirin, D. (2020). A Comparative study on temperature and relative humidity data of three caves in different climatic regions of Turkey, with notes on the distribution of Anatoliancave crickets (Insecta, Orthoptera, Rhaphidophoridae). *Journal of the Entomological Research Society*, 22(1), 53-73.

INTRODUCTION

Caves, naturally occurring underground spaces, range in size from single small rooms to interconnecting passages which can become minor caves in time, and host many unique biota that are highly interconnected and interdependent, and that fall into three different groups based on the amount of time they spend in the cave: Troglobites or cave dwellers, troglophiles or cave lovers, and trogloxenes or cave guests. Although cave ecosystems in which a diversity of habitats lies are often thought of as constant, they directly depend upon the surrounding surface environment that is very changeable. While cave ecosystems differ in terms of environmental conditions such as temperature, relative humidity, and light intensity, these conditions can change even in the entrance, twilight and dark zones of a cave (Cropley, 1965; Culver, 1982; De Freitas & Littlejohn, 1987; Gample, Dogwiler, & Mylroie, 2000; Culver & Pipan, 2009; Romero, 2009).

Caves are valued as natural underground laboratories by scientists since they can provide insight into evolutionary and ecological processes because of their geographical and hydrological isolation, by allowing rapid colonization (Vandel, 1964; Poulson & White, 1969; Sbordoni, 1980). Ecological studies on caves began with simple experimental observations towards the end of the 19th century (Crump, 1886; Leonard, 1889; Crump, 1890) and continued with increasing momentum (Di Russo, Carchini, & Sbordoni, 1994; Carchini, Rampini, & Sbordoni, 1994; Di Russo & Sbordoni 1998; Di Russo, Carchini, Rampini, Lucarelli, & Sbordoni, 1999, Yoder, Hobbs, & Hazelton, 2002; Weckerly, 2012). Especially in recent years methodological advances in speleology have enabled the development of biospeleology involving more detailed ecological studies in which temperature, RH, pressure, radon and CO_2 gas measurements of a given cave were taken, and air currents in the vertical and horizontal caves and the connection between surface and cave climate were examined (Cropley, 1965; De Freitas, Littlejohn, Clarkson, & Kristament, 1982; Ek & Gewelt, 1985; De Freitas & Littlejohn, 1987; Nepstad & Pisarowicz, 1989; Smithson, 1991; Forbes, 1998; Gample et al, 2000; Sanderson & Bourne, 2002; Gunn, 2004; Stoeva & Stoev, 2005; De Freitas, 2010; Gregoric, Zidansek, & Vaupotic, 2011). Surprisingly, despite the approximate presence of 40,000 caves in Turkey (Ozansoy & Mengi, 2006) there are few studies on cave biology. The majority of the studies carried out to date are faunistic rather than ecological (Kunt, Yağmur, Özkütük, Durmuş, & Anlaş, 2010).

So far, studies on relationships between ecological factors and the biology (reproduction, nutrition, physiology, etc.) of cave crickets have been restricted to North America and Europe. However, there are not any studies on the ecology of cave crickets in Anatolia. The distribution of cave crickets (Orthoptera, Rhaphidophoridae) remarkably differs among geographic regions in Anatolia. While only four species are distributed along northern Anatolia where the Black Sea climate prevails (Bey-Bienko, 1969; Di Russo, Rampini, & Landeck, 2007; Taylan, Mol, & Şirin, 2015), 14 species are found throughout southern and western Anatolia where the Mediterranean climate is dominant (Bolivar, 1899; Taylan, Di Russo, Rampini, & Cobolli, 2011; Rampini, Di Russo, Taylan, Gelosa, & Cobolli, 2012; Taylan, Di Russo, Cobolli, & Rampini, 2012; Gorochov & Ünal, 2015). However, cave crickets may not exist in the middle and eastern Anatolia where the Continental climate is present (Taylan et al, 2011) (Table 2, Fig. 1). Although some previous studies assumed that ancient central Anatolian lake systems (Taylan & Şirin, 2016), and climatic factors including temperature and RH in the cave ecological zones and the surface may affect the distribution of cave crickets (Taylan et al, 2011), there is no data-based explanation of the distribution pattern of different species of cave crickets in Anatolia. Therefore, this study aimed to reveal any possible relation between climatic conditions and distribution of cave crickets as model organisms in three caves selected to represent three climate types seen in Anatolia (Fig. 1), (i) by comparing the annual data of temperature and RH for each cave, cave zone and climate type, (ii) comparing the relationship between surface and cave climate in different climate regions, and (iii) evaluating possible effects on the biogeographic distribution of cave crickets.

Table 1. The data of surface and ecological zones of the study caves.

Caves	Measured data	Surface	Entrance zone	Twilight zone	Dark zone
Geyikbayırı cave	Annual mean temperature	18.98	15.06	15.65	16.64
	Annual mean relative humidity	50.64	98.53	98.91	98.94
	Weekly maximum mean temperature	32.99	20.83	17.2	17.61
	Weekly minimum mean temperature	5.06	7.78	14.22	15.06
	Weekly maximum mean relative humidity	77.8	99.0	99.0	99.0
	Weekly minimum mean relative humidity	18.37	97.0	97.0	98.0
Sipahiler cave	Annual mean temperature	11.10	9.79	10.08	9.76
	Annual mean relative humidity	78.65	78.61	99.85	98.98
	Weekly maximum mean temperature	23.6	15.14	12.1	10.72
	Weekly minimum mean temperature	-0.98	6.25	8.74	8.89
	Weekly maximum mean relative humidity	91.75	88.61	99.99	99.0
	Weekly minimum mean relative humidity	60.79	68.71	96.61	97.8
Tuluntaş cave	Annual mean temperature	11.81	11.02	9.43	10.09
	Annual mean relative humidity	57.12	98.60	98.92	98.99
	Weekly maximum mean temperature	30.23	16.98	10.6	10.56
	Weekly minimum mean temperature	-7.83	5.1	7.35	9.56
	Weekly maximum mean relative humidity	87.25	99.0	99.0	99.0
	Weekly minimum mean relative humidity	27.29	97.0	97.08	98.99

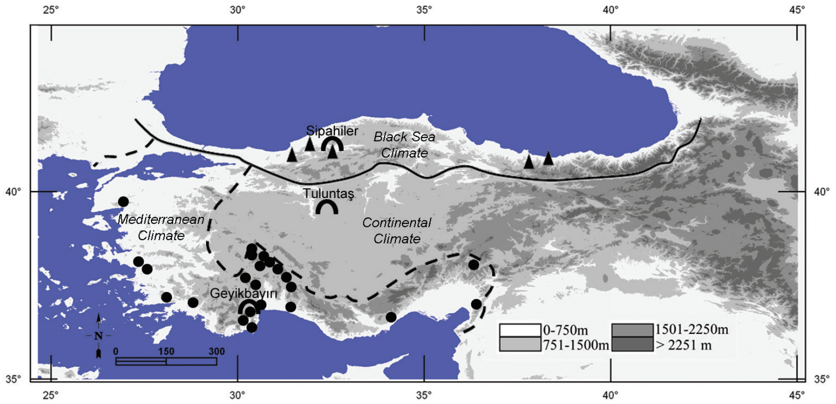


Fig. 1. Localities of the study caves and climatic regions in Turkey. The spots indicate the locations of the cave crickets.

MATERIAL AND METHODS

The climate characteristics in Turkey with reference to the locations of the studied caves

The climate mainly determined by the topography and other geographical factors such as landscape and altitude, along with atmospheric circulation patterns. Three different types of climate are found in the country; (i) Mediterranean climate with hot, moderately dry summers and mild to cool, wet winters, (ii) Black Sea climate with warm, wet summers and cool to cold, wet winters, and (iii) Continental climate with sharply contrasting seasons in that summers are extremely hot and dry and winters are especially severe. Mediterranean climate prevails in the coastal areas of Turkey bordering the Aegean Sea and the Mediterranean Sea, having both winter and late spring precipitation peaks which vary from 580 to 1.300 mm. Black Sea climate is seen in the coastal areas of the country which border the Black Sea and receive high precipitation (2000-2500 mm) throughout the year. About half of Turkey, which lies between the North Anatolian Mountains with 3942 m elevation and the South Anatolian Mountains (Taurus) with an elevation of 3000 m, is dominated by Continental climate with a precipitation peak in late spring or early summer, being between 295 and 770 mm for the central Anatolia, 321 and 1230 mm for the East Anatolia, and 331 and 821 mm for the south-eastern Anatolia (Atalay, 2000; Sensoy, Demircan, Ulupinar, & Balta, 2008; Deniz, Toros, & Incecik, 2011). Depending on location and elevation, the annual mean temperature (AMT) in Turkey varies from 3.6°C to 20.1°C, reaching a maximum in July (the Southern parts of Anatolia and the Mediterranean coasts) and a minimum in January (the East Anatolia). The annual mean precipitation in the country is around 648 mm, ranging from 295 (the central Anatolia) to 2220 mm (the eastern Black Sea coasts), and the precipitation occurs mostly during the winter months (Deniz et al, 2011). Three natural non-tourist caves, each from three different climatic regions and having horizontal structure and similar entrance size, were selected as the study caves: Geyikbayırı cave, Sipahiler cave and Tuluntaş cave which are located in the Mediterranean, the Black Sea and the Continental climate regions, respectively (Fig. 1).

The characteristics of the studied caves

Geyikbayırı cave is located in the village of Geyikbayırı about 26 km away from Antalya Province (N 36°52’ 37’’- E 30°28’ 21’’, entrance elevation is 550 m). The cave with a total length of 120 m is 7 m deep. It has completely developed in massive gray Permian limestone, with Triassic conglomeratic limestone and sandstone in its base, and has many travertine formations. This cave, which has almost completed its development process, was researched and mapped by General Directorate of Mineral Research and Exploration of Turkey (MTA) in 1982 (Gürcan, Yamaç, Tanındı, & Uygun, 2006). *Dolichopoda lycia* (Galvagni, 2006) is known to occur in this cave (Taylan & Şirin, 2016).

Sipahiler cave having dimensions of 338 m length and 32 m depth is situated (N 21°38’ 15’’- E 32°29’ 42’’, entrance elevation is 204 m), in Kayadibi, a village in the central district of Bartın Province (Gürcan et al, 2006). Although a detailed geological investigation of the cave has not yet been carried out, the cave is famous for its large-scale stalactites, stalagmites, and columns that form a wonderful spectacle. The Society of Anatolian Speleology Group (ASPEG) researched and mapped the cave in 2009. *Troglophilus aspegi* Taylan & Şirin, 2015 was reported from this cave (Taylan et al, 2015).

Tuluntaş cave, 15 km away from Ankara, is located in the village of İncek in Gölbaşı district of Ankara Province (N 39°46’ 26’’- E 32°40’ 59’’, entrance elevation is 1251 m), and is 549 m long and 10 m deep. The cave with stalagmites and stalactites has developed in Permo-Triassic limestone blocks which spread about 5-7.5 square km and have a thickness of 30-40 m. This cave has almost completed its development process~~,~~ and consists of a horizontal gallery and interconnecting passages. It was explored and mapped in a joint work of MTA and Cave Research Association of Turkey (MAD) in 1992 (Gürcan et al, 2006). No cave crickets were found in this cave.

Data loggers: their technical features and applications

Tt-Technic Ds 100 data loggers (Templog Electronic, Istanbul) were employed to continuously monitor air temperature and relative humidity (RH) at one-hour intervals. Battery-powered, these waterproof devices are small enough to be placed in environments such as caves and cold storages/rooms. They measure the temperature in the range of -20 ºC to +85 ºC with a standard error of ± 0.05 ºC and high measurement accuracy of 0.05 ºC, and RH in the range of zero to 100% with a standard error of ± 0.05% and a high measurement accuracy of 0.05%. A total of 12 data loggers were used in the present study, four data loggers in each cave. One device was placed in outside of cave as 10 m average distance among entrance zone to the cave to measure the surface temperature and RH, while the others were placed in the ecological zones (to entrance zone, twilight zone, and dark zone) (Culver & Pipan, 2009), with distance from entrance of cave; 10 m, 40 m, and 100 m, respectively to measure the temperature and RH of the cave atmosphere. The caves were visited on a three-monthly basis for data collection and battery replacement between the dates of January 15, 2012 and January 14, 2013.

Statistical analysis

With descriptive statistics, the maximum, minimum, average and standard deviations of temperature and humidity values on a weekly and annual basis were determined. Whether there is a difference in the humidity and temperature values of caves compared to climate characteristics was tested by one-way ANOVA. For multiple comparisons, Post-Hoc (Scheffe) test was used to find out from which caves the difference found resulted. The Pearson product-moment correlation coefficient test was used to refer to the correlation between surface and cave zones in terms of temperature and humidity values of each cave. Besides, descriptive statistical methods were used for comparison between each cave's zones. IBM SPSS Statistics 22.0 software was employed for all statistical analysis.

RESULTS

Daily air temperature and RH data were obtained from Geyikbayırı, Sipahiler and Tuluntaş caves over one year-study period. These data were analyzed based on weekly mean data. In order to make the data more understandable, atmospheric and ecological zones of the study caves were compared with each other for each study cave.

Comparison of temperature in surface and ecological zones among the caves

Whether there is a difference in temperature values among the caves (or among the climate types as each cave is in a separate climate) compared to climatic characteristics was tested by one-way ANOVA, and it was found that there was a statistically significant difference in all zones of the cave in each climate (Table 3). Scheffe's multiple comparison test was employed to determine which caves resulted in this difference. Accordingly, if the zones are evaluated separately:

Surface: Statistical analysis showed that in terms of surface zone temperature values Geyikbayırı cave was different from Sipahiler and Tuluntaş caves, but Sipahiler and Tuluntaş caves did not have a significant difference in terms of these parameters (Table 4). As the most southern cave, Geyikbayırı cave had the highest value of 18.98°C in terms of AMT. The AMT of Sipahiler cave, which is covered with temperate rain forests in the north, was 11.1 °C, while it was 11.81 °C in Tuluntaş cave, which is nearly located at the middle of Anatolia where the Continental climate is dominant (Table 1). The annual mean temperatures (AMTs) were negative in Tuluntaş cave for 12 weeks (minimum weekly mean -7.83 °C), negative temperatures lasted one week in Sipahiler cave (minimum weekly mean -0.98 °C), however negative values were never found in Geyikbayırı cave during the study year (minimum weekly mean 5.06°C) (Fig. 2A). The AMTs increased in the first 26-28 weeks of the year in three study caves, reaching maximum weekly values which were 32.99 °C for Geyikbayırı cave, 30.23 °C for Tuluntaş cave, and 23.6 °C for Sipahiler cave, while it decreased in the remainder of the year (Table 1, Fig. 2A).

Table 3. One-way ANOVA results of temperature (°C) values of cave zones compared to caves/climate characteristics.

		Sum of Squares	**df**	**Mean Square**	**F**	**p**
Surface	Between Groups	1972,862	2	986,431	12,980	,000
	Within Groups	11627,119	153	75,994		
	Total	13599,980	155			
Entrance zone	Between Groups	793,314	2	396,657	28,810	,000
	Within Groups	2106,494	153	13,768		
	Total	2899,808	155			
Twilight zone	Between Groups	1218,735	2	609,367	686,338	,000
	Within Groups	135,841	153	,888		
	Total	1354,576	155			
Dark zone	Between Groups	1564,857	2	782,428	2066,285	,000
	Within Groups	57,936	153	,379		
	Total	1622,792	155			

Entrance zone: Statistical analysis revealed that Geyikbayırı cave was different from Sipahiler and Tuluntaş caves in terms of entrance zone temperature values, but Sipahiler and Tuluntaş caves having no significant difference in terms of these parameters (Table 4). The AMT of this zone was determined as 15.06 °C in Geyikbayırı cave, 11.02 °C in Tuluntaş cave, and 9.79 °C in Sipahiler cave (Table 1). Although being the closest to the outside, negative temperatures were not observed in the entrance zone of all three study caves. Minimum values of 7.78 °C, 5.1 °C, and 6.25°C were observed in the 6th week for Geyikbayırı cave and the first week for Tuluntaş and Sipahiler caves, respectively (Table 1, Fig. 2B). The WMTs gradually increased in the first 30-38 weeks of the year, followed by a faster decline, reaching the value of the first week (Fig. 2B). Maximum values were measured in the 38th, 33rd and 30th week of the year as 20.83 °C, 15.14 °C, and 16.98 °C for Geyikbayırı, Sipahiler, and Tuluntaş caves, respectively (Table 1, Fig. 2B).

Twilight zone: It was found from statistical analysis that the three caves differed in terms of twilight zone temperature values (Table 4). The AMT of this zone was observed to be 15.65 °C in Geyikbayırı cave, 9.43 °C in Tuluntaş cave, and 10.08 °C in Sipahiler cave (Table 1). In this zone, the minimum weekly mean temperatures (WMTs) were higher than those of the entrance zone, in that they were 14.22 °C in Geyikbayırı cave in the 7th week, 7.35 °C in Tuluntaş cave in the 3rd week, and 8.74°C in Sipahiler cave in the 15th week (Table 1, Fig. 2C). These zonal temperatures exhibited a gradual increase during the first 39-41 weeks of the year and a rapid decrease after a shorter period (Fig. 2C). The WMTs reached a maximum of 17.20 °C in the 41st week, 12.10 °C in the 40th week, and 10.60 °C in the 39th week in Geyikbayırı, Sipahiler, and Tuluntaş caves, respectively (Table 1, Fig. 2C). There was a variation in the WMTs in this zone of all three caves from 2.98 °C to 3.36 °C (Fig. 2C).

Table 4. Post-Hoc (Scheffe) test results of temperature (°C) values of cave zones compared to caves/ climate characteristics. (1. Geyik Bayırı Cave, 2. Sipahiler Cave, 3. Tuluntaş Cave)

Dependent Variable		**(I) caves**	**(J) caves**	**Mean Difference (I-J)**	**p**		**Dependent Variable**		**(I) caves**	**(J) caves**	**Mean Difference (I-J)**	**p**
Surface	Scheffe	1,00	2,00	,000	,000		Twilight zone	Scheffe	1,00	2,00	,000	,000
			3,00	,000	,000					3,00	,000	,000
		2,00	1,00	,000	,000				2,00	1,00	,000	,000
			3,00	,917	,917					3,00	,003	,003
		3,00	1,00	,000	,000				3,00	1,00	,000	,000
			2,00	,917	,917					2,00	,003	,003
Entrance zone	Scheffe	1,00	2,00	,000	,000		Dark zone	Scheffe	1,00	2,00	,000	,000
			3,00	,000	,000					3,00	,000	,000
		2,00	1,00	,000	,000				1,00	1,00	,000	,000
			3,00	,245	,245					3,00	,025	,025
		3,00	1,00	,000	,000				3,00	1,00	,000	,000
			2,00	,245	,245					2,00	,025	,025

*The mean difference is significant at the 0.05 level.

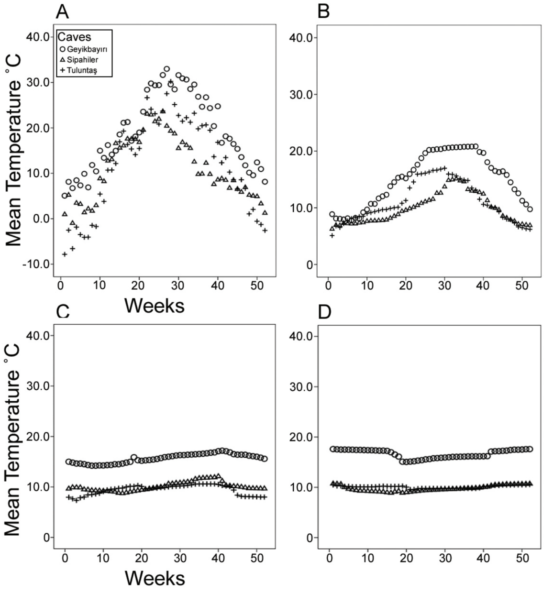


Fig. 2. Comparison of weekly mean temperatures of surface and ecological zones of the study caves (A: Surface, B: Entrance zone, C: Twilight zone, D: Dark zone).

Dark zone: Based on statistical analysis, Geyikbayırı, Sipahiler, and Tuluntaş caves were found to be different from each other in terms of temperature values of the dark zone (Table 4). The AMT of this zone was quite similar to that of the twilight zone, being 16.64 °C, 10.09 °C, and 9.76 °C in Geyikbayırı, Tuluntaş, and Sipahiler caves, respectively (Table 1). This zone exhibited a different pattern of temperature variation, such that the WMT decreased gradually in Geyikbayırı and Tuluntaş caves until the 15th and the 20th week, respectively, then showing a rapid decline in the former for 5 weeks and in the latter for 2 weeks, following a gradual increase in Geyikbayırı cave until the 41st week and in Tuluntaş cave until the 38th week, and a high increase during the following two weeks, and a gradual increase until the end of the year. In Sipahiler cave, the temperature of this zone decreased until the 16th week~~,~~ and gradually increased until the end of the year (Fig. 2D). In this zone, the minimum WMTs were higher than in the entrance zone and recorded as 15.06 °C in Geyikbayırı cave in the 20th week, 9.56°C in Tuluntaş cave in the 21st week, and 8.89 °C in Sipahiler cave in the 16th week (Table 1, Fig. 2D). Yet, the maximum WMTs reached 17.61°C in Geyikbayırı cave and 10.72 °C in Sipahiler cave in the first week, while 10.56 °C in Tuluntaş cave in the 52nd week of the year (Table 1, Fig. 2D). Variation in WMT was found to be in the range of 1.00°C to 2.55°C in all three study caves (Fig. 2D).

Temperature variations in surface and ecological zones of each study cave

Geyikbayırı cave: Pearson correlation analysis revealed a significant positive correlation between surface temperature values and entrance and twilight zone temperature values in this cave, whereas a significant but negative correlation was found between surface and dark zone. On the other hand, it was determined that entrance zone temperature values showed a significant and positive relationship with twilight zone temperature values, but a significant but negative correlation with dark zone temperature values. Furthermore, a significant negative correlation was found between twilight and dark zones temperature values, whereas a negative correlation was found between dark zone temperature values and other ecological zones (Table 7). In this cave, the temperature followed a very similar pattern in the atmospheric and entrance zones. The WMTs increased from 5.06 °C to 32.99 °C from the first week until the 27th week of the year in the surface, while from 7.78 °C to 20.83 °C from the 6th week through the 38th week in the entrance zone, followed by a gradual decrease reaching the initial temperatures in both zones. The WMTs of the twilight zone continued to fall from the first week through the 7th week (from 15.00°C to 14.22°C), reached 17.2 °C by gradually increasing from the 8th week to the 42nd week, and then dropped to 15.58 °C in the last week of the year (Fig. 3A). The WMTs of the dark zone were quite different from other zones during the study year, in that a WMT variation of only 0.36 °C occurred from the first week to the 15th week of the year (a drop from 17.61 °C to 17.25 °C), and subsequently another temperature drop of only 2.15 °C (15.15 °C from 17.25 °C) over the next 4 weeks was observed, followed by a gradual increase from the 20th week through the 41st week, and about a 1 °C increase (from 16.2 °C to 17.12 °C) between the 41st and 42nd weeks, and then a gradual increase until the end of the year (Fig. 3A).

Sipahiler cave: It was revealed by Pearson correlation analysis that a significant positive correlation between surface and entrance zones temperature values was found in this cave, but the correlation between surface and dark zones was significant but negative, yet there was no significant correlation between surface and twilight zones temperature values. On the other hand, a significant positive correlation was determined between temperature values of entrance and twilight zones, while entrance zone temperature values did not show a significant correlation with dark zone temperature values. In addition, there was a significant positive correlation between twilight and dark zones temperature values (Table 7). Sipahiler cave followed a pattern very similar to that of Geyikbayırı cave for temperature in the surface and entrance zones. The WMTs showed an increase from -0.98°C to 23.6 °C from the first week to the 26th week of the year in the atmospheric zone, while an increase from 6.25 °C to 15.14 °C was observed from the first week to the 33rd week in the entrance zone; however, they gradually dropped up to the initial temperature values in both zones in the remainder of the year (Fig. 3B). Negative temperatures lasted a week at the beginning of the year on the surface. In the twilight zone, WMTs dropped to 8.74 °C from 9.66 °C until the 16th week of the year, followed by a gradual increase of 3.36 °C until the 40th week, reaching a maximum, and then a drop until the end of the year (Table 1, Fig. 3B). The WMTs of the dark zone showed a decrease of 1.83 °C in the first 16 weeks of the year, reaching the lowest temperature value of 8.89 °C, and then a gradual increase from this week to the end of the year (Fig. 3B).

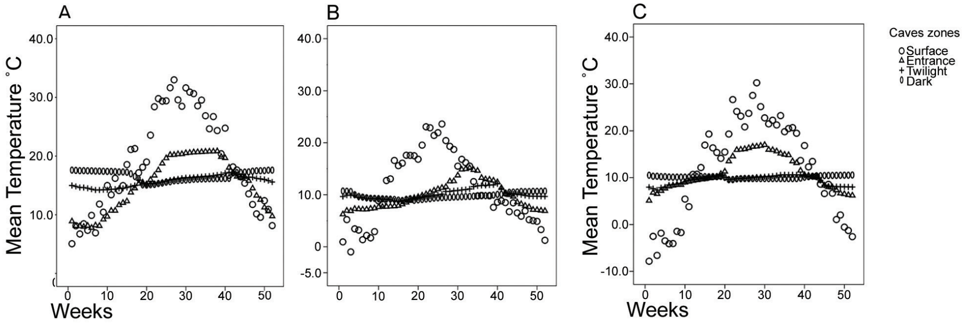


Fig. 3. Comparison of weekly mean temperature variations in surface and ecological zones of each study cave (A: Geyikbayırı Cave, B: Sipahiler Cave, C: Tuluntaş Cave)

Tuluntaş cave: With Pearson correlation analysis, it was revealed that there was a significant positive correlation between surface temperature values and entrance and twilight zones temperature values in this cave, whereas a significant but negative correlation was found between surface and dark zone temperature values. Also, it was determined that entrance zone temperature values showed a significant positive correlation with twilight zone temperature values~~,~~ and a significant negative correlation with dark zone temperature values. Additionally, whereas a significant negative correlation was found between twilight and dark zones temperature values, a negative correlation was found between dark zone temperature values and other ecological zones (Table 7). The WMTs of the atmospheric and entrance zones of this cave showed a similar pattern to Geyikbayırı and Sipahiler caves (Fig. 3C), such that they increased from -7.83°C to 30.23°C within the first 26 weeks of the year in the atmospheric zone, whereas an increase from 5.1°C to 16.98°C was recorded from the first week to the 30th week of the year in the entrance zone (Fig. 3C). Negative temperatures lasted 12 weeks in the atmospheric zone. In both zones, the WMTs dropped during the remainder of the year, reaching the initial values (Fig. 3C). In the twilight zone, a drop of 0.61°C in the WMTs was observed in the first 3 weeks of the year and subsequently, they increased from 7.35°C to 10.6°C until the 36th week, followed by a constant temperature for next 4 weeks, and then a gradual drop (Table 1, Fig. 3C). In the dark zone, the first 21 weeks of the year exhibited a drop of 0.95°C in the WMTs, reaching a minimum value of 9.56°C. The WMTs gradually increased during the remainder of the year (Fig. 3C).

Comparison of surface and ecological zones of the study caves in terms of RH

Using one-way ANOVA, it was tested whether there is a difference in RH values among the caves (or among the climate types as each cave is in a separate climate) compared to climatic characteristics. This analysis found a statistically significant difference in all zones of the cave in each climate except for the dark zone (Table 5). Scheffe's multiple comparison test was employed to determine which caves resulted in this difference. Accordingly, if the zones are evaluated separately:

Table 5. One-way ANOVA results of relative humidity (%) values of cave zones compared to caves/climate characteristics.

		Sum of Squares	df	Mean Square	F	p
Surface	Between Groups	22367,091	2	11183,546	57,719	,000
	Within Groups	29644,926	153	193,758		
	Total	52012,018	155			
Entrance zone	Between Groups	13807,776	2	6903,888	925,015	,000
	Within Groups	1141,922	153	7,464		
	Total	14949,698	155			
Twilight zone	Between Groups	30,066	2	15,033	85,221	,000
	Within Groups	26,989	153	,176		
	Total	57,055	155			
Dark zone	Between Groups	,087	2	,044	1,573	,211
	Within Groups	4,239	153	,028		
	Total	4,326	155			

Surface: Statistical analysis showed that Sipahiler cave was different from Geyikbayırı and Tuluntaş caves in terms of surface RH values, while Geyikbayırı and Tuluntaş caves did not have a significant difference in terms of surface RH (Table 6). A wide range of RH was recorded in this zone of all three study caves. While

RH levels of 20-80% were measured in Geyikbayırı and Tuluntaş caves, it ranged between 60% and 80% in Sipahiler cave (Fig. 4A). The weekly mean relative humidity (WMRH) showed an irregular decrease in the first 32 weeks and 37 weeks of the year in Geyikbayırı (minimum value 18.37%) and Tuluntaş (minimum value 27.29%) caves, respectively, and then increased until the end of the year (Table 1, Fig. 4A). However, it did not fluctuate so much over the year in Sipahiler cave (Fig. 4A).

Entrance zone: It was shown by statistical analysis that Sipahiler cave differed from Geyikbayırı and Tuluntaş caves in terms of entrance zone RH values, but Geyikbayırı and Tuluntaş caves having no significant difference (Table 6). The WMRH of this zone was around 95-100% in Geyikbayırı and Tuluntaş caves throughout the year, while it was between 71-90% in Sipahiler cave, a similar value to that of the atmospheric zone (Fig. 4B). Besides, a minimum WMRH of 71.03% was seen in Sipahiler cave in the 25th week of the year (Fig. 4B).

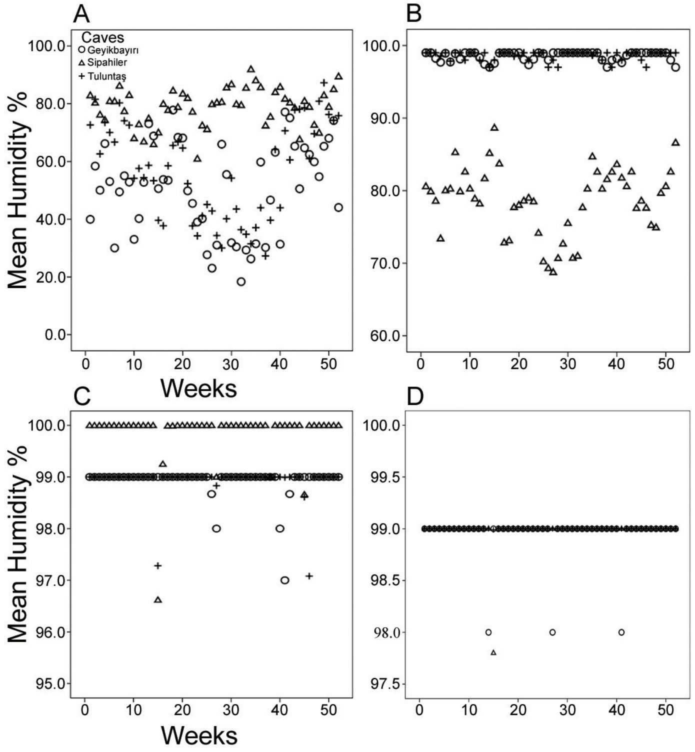


Fig. 4. Comparison of weekly mean RH of surface and ecological zones of the study caves (A: Surface, B: Entrance zone, C: Twilight zone, D: Dark zone).

Twilight zone: It was understood from statistical analysis that Sipahiler cave was different from Geyikbayırı and Tuluntaş caves in terms of twilight zone RH values, but Geyikbayırı and Tuluntaş caves did not have a significant difference (Table 6).

RH of this zone remained constant around 99.99% in Sipahiler cave throughout the year, while it was constantly 99% in Geyikbayırı (except for 26th and 42nd weeks) and Geyikbayırı caves (except for 15th, 27th, 41th, 45th and 46th weeks) (Fig. 4C).

Dark zone: Statistical analysis revealed no difference among three study caves in terms of dark zone RH values (Table 6). A constant RH of 99% prevailed in this zone of all three caves over the year except for a few extreme weeks (Fig. 4D).

RH variations in surface and ecological zones of each study cave

Geyikbayırı cave: Pearson correlation analysis revealed that there was no significant correlation between surface and ecological zones RH values in this cave. However, a significant positive correlation was found between twilight and dark zones RH values, while there was no significant correlation between other zones RH values (Table 8). The WMRH ranged between 18.37-77.8% in the surface, such that it was around 37.63% in the 25-40 weeks of the year, which cover the summer months, with a minimum WMRH of 18.37% in the 32nd week (Table 1, Fig. 5A), and the values of 30.03-77.8% were recorded in the other weeks. The WMRH of the other zones was nearly constant at a value of 97-99% throughout the year (Fig. 5A).

Sipahiler cave: With Pearson correlation analysis, no significant correlation was found between surface and ecological zones RH values in this cave. But, a significant negative correlation was seen between entrance and dark zones RH values, and there was a significant positive correlation between twilight and dark zones RH values (Table 8). The WMRH of 60-90% was observed in the surface and entrance zones over the year, a minimum WMRH of 18.37% is in the 32nd week (Table 1, Fig. 5B). However, it was constant in the other zones, are 99.99% and 99% in the twilight and dark zones, respectively (Fig. 5B).

Tuluntaş cave: Pearson correlation analysis revealed no significant correlation between surface and ecological zones RH values in this cave. However, it was found that there was a significant positive correlation between entrance and twilight zones RH values, and between twilight and dark zones RH values (Table 8). The WMRH of 80% in the first week gradually dropped to 27.29% in the 37th week, followed by an irregular increase to 80% until the end of the year (maximum 87.25%) on the surface (Fig. 5C). In the entrance and twilight zones, WMRH was around 97-99%, while it remained constant at a value of 99% in the dark zone (Fig. 5C).

Taken together, our data show within cave variations in temperature and RH among all three caves: (i) variations in temperature and RH decreased from the entrance zone to the dark zone, (ii) over winter period, temperature increased from the entrance to the dark zone, (iii) over summer period, temperature decreased from the entrance to the dark zone, (iv) the most distinct temperature variation was measured in Continental climate (Fig. 1), and (v) the greatest similarity of climatic conditions between cave and climate type was observed in the Black Sea region, therefore cave crickets species have widened distribution and limited speciation in this region.

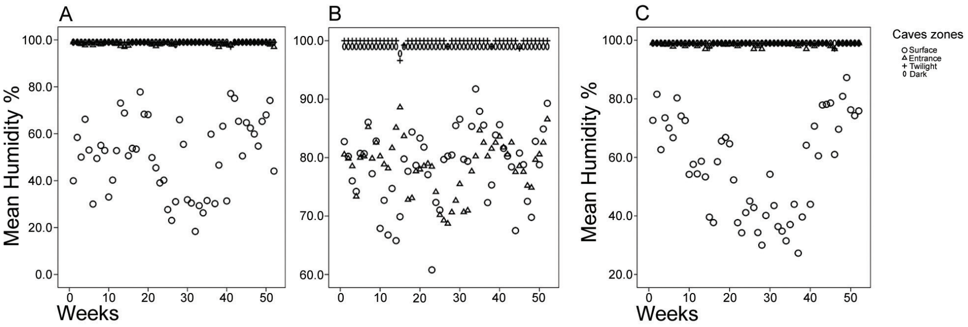


Fig. 5. Comparison of weekly mean RH variations in surface and ecological zones of each study cave (A: Geyikbayırı Cave, B: Sipahiler Cave, C: Tuluntaş~~ş~~ Cave).

CONCLUSIONS AND DISCUSSION

This study is the first report on a comparative evaluation of temperature and RH data obtained from three caves which represent three climate types, Mediterranean, Black Sea, and Continental climate, present in Anatolia. Results show that the temperature and RH data of the zones of the given caves are generally similar in the three caves located in different geographical regions (Fig. 2, Fig. 4). In spite of this similarity, however, it appears that there are some conspicuous differences among many parameters (the number of weeks with the negative temperature values, WMTs and RH, etc.) when considered in detail (Table 1, Fig. 2, Fig. 4). Ecological studies concerning annual temperature and RH on the existing caves in Turkey are limited. A study by Bekaroğlu & Yiğitbaşıoğlu (2010) provided annual temperature values for Karaca cave (Gümüşhane Province) in the Black Sea Region; however, in their study data loggers were not placed in the ecological zones, but in the rooms which are visited by people mostly, since it is a public cave which opens for eight months to tourists (through April to November). Additionally, although this cave with an altitude of 1550 m is geographically in the Black Sea Region, its location is closer to the Eastern Anatolia Region where typical Black Sea climate is not observed, as supported by the temperature values of the surface of this cave which were always negative from January to the end of March. When the temperature data are evaluated, it is seen that Karaca cave is more similar to Tuluntaş cave in the region with a Continental climate in our study, with some differences, such that AMT values of Karaca cave are 2-4 °C higher than Tuluntaş cave (Karaca cave's room I 13.38 °C vs. Tuluntaş cave's entrance zone 11.81 °C; Karaca cave's rooms II and III 13.47-13.39 °C vs. Tuluntaş cave's twilight zone 9.43 °C; Karaca cave's room IV 12.82 °C vs. Tuluntaş cave's dark zone 10.09 °C) (Bekaroğlu & Yiğitbaşıoğlu, 2010). Once again concerning Karaca cave, the highest temperatures were measured in the 35th-40th weeks for room I, 31st-38th weeks for room II, 32nd-37th weeks for room III, and 26th-30th weeks for room IV (Bekaroğlu & Yiğitbaşıoğlu, 2010), but the maximum values were observed in the 22nd-33rd weeks of the year in the zones of Tuluntaş cave. This situation in

Karaca cave may result from its structure as well as such anthropogenic conditions as lighting, electricity cabling, tourist paths, and open to visitors for 8 months of the year.

When the number of species and geographical distribution of cave crickets in Turkey is taken into account, a remarkable distribution is noted: There are four species (*Dolichopoda euxina* Semenov 1901, *Dolichopoda noctivaga* Di Russo & Rampini, 2007, *Troglophilus tatyanae* Di Russo & Rampini 2007, *Troglophilus aspegi* Taylan & Sirin 2015) spread throughout northern Anatolia where the Black Sea climate prevails, whereas 14 species (*Dolichopoda aranea* Bolivar 1899, *D*. *pusilla* Bolivar 1899, *D*. *sbordonii* Di Russo & Rampini, 2006, *D*. *lycia* (Galvagni, 2006), *D*. *sutini* Rampini & Taylan 2012, *D. fortuita* Gorochov & Ünal, 2015, *Troglophilus escalerai* Bolivar, 1899, *T*. *adamovici* Us 1974, *T*. *gajaci* Us 1974, *T*. *bicakcii* Rampini & Di Russo, 2003, *T*. *alanyaensis* Taylan, Di Russo, Cobolli & Rampini, 2012, *T*. *fethiyensis* Taylan, Di Russo, Cobolli & Rampini, 2012, *T*. *ferzenensis* Taylan, Di Russo, Cobolli & Rampini, 2012, *T*. *ozeli* Taylan, Di Russo, Cobolli & Rampini, 2012) were reported from southern and western Anatolia with a Mediterranean climate (Bolivar, 1899; Us, 1974; Rampini & Di Russo, 2003; Di Russo & Rampini, 2006; Galvagni, 2006; Di Russo et al, 2007; Rampini & Di Russo, 2008; Taylan, 2011; Taylan et al, 2011; Rampini et al, 2012; Taylan et al, 2012; Gorochov & Ünal, 2015; Taylan & Şirin, 2016) (Table 2).

Intriguingly, to date, no cave crickets have been found in Central Anatolia where Continental climate is dominant. Diversity and distribution patterns of species groups (Heller & Sevgili, 2005; Şirin, Helversen, & Çıplak, 2010; Şirin, Mol, & Çıplak, 2011; Mol & Zeybekoglu, 2013; Mol, Taylan, & Şirin, 2015), genera (Demirsoy, Salman, & Sevgili, 2002; Sevgili, 2004; Sevgili, Çağlar, & Sağlam, 2010; Taylan, Di Russo, Rampini, & Ketmaier, 2013; Taylan & Şirin, 2016; Ünal, 2017) or larger groups (Demirsoy, 1977; Şirin, Eren, & Çıplak, 2010; Taylan et al, 2011; Ünal, 2016) in Anatolian Orthoptera have been proposed regardless of annual climatic data until this study.

It can be seen from the obtained results that RH values of the twilight and dark zones are almost the same in the three study caves, while RH values (almost 100%) of the entrance zone of Geyikbayırı cave in the Mediterranean climate region are similar to those of Tuluntaş cave in the Continental climate region, but the entrance zone of Sipahiler cave in the Black Sea climate region has an RH of 60-90% (Fig. 5B). As to surface, RH is between 20% and 80% in Geyikbayırı and Tuluntaş caves, while it ranges from 60% to 90% in Sipahiler cave. Although RH values of Geyikbayırı and Tuluntaş caves seem to be similar, in fact, the values of Geyikbayırı are steady throughout the year, whereas Tuluntaş cave has fluctuating RH values, with a decline from 80% to 20% towards the middle of the year, and increase to 80% again at the end of the year. Hence, if RH values of the three cave zones supported cave cricket populations, they could be expected in Tuluntaş and other caves in the Continental climate region. In this case, one can say that surface-climatic conditions (annual distribution of negative temperature and RH values) significantly influence the distribution of cave crickets (Table 2).

Table 2. Cave crickets, their localities and climatic zones in Anatolia.

Taxa	Localities	Climate type		
		Mediterranean	Black Sea	Continental
Dolichopoda aranea Bolivar, 1899	K.Maraş, Yenidje-kale	+	-	-
D. pusilla Bolivar, 1899	Hatay, Akbez Cave	+	-	-
D. euxina Semenov, 1901	Artvin, in forest zone	-	+	-
D. sbordonii Di Russo & Rampini, 2006	Antalya, Karaini cave, Kocain cave, Tabak cave	+	-	-
D. lycia (Galvagni, 2006)	Antalya, Kemer, Gedelma Cave, Geyikbayırı cave	+	-	-
D. noctivaga Di Russo & Rampini, 2007	Artvin, Erzurum, Bartın, Bolu, Karabük provinces	-	+	-
D. sutini Taylan & Sirin 2016	İzmir, Selçuk, Sutini Cave, Aydın, Aşıkali Cave	+	-	-
D. fortuita Gorochov & Ünal, 2015	Antalya, Göynük	+	-	-
Troglophilus escalerai Bolivar, 1899	K.Maraş, Yenidje-kale, Döngel Cave	+	-	-
T. gajaci Us, 1974	Mersin, Cennet Cave, Alanya Kadıini cave	+	-	-
T. adamovici Us, 1974	Isparta Zindan Cave	+	-	-
T. bicakcii Rampini & Di Russo, 2003	Konya Derebucak, Bıçakçıini cave, Balatini cave, Feyzullah Cave, Suluin cave	+	-	-
T. tatyanae Di Russo & Rampini 2007	Artvin province, forest zone	-	+	-
T. alanyaensis Taylan, Di Russo, Cobolli & Rampini, 2012	Alanya, Dim cave	+	-	-
T. ozeli Taylan, Di Russo, Cobolli & Rampini, 2012	Balıkesir, Havran cave	+	-	-
T. ferzenensis Taylan, Di Russo, Cobolli & Rampini, 2012	Konya, Seydişehir, Ferzene cave	+	-	-
T. fethiyensis Taylan, Di Russo, Cobolli & Rampini, 2012	Muğla Fethiye, Güroluk cave	+	-	-
T. aspegi Taylan & Sirin 2015	Bartın, Sipahiler cave	-	+	-

*Data from Bolivar 1899, Semenov 1901, Us 1974,Rampini and Di Russo 2003, Di Russo et al. 2007, Taylan 2011, Taylan et al. 2012, Gorochov and Ünal 2015, Taylan et al. 2015, Taylan and Şirin 2016.

Cave crickets are troglophiles, and spend most of their lives in caves; however, they are reported to go out the cave at night for feeding purposes when bat guano (bat droppings) as source of energy and nutrients is not found or occurs in small amounts in the cave (Di Russo, Vellei, Carchini, & Sbordoni, 1987; Di Russo & Sbordoni, 1998). Therefore, more realistic scenarios about the diversity and distribution of cave crickets can be produced by considering both in-cave conditions and surface climatic conditions together. Among the studied three caves, Geyikbayırı Cave contains one cave cricket species (*Dolichopoda lycia* (Galvagni, 2006) and Sipahiler Cave (*Troglophilus aspegi* Taylan & Şirin, 2015), while Tuluntaş cave does not contain any cave crickets species. The most species density of *D. lycia* observed in the twilight zone of Geyikbayırı cave. On the other hand, most species density of *T. aspegi* observed in the entrance zone (first room) and the twilight zone of Sipahiler Cave which has similarity to surface RH with Black Sea region. However, *T. aspegi* (Sipahiler Cave) species has a wider distribution zone than *D. lycia* (Galvagni, 2006) *species* (Geyikbayırı Cave) in the caves zones. The specimens of *T. aspegi* Taylan & Şirin, 2015 have distribution in all zones and *main galleries* of the cave while the specimens of *D. lycia* (Galvagni, 2006) have limited distribution in the *side galleries* of twilight zone.

From the data of this study, RH of the atmospheric zone of Sipahiler cave in the Black Sea climate region appears to be very close to that surface of the cave, especially of the entrance zone (Table 1, Fig. 5), being an expected situation for the Black Sea Region which has a temperate rainforest characteristic. As a predominately-forested region in Turkey (Doğanay & Doğanay, 2004), the Black Sea Region generally has a forest structure consisting of non-evergreen trees (*Fagus* sp., *Carpinus* sp., *Alnus* spp. etc) at altitudes of 200 m to 1200 m, and coniferous trees resistant to harsh climatic conditions above 1200 m (Atalay, Tetik, & Yılmaz, 1985). Dense and moist forests in this region allow cave crickets to move within the forests, thereby to expand their distribution areas and to exchange genes with individuals in different areas, explaining the presence of *Dolichopoda noctivaga* Di Russo & Rampini, 2007, almost the provinces on whole Black Sea Region (Bolu, Karabük, Bartın, Ordu, Artvin). RH of in-cave zones of Geyikbayırı cave in the Mediterranean climate region is higher than that of its atmospheric zone (Fig. 5, Table 1). Long periods with low or high temperatures outside the cave (Fig. 2A, Fig. 3A) may dissuade cave crickets from their visits to outside the cave (Poulson, Lavoie, & Helf, 1995; Helf, 2003; Taylor, Krejca, & Denight, 2005; Lavoie, Helf, & Poulson, 2007; Helf, Philippi, Moore, & Scoggins, 2015). Furthermore, the vegetation of the Mediterranean Region is maquis at lower altitudes, arid-climate-loving Calabrian pine (*Pinus brutia*) forests at altitudes of 250-300 m to 1200 m, followed by cedar (*Cedrus libani*) forests above 1200 m (Kürschner, Parolly, & Raab-Straube, 1982). It may be said that since the surface temperature values are very high at certain periods of the year, and RH remains at low levels from time to time, and the vegetation that will allow them to spread does not exist, cave crickets in this region are isolated to their caves, thus leading to restricted gene exchange among the populations, and correspondingly speciation.

Although it is necessary to examine more caves in a longer period, we can make some assumptions from this study based on data logging. Our study reveals that distinct variations in temperature and RH lead to minute changes in the dark zone in that a 50 ºC variation drops the temperature of the dark zone by 1-2 ºC, suggesting that the cave ecosystem tolerates considerable changes in the outdoor atmosphere. An assumption can be made from this important finding: As it is known, cold-loving (cryophilic) organisms tend to gradually move towards the higher parts of mountains as a result of global warming (Hewitt, 1996, 1999, 2000; Taberlet, Fumagalli, Wust-Saucy, & Cosson, 1998). Like this vertical translocation, cave crickets may be passing from the entrance zone to the dark zone within their caves in response to warming, as revealed by this study that temperature and RH gradually drop from the entrance zone towards the dark zone (horizontal translocation) which becomes almost a stable ecological zone (Fig. 3D, Fig. 5D). Concordantly, and the high tolerance of dark zone to temperature variations makes caves important shelters for many invertebrates, especially troglophiles that mostly inhabit entrance and twilight zones of a cave (Reddell, 2005; Romero, 2009), against the negative effects of global warming.

ACKNOWLEDGEMENTS

We thank Anıl Alkan and Ceyhun Uludağ from The Society of Anatolian Speleology Group-Aspeg, Semih Tan and Nezihi Ekizoğlu from Akdeniz University Cave Research Group-Akumak, and Ferdi Uğurlu from Hacettepe University Cave Research Group-Humak for their help in the field studies. Dr. Gökhan GÜNEŞ from Hakkari University is appreciated for his help with statistics. This work was supported by TUBITAK BİDEB-2218 (Ankara, Turkey) post-doctoral scholarship [Grant number 1929B011100092].

REFERENCES

Atalay, I. (2000). *Geography of Turkey*. Inkilap Publishing, Istanbul, Turkey.

Atalay, I., Tetik, M., & Yılmaz, O. (1985). Ecosystems of North-eastern Anatolia. *Aegean Geographical Journal*, 3, 16-56.

Bekaroğlu, E. & Yiğitbaşıoğlu, H. (2010). Assessment of one-year-long temperature record of Karaca Cave (Gumushane, NE Turkey). *Ankara University Journal of Environmental Sciences*, 2, 1-12.

Bey-Bienko, G. (1969). *On the cavernicolous grasshoppers of the genus Dolichopoda Bol. (Orthoptera) from the Caucasus*. 4th International Congress of Speleology, Jugoslavia, p. 73-74.

Bolivar, I. (1899). Orthopteres du Voyage de Martinez Escelera dans L'Asie Mineure. *Annales de la Société Entomologyique de Belgique*, 43, 583-607.

Carchini, G., Rampini, M., & Sbordoni, V. (1994). Life cycle and population ecology of the cave cricket *Dolichopoda geniculata* (Costa) from Valmarino cave (Central Italy). *International Journal of Speleology*, 23, 203-218.

Cropley, J.B. (1965). Influence of surface conditions on temperatures in large cave systems. *Bulletin of the National Speleological Society*, 27, 1-10.

Crump, M.H. (1886). Air from a cave for house-cooling. *Science*, 8, 413.

Crump, M.H. (1890). Experiments with cave air. *Science,* 15, 290-291.

Culver, D.C. (1982). *Cave life: Evolution and ecology.* Cambridge, Massachuscits, Harvard University Press, 189 p, USA.

Culver, D.C. & Pipan, T. (2009). *The biology of caves and other subterranean habitats.* New York, Oxford University Press, 256 p, USA.

De Freitas, C.R. (2010). The role and importance of cave microclimate in the sustainable use and management of show caves. *Acta Carsologica*, 39, 477-489.

De Freitas, C.R. & Littlejohn, R.N. (1987). Cave climate: Assessment of heat and moisture exchange. *International Journal of Climatology*, 7, 553-569.

De Freitas, C.R., Littlejohn, R.N., Clarkson, T.S., & Kristament, I.S. (1982). Cave climate: Assessment of airflow and ventilation. *International Journal of Climatology*, 2, 383-397.

Demirsoy, A. (1977). *The determination and taxonomical studies of the Califera (Insecta, Orthoptera) Fauna of Turkey*. Ataturk University Press, 252 p, Atatürk University, Erzurum, Turkey.

Demirsoy, A., Salman, S., & Sevgili, H. (2002). *Novadrymadusa*, a new genus of bushcricket with a new species and notes on related genera (Orthoptera: Tettigoniidae). *Journal of Orthoptera Research*, 11, 175-183.

Deniz, A., Toros, H., & İncecik, S. (2011). Spatial variations of climate indices in Turkey. *International Journal of Climatology*, 31, 394-403.

Di Russo, C., Carchini, G., Rampini, M., Lucarelli, M., & Sbordoni, V. (1999). Long term stability of a terrestrial cave community. *International Journal of Speleology*, 26 (1-2), 75-88.

Di Russo, C., Carchini, G., & Sbordoni, V. (1994). Life-history variation in *Dolichopoda* cave crickets. In: H.V. Dands (Ed.). *Insect life-cycle polimorphism* (pp. 205-226). Kluwer Academic Publ., Netherlands.

Di Russo, C. & Rampini, M. (2006). A new species of *Dolichopoda* from caves of southern Turkey (Orthoptera, Rhaphihophoridae). *Fragmenta Entomologica, Roma*, 38, 7-14.

Di Russo, C., Rampini, M., & Landeck, I. (2007). The cave crickets of northeast Turkey and transCaucasian regions, with descriptions of two new species of the genera *Dolichopoda* and *Troglophilus* (Orthoptera, Rhaphidophoridae). *Journal of Orthoptera Research*, 16, 67-76.

Di Russo, C. & Sbordoni, V. (1998). Gryllacridoidea. In: C. Juberthie, V. Decu (Eds.). *Encyclopedia biospeleologica* (pp. 989-1001). Moulis, Bucarest.

Di Russo, C., Vellei, A., Carchini, G., & Sbordoni, V. (1987). Life cycle and age structure of *Dolichopoda* populations (Orthoptera, Rhaphidophoridae) from natural and artificial cave habitats. *Bolletino di Zoologia*, 54, 337-340.

Doğanay, H. & Doğanay, S. (2004). Forest fires and measures to be taken in Turkey. *The East African Geographical Review*, 11, 31-48.

Ek, C. & Gewelt, M. (1985). Carbon dioxide in cave atmospheres. New results in Belgium and comparison with some other countries. *Earth Surface Processes and Landforms*, 10, 173-187.

Forbes, J. (1998). Air temperature and relative humidity study: Torgac Cave, New Mexico. *Journal of Cave and Karst Studies*, 60, 27-32.

Galvagni, A. (2006). Nuovo Genere e nuova specie di Dolichopodinae dell' Anatolia sud-occidentale: Hellerina lycia n. sp. (Insecta Orthoptera Rhaphidophoridae). *Accademia Roveretana degli Agiati*, 8, 75-83.

Gample, D.W., Dogwiler, J.T., & Mylroie, J. (2000). Field assessment of the microclimatology of tropical flank margin caves. *Climate Research*, 16, 37-50.

Gorochov, A.V. & Ünal, M. (2015). A new species of the genus *Dolichopoda* (Orthoptera: Rhaphidophoridae) from Teke peninsula, Turkey. *Proceedings of the Zoological Institute of the Russian Academy of Sciences*, 319, 126-131.

Gregoric, A., Zidansek, A., & Vaupotic, J. (2011). Dependence of radon levels in Postanja Cave on outside air temperature. *Natural Hazards and Earth System Sciences*, 11, 1523-1528.

Gunn, J. (2004). *Encyclopedia of caves and karst science*. 1940 p., Fitzroy, Dearborn, New York-London.

Gürcan, G., Yamaç, A., Tanındı, O., & Uygun, D. (2006). *Türkiye Mağaralar Envanteri.* Vol a/b, 1220 p., Ege Publishing, Istanbul.

Helf, K.L. (2003). *Foraging Ecology of the Cave Cricket Hadenoecus subterraneus: Effects of Climate, Ontogeny, and Predation*. PhD Thesis, 170 p, University of Illinois, Chicago, USA.

Helf, K.L., Philippi, T., Moore, B.J., & Scoggins, L. (2015). *A protocol for monitoring cave crickets (Hadenoecus subterraneus) at Mammoth Cave National Park: Version 1.0. Natural Resource Report NPS/CUPN/NRR*, *National Park Service,* 934 p., Fort Collins, Colorado, USA.

Heller, K.G. & Sevgili, H. (2005). Systematics and bioacoustics of the Poecilimon sanctipauli-group (Orthoptera, Tettigonioidea, Phaneropteridae). *European Journal of Entomology*, 102, 265-277.

Hewitt, G.M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, 58, 247-276.

Hewitt, G.M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, 68, 87-112.

Hewitt, G.M. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405, 907-913.

Kunt, K.B., Yağmur, E.A., Özkütük, S., Durmuş H., & Anlaş, S. (2010). Checklist of the cave dwelling invertebrates (Animalia) of Turkey. *Biological Diversity and Conservation*, 3, 26-41.

Kürschner, H., Parolly, G., & Raab-Straube, E.V. (1982). Phytosociological studies on high mountain plant communities of the Taurus Mountains (Turkey). 3. Snow-patch and meltwater communities. *Feddes Repertorium*, 109, 581-616.

Lavoie, K.H., Helf, K.L., & Poulson, T.L. (2007). The biology and ecology of North American cave crickets. *Journal of Cave and Karst Studies*, 69, 114-134.

Leonard, W.H. (1889). Cave-air for ventilation. *Science*, 14, 395.

Mol, A. & Zeybekoğlu, U. (2013). Distribution and taxonomy of Gomphocerinae (Orthoptera, Acrididae) grasshoppers in the Anatolian Black Sea basin and check list of Turkey subfamily fauna. *Journal of the Entomological Research Society*, 15, 69-102.

Mol, A., Taylan, M.S., & Şirin, D. (2015). Contribution to the knowledge of two endemic species of Anatolian Gomphocerinae Fieber, 1853 (Orthoptera: Acrididae). *Zootaxa*, 3972, 482-494.

Nepstad, J. & Pisarowicz, J. (1989). Wind cave temperature and humidity variations. *Bulletin of the National Speleological Society*, 51, 125-128.

Ozansoy, C. & Mengi, H. (2006). *Mağarabilimi ve Mağaracılık*. p. 337, Tubitak publishing, Ankara, Turkey.

Poulson, T. L., Lavoie, K.H., & Helf, K.L. (1995). Long-term effects of weather on the cricket (*Hadenoecus subterraneus*, Orthoptera, Rhaphidophoridae) guano community in Mammoth Cave National Park. *American Midland Naturalist*, 134, 226-236.

Poulson, T.L. & White, W.B. (1969). The cave environment. *Science*, 165, 971-981.

Rampini, M. & Di Russo, C. (2003). Una nuova specie di Troglophilus di Turchia (Orthoptera, Rhaphidophoridae). *Fragmenta Entomologica, Roma*, 34, 235-247.

Rampini, M. & Di Russo, C. (2008). On southern Anatolian *Dolichopoda* Bolivar, 1880 with taxonomic notes on the genus *Hellerina* Galvagni, 2006 (Orthoptera, Rhaphidophoridae). *Fragmenta Entomologica, Roma,* 40, 229-236.

Rampini, M., Di Russo, C., Taylan, M.S., Gelosa, A., & Cobolli, M. (2012). Four new species of *Dolichopoda* Bolivar, 1880 from southern Sporades and western Turkey (Orthoptera, Rhaphidophoridae, Dolichopodainae). *Zookeys*, 201, 41-56.

Reddell, J.R. (2005). Spiders and related groups. In D.C. Culver, W.B. White (Eds.). *Encyclopedia of caves* (pp. 786-797). Elsevier, Amsterdam, The Netherlands.

Romero, A. (2009). *Cave biology: Life in darkness ecology, biodiversity and conservation*. 291 p. Cambridge University Press, Cambridge, USA.

Sanderson, K. & Bourne, S. (2002). Cave temperatures at Naracoorte Caves. *Helictite*, 38, 7-10.

Sbordoni, V. (1980). Strategie adattive negli animali cavernicoli: uno studio di genetica ed ecologia di popolazione. *Contributi del Centro Linceo Interdisciplinare di Accademia Nazionale dei Lincei,* 51, 61-100.

Sensoy, S., Demircan, M., Ulupinar, U., & Balta, I. (2008). *Turkish State Meteorological Service: Climate of Turkey*. Retrieved from http://www.dmi.gov.tr/iklim/iklim.aspx.

Sevgili, H. (2004). Review of the genus Leptophyes of Turkey with the description of a new species (Orthoptera: Phaneropterinae). *Transactions of the American Entomological Society*, 130, 95-112.

Sevgili, H., Çaglar, S.S., & Sağlam, K. (2010). Re-evaluation of the genus Phonochorion (Orthoptera. Tettigoniidae: Phaneropterinae). *European Journal of Entomology*, 107, 631-645.

Smithson, P.A. (1991). Inter-relationships between cave and outside air temperatures. *Theoretical and Applied Climatology*, 44, 65-73.

Stoeva, V. & Stoev, A. (2005). Cave air temperature response to climate and solar and geomagnetic activity. *Memorie della Società Astronomica Italiana*, 76, 10-42.

Şirin, D., Eren, O., & Çıplak, B. (2010). Grasshopper diversity and abundance in relation to elevation and vegetation from a snapshot in Mediterranean Anatolia: role of latitudinal position in altitudinal differences. *Journal of Natural History*, 44, 1343-1363.

Şirin, D., Helversen, O.V., & Çıplak, B. (2010). *Chorthippus brunneus* subgroup (Orthoptera, Gomphocerinae) in Anatolia with description of two new species: data suggest an Anatolian origin for the lineage. *Zootaxa*, 2410, 1-28.

Şirin, D., Mol, A., & Çıplak, B. (2011). *Myrmeleotettix* Bolivar (Orthoptera, Gomphocerinae) in Anatolia on the basis of morphological and behavioural characters: data suggest a new species from southern end of the Anatolian refugium. *Zootaxa*, 2917, 29-47.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., & Cosson, J.F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, 7, 453-464.

Taylan, M.S. (2011). *Cave crickets of Turkey (Orthoptera, Rhaphidophoridae): Their taxonomy, phylogeny and biogeography in the light of morphological and molecular data*. 203 p. Unpublished PhD Thesis, Akdeniz University, Antalya, Turkey.

Taylan, M.S., Di Russo, C., Cobolli, M., & Rampini, M. (2012). The new species of the Troglophilus Krauss 1879 (Orthoptera, Rhaphidophoridae) from western and southern Anatolian caves, Turkey. *Zootaxa*, 3597, 33-40.

Taylan, M.S., Di Russo, C., Rampini, M., & Cobolli, M. (2011). The Dolichopodainae and Troglophilinae cave crickets of Turkey: an update of taxonomy and geographic distribution (Orthoptera, Rhaphidophoridae). *Zootaxa*, 2829, 59-68.

Taylan, M.S., Di Russo, C., Rampini, M., & Ketmaier, V. (2013). Molecular systematics of the genus *Troglophilus* (Rhaphidophoridae, Orthoptera) in Turkey: mitochondrial 16S rDNA evidences. *Zookeys*, 257, 33-46.

Taylan, M.S., Mol, A., & Şirin, D. (2015). Description of a new species of the genus *Troglophilus* Krauss, 1879 (Orthoptera: Rhaphidophoridae) from northern Anatolia, Turkey. *Journal of the Entomological Research Society*, 172, 51-57.

Taylan, M.S. & Şirin, D. (2016). Speciation of the genus *Dolichopoda* in Anatolia with reference to the role of ancient central lake system. *Insect Systematics and Evolution*. 473, 267-283.

Taylor, S.J., Krejca, J.K., & Denight, M.L. (2005). Foraging range and habitat use of *Ceuthophilus secretus* (Orthoptera: Rhaphidophoridae), a key trogloxene in central Texas cave communities. *American Midland Naturalist*, 154, 97-114.

Us, P.A. (1974). Cave Orthoptera (Saltatoria: Rhaphidophoridae and Gryllidae) collected by Dr. Jean Gajac in Yugoslavia, Greece and Turkey. *Entomologist's Monthly Magazine*, 110, 182-193.

Ünal, M. (2016). Pamphagidae (Orthoptera: Acridoidea) from the Palaearctic region: Taxonomy, classification, keys to genera and a review of the tribe Nocarodeini I. Bolívar. *Zootaxa*, 4206, 1-23.

Ünal, M. (2017). Revision of the genus *Bradyporus* Charpentier, 1825 (Orthoptera: Tettigoniidae: Bradyporinae). *Zootaxa*, 4272, 491-528.

Vandel, A. (1964). *Biospeologie: la biologie des animaux cavernicoles*. 620 p., Gauthier-Villars, Paris, France.

Weckerly, F.W. (2012). Cave cricket exit counts: environmental influences and duration of surveys. *Journal of Cave and Karst Studies*, 74, 1-6.

Yoder, J.A., Hobbs, III, H.H., & Hazelton, M.C. (2002). Aggregate protection against dehydration in adult females of the cave cricket, *Hadenoecus cumberlandicus*. *Journal of Cave and Karst Studies*, 64(2), 140-144.

Received: April 24, 2019 *Accepted: February 13, 2020*

J. Entomol. Res. Soc., 22(1): 75-82, 2020
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

Description of a New Species of *Ericydnus* (Haliday, 1832) (Hymenoptera: Encyrtidae: Tetracneminae) from India

Manendra KANERIA[1*]   Sudhir SINGH[2]

[1*,2]Forest Entomology Discipline, Forest Protection Division, Forest Research Institute, New Forest, Dehradun 248006, Uttarakhand, INDIA
e-mails: [1*]kaneria.manendra@gmail.com, [2]ssandot2@gmail.com
ORCID IDs: [1*]0000-0003-3882-0250, [2]0000-0001-5504-7509

ABSTRACT

A new species, *Ericydnus sheopurensis* Kaneria & Singh sp. nov. of Encyrtidae (Hymenoptera: Chalcidoidea) is described from Madhya Pradesh, India. Female of the described species is illustrated. All type materials are deposited to National Forest Insect Collection at the Forest Protection Division, Forest Research Institute, Dehradun, India.

Key words: Madhya Pradesh, *Ericydnus sheopurensis*, *Cordia myxa* L., new species.

Kaneria, M. & Singh, S. (2020). Description of a new species of *Ericydnus* (Haliday, 1832) (Hymenoptera: Encyrtidae: Tetracneminae) from India. *Journal of the Entomological Research Society*, 22(1), 75-82.

INTRODUCTION

The genus *Ericydnus* was erected by Haliday, in Curtis 1832 (Walker, 1837; Graham, 1991) with types species *Ericydnus paludatus*, which is erroneously accredited to Walker (Gahan & Fagan 1923; Kerrich, 1966; 1967). This genus belongs to the tribe Ericydnini (Hoffer, 1955), which is closely related to the tribe Anagyrini (Noyes, 2000). At present, a total of 33 species are known worldwide, mainly (32 species) from Palearctic (Noyes, 2018). Only one species *Ericydnus lamasi* (Domenichini, 1951) is described from Neotropical region. The following species are described from Palearctic regions:*E. longicornis* (Dalman, 1820), *E. ventralis* (Dalman, 1820), *E. strigosus* (Nees, 1834), *E. sipylus* (Walker, 1837), *E. baleus* (Walker, 1838), *E. apterogenes* Mayr, 1876, *E. metriocerus* Masi, 1921, *E. robustior* Mercet, 1921, *E. aeneus* Nikolskaya, 1952, *E. caudatus* Erdös, 1957, *E. heliococci* Trjapitzin & Herthevtzian, 1972, *E. danatensis* Myartseva, 1980, *E. karakalensis* Myartseva, 1980, *E. niger* Myartseva, 1980, *E. tamaricicola* Myartseva, 1980, *E. turkmenicus* Myartseva, 1980, *E. bischoffi* Trjapitzin, 1982, *E. elizabethae* Trjapitzin, 1982, *E. theron* Trjapitzin, 1982, *E. beybienkoae* Sharkov, 1983, *E. dzhanokmenae* Sharkov, 1986, *E. peliococci* Myartseva & Kharchenko, 1988, *E. samadae* Myartseva & Kharchenko, 1988, *E. pilosulus* Graham, 1991, *E. scutellus* Xu, 2000, *E. luka* Japoshvili, 2007, *E. nino* Japoshvil, 2007, *E. novosibiricus* Sugonjaev & Gavrilyuk, 2012, *E. gigas* Liu, Wang & Li, 2013, *E. huangi* Liu, Wang & Li, 2013, *E. infuscatus* Liu, Wang & Li, 2013, *E. liaoi* Liu, Wang & Li, 2013. *Ericydnus sheopurensis* Kaneria & Singh, sp. nov. is the first described species from Oriental region.

A revisionary work has done on Chinese species by Liu et al, 2013. Major hosts of *Ericydnus* belong to Pseudococcidae (Hemiptera). In the present study, a new species described collected from leaves of *Cordia myxa* Linnaeus which is broad-leaved deciduous tree distributed rom dry deciduous to moist deciduous forest. It is commonly known as Indian cherry and has numerous medicinal properties used in dysentery, cholera, headache etc.

MATERIAL AND METHODS

Leaves of *Cordia myxa* L. and an indet. plant infested with pseudococcids were collected during April 2016 and November 2017 from Sheopur and Gwalior, respectively in state of Madhya Pradesh. They were reared in plastic containers mouthed with fine muslin cloth. Emerged parasitoids were collected with the help of aspirator. These specimens were killed using ethyl acetate fumes and transferred in the 80% ethanol. The specimens were cleaned and dried using HMDS (Hexamethyldisilazane) technique (Brown, 1993). Dried specimens were photographed using Automontage System with Micropublisher Q-Imaging 5.0 RTV camera mounted on Olympus SZX-16 stereozoom microscope. Slide mounted parts were photographed using Nikon Digital Sight DS-Fi1 with NIS-Br software (Nikon) mounted on Nikon Optiphot compound microscope. Morphometric measurements were taken from paratype while, body colour and sculpture was noted from holotype. The morphological terminology interpretations used follow Noyes & Hayat, 1984 and Singh & Agarwal, 1993. not found in the references

The abbreviations are used in the text as following:

POL = Minimum distance between posterior ocelli, OCL = Minimum distance between a posterior ocellus and the occipital margin, OOL = Minimum distance between a posterior ocellus and the corresponding eye margin, ITD = Distance between the toruli.

TMD = Distance between torulus and mouth margin, TED = Shortest distance between torulus and eye margin, F1, F2, etc. = Funicle segments 1, 2, etc.

Measurements of whole body length are in mm (milimetre) and other body parts described are in µm.

RESULTS

Genus *Ericydnus* Haliday

Ericydnus Haliday, in Curtis, 1832. Type species *Ericydnus paludatus* Haliday [recte walker, 1837], by designation of Westwood, 1840.

Grandoriella Domenichini, 1951. Type species *Grandoriella lamasi* Domenichini, by monotypy and original designation. Synonymy by Noyes, 1980.

Ericydnus sheopurensis Kaneria & Singh, 2020 sp. nov.

Description

Material examined: Holotype: 1♀ (on card), INDIA: Madhya Pradesh, Sheopur, 24.04.2016, Manendra Kaneria. Paratype: 1♀ (on slide under 8 cover slips, thorax missing), data same as for holotype and 3♀♀ (on card), INDIA: Madhya Pradesh, Gwalior, 16.10.2017, Manish Kaneria.

Female: Body length, 0.72-0.75 mm.

Body (Fig. 1a) dark brown to black; head completely black, frontovertex with sparsely arranged silvery white setae and with irregular polygonal raised reticulate sculpture, wider towards the scrobal margin and narrower around the ocellar triangle; para scrobal area with elongated reticulate and interantennal prominence with faint rugose sculpture frontovertex; scrobal and interantennal prominence area with light violet reflection. Gena and malar space smooth. Eyes chocolate brown with conspicuous white setae, inner margin lined with 20-25 white setae. Antenna light brown (Fig. 3b), scape with two white spots, one at ventral basal half and second at distal fourth; distal half of pedicel white; F1-F6 gradually becoming lighter; clava brown. Mandible (Fig. 1c) pale brown; maxillary and labial palpi testaceous.

Mesosoma dorsally black, laterally and ventrally dark brown, tegulae off white; metasoma dark brown dorsally, lighter laterally and ventrally. Pronotum shiny with irregular reticulate sculpture, 15-18 silvery white setae arranged in two rows transversely. Mesoscutum with fine reticulate sculpture, with light greenish reflection, 44-48 pale white setae arranged in five rows transversely.

Axillae with fine reticulation. Scutellum with elongate reticulate sculpture, 14-18 black setae sparsely arranged. Fore and hind wings hyaline.

Fore leg (Fig. 4c) whitish with tarsal segments light brown; middle leg light brown with brown coxa; hind leg with coxa and basal two-thirds of femur brown, rest light brown. Metasoma brown with violet reflection.

Head in dorsal view (Fig. 2a), 0.52x as long as wide (160:306); 2.8x as wide as frontovertex at the level of anterior ocellus (306:110); ocelli arranged in right angle triangle; eyes touching occipital margin; 1.7x as long as wide (143: 86). Frontovertex 0.96s as long as wide (110:115); POL 2.9x, OCL 0.84, and OOL 0.79x as long as diameter of middle ocellus (55:16:15:19). In frontal view (Fig. 1b), head 0.96x as long as wide (294:306); scrobe deep, inverted U-shaped and meeting dorsally; eye 2.2x as long as wide (203:93); toruli touching the line joining lower eye margin, 1.4x as high as wide (33:24); ITD 1.5x, TMD 0.82x, TED 1.4x length of a torulus (48:27:45:33); Mandible (Fig. 1c) bidentate and acute; maxillary palpi 4 segmented and labial palpi 3-segmented.

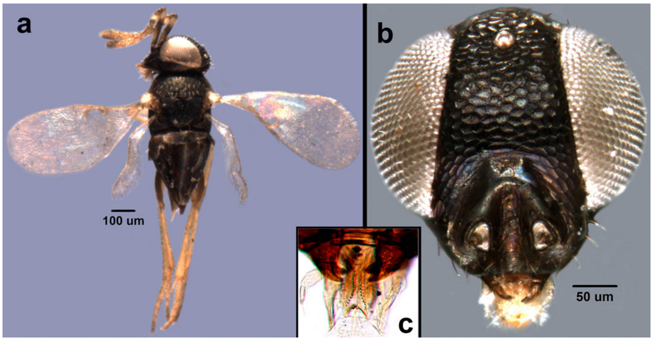


Fig. 1. *Ericydnus sheopurensis* sp. nov. ♀; a. Body in dorsal view, b. Head in frontal view, c. Mandible.

In lateral view (Fig. 2b), head 1.32x as high as wide (285:215); eye 1.32x as high as wide (197:149); gena 0.23x as wide as eye width (34:149); malar space 0.24x as long as head height (70:290).

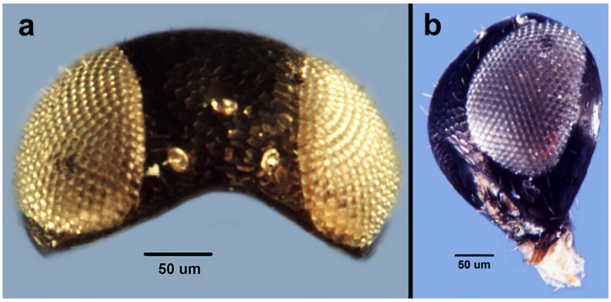
Antenna (Fig. 3b) with scape cylindrical, 6.1x as long as wide (176:29); pedicel conical, 2.33x as long as wide (70:30); all funicle segments distinctly longer than wide; F1-F5 narrower than and F6 as wide as pedicel, F1 1.8x (36:20), F2 1.8x (36:20), F3 1.6x (38:23), F4 1.7x (39:23) F5 1.6x (41:25) and F6 1.4x (45:32) as long as wide; clava three-segmented with parallel sutures, slightly pointed and tapering towards apex; 3.1x as long as wide (136:44).

Mesosoma (Fig. 3a) 0.92x as long as wide (238:259); pronotum 11.3x as wide as long (259:23); mesoscutum 2.4x as wide as long (275:116); scutellum 0.95x as long as broad (143:151); propodeum narrow, 24x as wide as long (265:11). Forewing (Fig. 4a, b), extending beyond apex of gaster; 2.7x as long as wide (810:295); linea calva interrupted with 3-4 rows of setae; marginal vein 4x as long as stigmal vein (346:86); postmarginal vein 0.44x as long as marginal vein (154:346). Middle leg (Fig. 4c), with tibial spur as long as basitarsus (92:92).

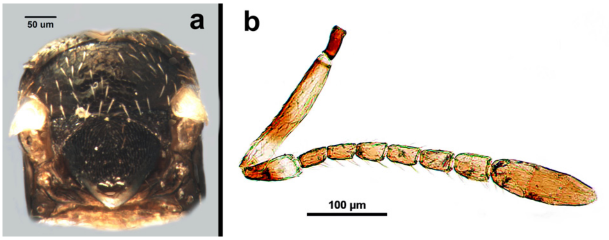
Metasoma (Fig. 1a), pointed at apex; 1.34x as long as wide (340:253); hypopygium reaches apex of gaster; ovipositor (Fig. 5k.) 0.58x as long as midtibia (227:389).

Host: Pseudococcid on *Cordia myxa* L. (Boraginaceae) and an indet. plant.

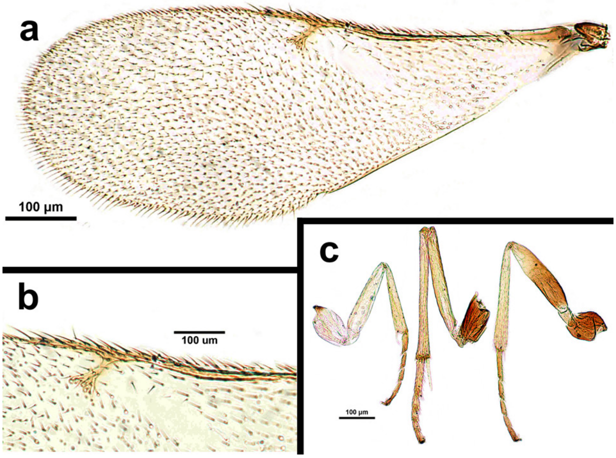
Etymology: The species name is derived from that of its type locality Sheopur (Madhya Pradesh).



Figs. 2. *Ericydnus sheopurensis* sp. nov. ♀; a. Head in dorsal view, b. Head in lateral view.



Figs. 3. *Ericydnus sheopurensis* sp. nov. ♀; a. Mesosoma, b. Antenna.



Figs. 4. *Ericydnus sheopurensis* sp. nov. ♀; a. Fore wing, b. Fore wing venation,c. Fore leg, mid leg, hind leg.

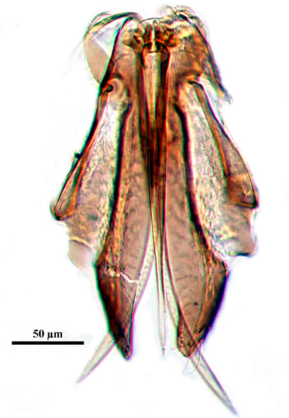


Fig. 5. *Ericydnus sheopurensis* sp. nov. ♀; Ovipositor.

Diagnosis

Body black to brown; varying from elongate to robust; occipital margin acute to sharpe; eye narrower than frontovertex width; toruli separated from mouth by less than their own length; scape subcylindrical or cylindrical funicle six segmented; clava entire or three segmented; mandible sharp and bidentate; tegulae dark brown to off white; fore wing normally developed and hyaline or infuscate; postmarginal vein more than one and one-half times as long as stigmal vein; marginal vein longer than stigmal and marginal vein not broader than long; coaxe varying from yellowish brown to dark brown; hypopygium reaching apex of gaster or extending past.

DISCUSSION

In the keys by Kerrich, 1967 and Liu et al, 2013 *E. sheopurensis* sp. nov. runs to *ventralis* (Dalman). But *sheopurensis* is distinctly different from it by the following characters (characters given in brackets are for *E. ventralis*): tegulae off white (tegulae black); fore coxae dull white (fore coxae black); antenna pale brown (dark brown); head 2.5x as wide as frontovertex (2.1x), ocelli forming acute angle (about 90^0); OOL 0.79x (0.33x) and OCL 0.84x (2.3x) posterior ocellus diameter; eyes reaching occipital margin (not reaching); antenna with scape cylindrical (slightly expanded and flattened) 1.3x as long as clava (1.6x), all funicular segments shorter than pedicel (only F6 shorter), scutellum slightly convex from side to side, 0.95x as long as broad (almost flat, 1.2x as long as broad); forewing with linea calva obliterated (not obliterated); gaster dark brown with violet shine (black with bluish green reflection).

ACKNOWLEDGEMENTS

Author is highly thankful to UGC, New Delhi for providing fellowship. We thank Dr. Savita, the Director, Forest Research Institute, for providing the laboratory facility to carry out of the present work.

REFERENCES

Brown, B.V. (1993). A further chemical alternative to critical-point-drying for preparing small (or large) flies. *Fly Times,* 11, 10.

Curtis, J. (1832). *British entomology,* 9, 395.

Dalman, J.W. (1820). Försök till Uppställning af Insect-familjen Pteromalini, i synnerhet med afseen de på de i Sverige funne Arter. *Kungliga Svenska Vetenskapsakademiens Handlingar,* 41(1), 166.

Domenichini, G. (1951). Parassiti e iperparassiti di *Pseudococcus citri* Risso in Italia e nel Peru. *Bollettino di Zoologia Agraria e Bachicoltura, Milano,* 17, 173.

Erdös, J. (1957). Series Encyrtidarum novarum hungaricarum. *Acta Zoologica Academiae Scientiarum Hungaricae,* 3(1, 2), 23.

Gahan, A. B. & Fagan, M. M. (1923). The type species of the genera of Chalcidoidea or chalcidflies. *Bulletin of the United States National Museum,* 124, 1-173.

Graham, M.W.R. de V. (1991). Revision of western European species of *Ericydnus* Haliday (Hym., Encyrtidae) including one species new to science. *Entomologist's Monthly Magazine,* 127, 177-189.

Hoffer, A. (1955). The phylogeny and taxonomy of the family Encyrtidae (Hym., Chalcidoidea). *Acta Musei Nationalis Pragae,* 11(B), 1-22.

Japoshvili, G.O. (2007). New records of Encyrtidae (Hymenoptera, Chalcidodiea [sic]) with the description of three new species from Georgia. *Caucasian Entomological Bulletin,* 3(1), 82-83.

Kerrich, G.J. (1966). On the synonymy of the holarctic species of the genus *Ericydnus* Walker (Hym., Chalc. Encyrtidae). *Opuscula Entomologica, Lund*, 31(1/2), 119-120.

Kerrich, G.J. (1967). On the classification of the anagyrine Encyrtidae, with a revision of some of the genera (Hymenoptera: Chalcidoidea). *Bulletin of the British Museum (Natural History) (Entomology),* 20(5), 143-250.

Liu, X.W., Wang, Y., Li, C.D., & Zhang, Y.Z. (2013). On the Chinese species of *Ericydnus* Haliday (Hymenoptera: Encyrtidae). *Oriental Insects,* 47(1), 23-40.

Masi, L. (1921). Chalcididae raccolte in Cirenaica dal Dott. V. Zanon. *Annali del Museo Civico di Storia Naturale Giacomo Doria. Genova,* 49, 182.

Mayr, G. (1876). Die europäischen Encyrtiden.*Verhandlungen der Zoologisch Botanischen Gesellschaft in Wien,* 25, 76.

Mercet, R.G. (1921). *Fauna Iberica. Himenopteros Fam. Encírtidos.* pp.164 Museo Nacional de Ciencas Naturales, Madrid.

Myartseva, S.N. (1980). Family Encyrtidae. In O.D. Niyazova (Ed.). *Ecological-faunistical complex of insects of South Western Turkmenia* (pp. 87-90). Ashkhabad.

Myartseva, S.N. & Kharchenko, G.A. (1988). Two new *Ericydnus* spp. Walker (Hymenoptera: Encyrtidae) which are parasites of the mealybug (Homoptera: Pseudococcidae). *Izvestiya Akademii Nauk Turkmenskoy SSR (Seriya Biologicheskikh Nauk),* 1988(4), 60-61.

Nees Ab & Esenbeck, C.G. (1834). *Hymenopterorum Ichneumonibus Affinium Monographiae, Genera Europaea et Species Illustrantes:* Volumen secundum. Stuttgartiae et Tubingen.

Nikol'skaya, M. (1952). Chalcids of the fauna of the USSR (Chalcidoidea). *Opredeliteli po Faune SSSR,* 44, 357. Zoologicheskim Institutom Akademii Nauk SSSR, Moscow and Leningrad.

Noyes, J.S. (1980). A review of the genera of Neotropical Encyrtidae (Hymenoptera: Chalcidoidea). *Bulletin of the British Museum (Natural History) (Entomology),* 41, 107-253.

Noyes, J.S. (2000). Encyrtidae of Costa Rica (Hymenoptera: Chalcidoidea), 1. The subfamily Tetracneminae, parasitoids of mealybugs (Homoptera: Pseudococcidae). *Memoirs of the American Entomological Institute,* 62, 1-355.

Noyes, J.S. (2018, July 10). Universal Chalcidoidea Database. Retrieved from http://www.nhm.ac.uk/chalcidoids.htm

Noyes, J.S. & Hayat, M. (1984). A review of the genera of Indo-Pacific Encyrtidae (Hymenoptera: Chalcidoidea). *Bulletin of the British Museum (Natural History) (Entomology),* 48, 131-395.

Sharkov, A.V. (1983). New species of encyrtids (Hymenoptera, Encyrtidae) from the Far East. *Entomologicheskoe Obozrenie,* 62(4), 789-791.

Sharkov, A.V. (1986). New species of encyrtids (Hymenoptera, Encyrtidae) from Primorsky Kray and Sakhalin.*Trudy Zoologicheskogo Instituta. Akademiya Nauk SSSR. Leningrad,*159, 67-68.

Singh, S. & Agarwal, M.M. (1993). Taxonomic studies on Indian encyrtid parasites (Hymenoptera: Encyrtidae) from north-eastern region. *Aligarh Muslim University Zoological Publications, Indian Insect Types,* 14, 18-19.

Sugonjaev, E.S. & Gavrilyuk, A.V. (2012). A new species of chalcid-wasp genus *Ericydnus* Walker (Hymenoptera, Chalcidoidea, Encyrtidae) from west Siberia. *Entomologicheskoe Obozrenie,* 91(1), 160-163.

Trjapitzin, V.A. (1982). New species of the parasitic Hymenoptera genus *Ericydnus* (Hymenoptera, Encyrtidae) of the European fauna. *Vestnik Zoologii,* Kiev (6), 15-17

Trjapitzin, V.A. & Herthevtzian, E.K. (1972). New species of the genus *Ericydnus* Walker, 1837 (Hymenoptera, Encyrtidae) in the Armenian fauna. *Doklady Akademii Nauk Armyanskoy SSR*, 54(5), 277-279.

Walker, F. (1837). Monographia Chalciditum. *Entomologist's Magazine*, 4(5), 439-461.

Walker, F. (1838). Monographia Chalciditum. *Entomologist's Magazine*, 5(5), 428.

Westwood, J.O. (1840). *Synopsis of the genera of British insects*. In *A Introduction to the Modem Classification of Insects. (*Volume 1, 2) (Appendix) 158 pp. London.

Xu, Z.H., Wu, G.Y., & Lou, J.X. (2000). Notes on two genera new to China with three new species of parasitic wasps (Hymenoptera: Encyrtidae) on scale insects. *Entomotaxonomia,* 22(4), 285-286, 289.

Received: June 03, 2019 *Accepted: February 14, 2020*

J. Entomol. Res. Soc., 22(1): 83-99, 2020 *Research Article*
Print ISSN:1302-0250 *Online ISSN:2651-3579*

The First Faunistic Data on the Leaf Beetles (Coleoptera: Chrysomelidae) of 26 Ağustos Nature Park, Afyonkarahisar, Turkey

Ali GÖK[1*] Kadir BOSTAN[2*]

[1,2*]Süleyman Demirel University, Science and Art
Faculty, Department of Biology, 32260, Isparta, TURKEY
e-mails: [1]aligok32@gmail.com, [2]kadirbostan@yahoo.com
ORCID ID: [1] 0000-0003-2931-7151, [2] 0000-0002-6342-0251

ABSTRACT

The first data on faunistic composition of leaf beetles of 26 Ağustos Nature Park, Afyonkarahisar, Turkey are presented. The field researches were conducted from April to November 2017. During this study, a total of 46 leaf beetles species belonging to 23 genera in 6 subfamilies were identified, all of which are recorded for the first time in the study area. Galerucinae was identified as the richest subfamily with 27 species. About 42.15% of the leaf beetle abundance was represented by three species of Alticini: *Chaetocnema concinna* (17.92%), *Altica carduorum* (13.01%) and *Chaetocnema mannerheimi* (11.22%). Eleven species were represented with only one or two specimens in the study area, and they were considered as rare taxa: *Labidostomis cyanicornis*, *Cryptocephalus moraei*, *Leptinotarsa decemlineata*, *Aphthona pygmaea*, *Neocrepidodera ferruginea*, *Phyllotreta procera*, *Ph*. *vittula*, *Psylliodes circumdata*, *Ps*. *napi*, *Galeruca interrupta* and *Cassida seraphina* (0.09%). In addition, short notes about host plant, phenology and abundance of each species are presented.

Key words: Coleoptera, Chrysomelidae, Fauna, 26 Ağustos Nature Park, Afyonkarahisar.

Gök, A. & Bostan, K. (2020). The first faunistic data on the leaf beetles (Coleoptera: Chrysomelidae) of 26 Ağustos Nature Park, Afyonkarahisar, Turkey. *Journal of the Entomological Research Society,* 22(1), 83-99.

INTRODUCTION

The Chrysomelidae, known as the leaf beetles, is considered one of the most important groups of phytophagous beetles due to the number of species, their abundance and relationship with plants (Jolivet & Verma, 2002). Leaf beetles are among the most conspicuous beetles on plants, and perhaps they are best known for their phytophagous habit (Clark et al, 2004).

26 Ağustos Nature Park is situated to the west of Afyonkarahisar, about 17 km from the city center. It is a nature park with an area of 65 hectares located in Akören town of Sinanpaşa, at the intersection of Afyonkarahisar, Antalya and İzmir roads. The Nature Park includes about 87 plant species and there is a small lake in the park (Kervankıran, 2013). 26 Ağustos Nature Park was declared as nature park in 2008 due to camping, trekking, wildlife observations (especially aquatic birds) and scientific excursions.

There are no published records on the leaf beetle fauna of 26 Ağustos Nature Park until now. Therefore, the objectives of the present paper are to investigate the leaf beetle fauna in 26 Ağustos Nature Park and to give short information about host plants, phenology and abundance of some species.

MATERIAL AND METHODS

The specimens were collected in the period from April to November in 2017. The adult beetles were collected by sweeping from various herbaceous and bushes vegetations using an entomological net and aspirator. The specimens were killed with ethyl acetate in plastic bottles, and taken to the laboratory for dissection and identification. All specimens were deposited at the Biology Department of Süleyman Demirel University (Isparta, Turkey). Plants showing feeding marks on the leaves by the adults were considered as a host plant. Identification was performed mainly based on the keys of Gruev & Tomov (1986), Warchałowski (2003, 2010), Čížek & Doguet (2008), Sekerka (2010), Konstantinov, Baselga, Grebennikov, Prena, & Lingafelter (2011), Bezdek & Baselga (2015), Rheinheimer & Hassler (2018). The subfamilies and genera are listed according to the Catalogue of the Palaearctic Coleoptera (Löbl & Smetana, 2010).

RESULTS

During the fieldworks conducted in 2017, a total number of 1060 leaf beetle specimens were collected, representing 46 species in 26 genera, and 6 subfamilies. The total number of individuals, relative abundances and the activity periods of all species collected from the area are given in Table 1. Also, 38 of 46 identified species are reported for the first time from Afyonkarahisar (see, (*) in Table 1). In addition, short notes about each species are presented.

Table 1. Leaf beetle species of 26 Ağustos Nature Park, total number of collected specimens, relative abundance, activity periods and host plants. The asterisk (*) refers to the species registered for the first time from Afyonkarahisar Province.

Species	Total Number	Relative Abundance (%)	Activity Periods	Host Plant
Subfamily: Criocerinae				
Tribe: Lemini				
*Oulema rufocyanea**	37	3,49	APR, MAY, JUN, JUL, AUG, NOV	-
Subfamily: Cassidinae				
Tribe: Cassidini				
*Cassida nobilis**	3	1,89	APR, MAY, JUN, JUL	*Amaranthus* sp.
*Cassida pannonica**	26	0,28	APR, MAY, AUG	-
*Cassida rubiginosa**	1	2,45	APR, MAY, JUN	*Cirsium* sp.
*Cassida seraphina**	11	0,09	APR	-
*Hypocassida cornea**	10	1,04	APR, JUN, AUG, SEP	-
Subfamily: Chrysomelinae				
Tribe: Chyrsomelini				
*Chrysomela populi**	4	0,38	APR, JUL	*Populus nigra* L.
Colaphellus sophiae	27	2,55	APR, JUN	*Descurainia sophia* (L.)
Entomoscelis adonidis	2	0,19	JUN	*Papaver* sp.
*Gastrophysa polygoni**	109	10,28	APR, JUN, JUL, AUG, OCT, NOV	*Polygonum* sp.
Leptinotarsa decemlineata	1	0,09	MAY	-
*Plagiodera versicolora**	18	1,7	APR, MAY, JUN	*Salix* sp.
Subfamily: Galerucinae				
Tribe: Alticini				
*Altica carduorum**	138	13,01	APR, MAY, JUN, JUL, AUG, OCT, NOV	-
*Altica oleracea**	26	2,45	APR, MAY, JUN, JUL, AUG, NOV	-
*Aphthona pygmaea**	1	0,09	APR	*Euphorbia* sp.
*Chaetocnema concinna**	190	17,92	APR – NOV	*Lycopus europaeus* L.
*Chaetocnema coyei**	10	0,94	APR, JUN, AUG, NOV	-
*Chaetocnema mannerheimi**	119	11,22	APR – NOV	-
*Chaetocnema obesa**	2	0,19	APR, JUN	-
*Crepidodera aurata**	25	2,36	APR, MAY, JUN, AUG	*Populus nigra* L., *Salix* sp.
*Longitarsus bertii**	4	0,38	APR, NOV	-
*Longitarsus fallax**	20	2,36	APR, JUN, NOV	-
*Longitarsus fuscoaeneus**	4	0,38	APR, JUN, NOV	-
*Longitarsus kutscherae**	17	1,6	APR, MAY, JUN, JUL, OCT	*Plantago* sp.
*Longitarsus longipennis**	16	1,51	APR, JUN, SEP, NOV	-
*Longitarsus lycopi**	28	2,64	APR	*Amaranthus* sp.

Table 1. Continued.

Species	**Total Number**	**Relative Abundance (%)**	**Activity Periods**	**Host Plant**
*Longitarsus pellucidus**	37	3,49	JUN, AUG, SEP, OCT, NOV	-
*Neocrepidodera ferruginea**	1	0,09	JUN	-
Phyllotreta atra	6	0,57	APR, JUN	-
Phyllotreta corrugata	13	1,23	APR, AUG	*Diplotaxis tenuifolia* (L.)
*Phyllotreta nemorum**	20	1,89	APR, MAY	-
*Phyllotreta ochripes**	25	2,36	APR, MAY, JUN, NOV	-
*Phyllotreta procera**	1	0,09	APR	-
*Phyllotreta vittula**	2	0,19	APR	*Cardaria draba* (L.)
*Psylliodes circumdata**	1	0,09	JUL	-
*Psylliodes napi**	1	0,09	JUL	-
*Psylliodes tricolor**	20	1,89	APR, JUN, JUL, NOV	-
Tribe: Galerucini				
*Galeruca interrupta**	1	0,09	APR	-
Tribe: Luperini				
*Luperus xanthopoda**	29	2,74	APR, MAY, JUN, JUL, NOV	*Betula* sp., *Prunus* sp., *Salix babylonica* L.
Subfamily: Cryptocephalinae				
Tribe: Clytrini				
*Labidostomis cyanicornis**	1	0,09	JUL	-
*Labidostomis oertzeni**	7	0,66	JUN	*Populus nigra* L.
Tribe: Cryptocephalini				
Cryptocephalus connexus	20	1,89	JUL, AUG	-
*Cryptocephalus duplicatus**	2	0,19	JUN, JUL	-
Cryptocephalus moraei	1	0,09	JUN	-
Pachybrachis fimbriolatus	18	1,7	MAY, JUN, JUL	-
Subfamily: Eumolpinae				
Tribe: Bromiini				
*Pachnephorus villosus**	5	0,47	APR, MAY, JUN, JUL	-
TOTAL	1060			

Subfamily: Criocerinae Latreille, 1804

Tribe: Lemini Gyllenhal, 1813

Oulema rufocyanea (Suffrian, 1847)

Material examined: 15.04.2017, 5♂♂, 5♀♀; 29.04.2017, 1♂; 13.05.2017, 7♂♂, 4♀♀; 07.06.2017, 3♂♂, 2♀♀; 01.07.2017, 5♂♂, 3♀♀; 06.08.2017, 1♂; 11.11.2017, 1♀.

Notes: This species was known only from Ankara in Turkey (Bezděk & Baselga, 2015). It was common in the study area and active from April to November. This species is morphologically very similar to *Oulema melanopus*. It can easily be misidentified as *O. melanopus*. It is very likely that some records of *O. melanopus* in Turkey refer to *O. rufocyanea*.

Subfamily: Cassidinae Gyllenhal, 1813

Tribe: Cassidini Gyllenhal, 1813

Cassida nobilis Linnaeus, 1758

Material examined: 15.04.2017, 3♂♂; 29.04.2017, 2♂♂; 13.05.2017, 1♂; 07.06.2017, 1♀; 21.06.2017, 1♂, 1♀; 01.07.2017, 1♂.

Notes: A very common and widely distributed species throughout Turkey (Ekiz, Aslan & Gök, 2013). It was found in small numbers in the park during April-July and collected on *Amaranthus* sp.

Cassida pannonica Suffrian, 1844

Material examined: 29.04.2017, 1♂; 13.05.2017, 1♂; 27.08.2017, 1♂.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013), but not abundant in the park. It was a rare species represented with only three specimens collected on herbaceous vegetations dominated by Asteraceae in April-August.

Cassida rubiginosa Müller, 1776

Material examined: 15.04.2017, 4♂♂, 1♀; 29.04.2017, 8♂♂, 1♀; 13.05.2017, 4♂♂, 2♀♀; 07.06.2017, 3♂♂, 2♀♀; 21.06.2017, 1♂.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was also common in the park and found in large numbers on *Cirsium* sp. during April-June.

Cassida seraphina Ménétriés, 1836

Material examined: 15.04.2017, 1♂.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was a very rare species in the park, represented with only one male specimen in April. The specimen was collected on herbaceous vegetation.

Hypocassida cornea (Marseul, 1868)

Material examined: 15.04.2017, 1♂; 07.06.2017, 7♂♂; 06.08.2017, 1♂, 1♀; 30.09.2017, 1♂.

Notes: Known from only Artvin province, in Turkey (Gök & Turantepe, 2019). It was found in relatively small numbers in the park during April-September. It was a species often confused with *Hypocassida subferruginea* morphologically.

Subfamily: Chrysomelinae Latreille, 1802

Tribe: Chrysomelini Latreille, 1802

Chrysomela populi Linnaeus, 1758

Material examined: 15.04.2017, 1♂, 1♀; 01.07.2017, 2♂♂.

Notes: A very common and widely distributed species in Turkey (Ekiz et al, 2013); however, it was a rare species represented with only four specimens in the park during April and July. It was collected from leaves of *Populus nigra*, one of its main hosts.

Colaphellus sophiae (Schaller, 1783)

Material examined: 15.04.2017, 14♂♂, 12♀♀; 21.06.2017, 1♂.

Notes: A very common and widely distributed species in Turkey, except southeast Anatolia region (Ekiz et al, 2013). It was found in large numbers in the park during April-June and collected on *Descurainia sophia*.

Entomoscelis adonidis (Pallas, 1771)

Material examined: 07.06.2017, 1♂; 21.06.2017, 1♀.

Notes: A very common and widely distributed species in Turkey (Ekiz et al, 2013); however, it was a rare species represented with only two specimens in the park. It was collected on leaves of *Papaver* sp.

Gastrophysa polygoni (Linnaeus, 1758)

Material examined: 15.04.2017, 3♂♂, 5♀♀; 29.04.2017, 14♂♂, 12♀♀; 07.06.2017, 2♂♂, 2♀♀; 21.06.2017, 22♂♂, 10♀♀; 01.07.2017, 7♂♂, 3♀♀; 23.07.2017, 8♂♂, 4♀♀; 06.08.2017, 2♂♂; 27.08.2017, 3♂♂; 21.10.2017, 3♂♂, 5♀♀; 25.11.2017, 3♂♂, 1♀.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was also one of the most common species in the park and found from April to November. The specimens were collected on *Polygonum* sp.

Leptinotarsa decemlineata (Say, 1824)

Material examined: 13.05.2017, 1♂.

Notes: This species was restricted to western North America: Colorado and surroundings. Because it feeds on *Solanum* sp. it has been introduced accidentally to all regions of the world where potatoes are grown. Outside potato plantations it is found on other species of *Solanum*, but generally rare. It is a cosmopolitan species throughout the world (Winkelman & Debreuil, 2008) and similarly widespread in Turkey (Ekiz et al, 2013). But it was rarely found in the park and represented with only one male specimen.

Plagiodera versicolora **(Laicharting, 1781)**

Material examined: 29.04.2017, 4♂♂, 7♀♀; 13.05.2017, 2♂♂; 07.06.2017, 4♂♂, 1♀.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was found in large numbers between April-June. Adults have been collected on *Salix* sp., one of the main host plants.

Subfamily: Galerucinae Latreille, 1802

Tribe: Alticini Spinola, 1844

Altica carduorum **Guérin-Méneville, 1858**

Material examined: 15.04.2017, 2♂♂, 13♀♀; 29.04.2017, 21♂♂, 21♀♀; 13.05.2017, 15♂♂, 32♀♀; 07.06.2017, 3♂♂, 1♀; 21.06.2017, 1♂, 3♀♀; 01.07.2017, 4♂♂, 7♀♀; 06.08.2017, 1♂, 4♀♀; 21.10.2017, 2♀♀; 11.11.2017, 1♂, 2♀♀; 25.11.2017, 1♂, 4♀♀.

Notes: A widely distributed species in Turkey, especially in central Anatolian parts (Ekiz et al, 2013). It was one of the most common and abundant species of the park and found from April to November.

Altica oleracea **(Linnaeus, 1758)**

Material examined: 29.04.2017, 1♂, 7♀♀; 13.05.2017, 6♀♀; 07.06.2017, 5♀♀; 01.07.2017, 2♀♀; 06.08.2017, 2♀♀; 11.11.2017, 1♂, 2♀♀.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was found in large numbers from April to November.

Aphthona pygmaea **(Kutschera, 1861)**

Material examined: 29.04.2017, 1♀.

Notes: Known from Edirne, İzmir, Aydın, Sakarya, Isparta, Antalya, Mersin, Adana, Erzincan and Erzurum in Turkey (Ekiz et al, 2013). Although it was a common species on *Euphorbia* spp., the main host plants, it was rarely found in the park and represented with only one female specimen in April.

Chaetocnema concinna **(Marsham, 1802)**

Material examined: 15.04.2017, 24♂♂, 22♀♀; 29.04.2017, 30♂♂, 29♀♀; 13.05.2017, 9♂♂, 5♀♀; 07.06.2017, 5♂♂, 4♀♀; 21.06.2017, 2♂♂, 5♀♀; 01.07.2017, 1♂, 3♀♀; 23.07.2017, 1♂; 06.08.2017, 14♂♂, 17♀♀; 27.08.2017, 1♂, 1♀; 30.09.2017, 4♀♀; 21.10.2017, 1♂, 8♀♀; 11.11.2017, 2♀♀; 25.11.2017, 1♂, 1♀.

Notes: Known from Edirne, Kocaeli, Eskişehir, Isparta, Antalya, Ankara, Nevşehir, Samsun, Amasya, Bayburt and Erzurum in Turkey (Ekiz et al, 2013). It was one of the most common and abundant species of the park and found from April to November. It was collected frequently in moist open areas on various species of Poaceae.

Chaetocnema coyei (Allard, 1864)

Material examined: 15.04.2017, 2♂♂; 07.06.2017, 4♂♂, 1♀; 27.08.2017, 1♂, 1♀; 11.11.2017, 1♀.

Notes: A very common and widely distributed species in Turkey (Ekiz et al, 2013), but not abundant in the study area.

Chaetocnema mannerheimi (Gyllenhal, 1827)

Material examined: 15.04.2017, 8♂♂, 8♀♀; 29.04.2017, 24♂♂, 8♀♀; 13.05.2017, 3♂♂, 10♀♀; 07.06.2017, 2♂♂, 12♀♀; 21.06.2017, 3♀♀; 01.07.2017, 4♂♂, 1♀; 23.07.2017, 3♂♂, 4♀♀; 06.08.2017, 3♂♂, 11♀♀; 30.09.2017, 2♂♂, 3♀♀; 21.10.2017, 4♂♂, 3♀♀; 11.11.2017, 1♂; 25.11.2017, 2♂♂.

Notes: Known from Edirne, Eskişehir, Ankara and Konya in Turkey (Ekiz et al, 2013). It was one of the most common and abundant species of the study area and found from April to November. Specimens were collected on *Phragmites australis* and various herbaceous plants.

Chaetocnema obesa (Boieldieu, 1859)

Material examined: 29.04.2017, 1♂; 07.06.2017, 1♀.

Notes: This species generally occurs in central parts of Turkey (Ekiz et al, 2013). It was very rare in the area, and only two specimens were found during April-June.

Crepidodera aurata (Marsham, 1802)

Material examined: 15.04.2017, 3♂♂, 5♀♀; 29.04.2017, 5♂♂, 2♀♀; 13.05.2017, 1♀; 07.06.2017, 3♂♂, 4♀♀; 27.08.2017, 1♂.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was collected on leaves of *Salix* sp. and *Populus* sp., the main host plants, between April and August.

Longitarsus bertii Leonardi, 1973

Material examined: 29.04.2017, 1♂, 1♀; 21.10.2017, 1♂, 1♀.

Notes: Known from Aydın, Isparta, Antalya, Konya, Aksaray, Gümüşhane and Erzurum in Turkey (Ekiz et al, 2013). It was a rare species in the park, represented with only four specimens in April and October.

Longitarsus fallax Weise, 1888

Material examined: 29.04.2017, 5♂♂, 7♀♀; 07.06.2017, 2♀♀; 11.11.2017, 5♂♂, 1♀.

Notes: A common species in Turkey (Ekiz et al, 2013). It was found in large numbers in the park in April, June and November.

Longitarsus fuscoaeneus Redtenbacher, 1849

Material examined: 29.04.2017, 1♀; 07.06.2017, 1♀; 11.11.2017, 1♂, 1♀.

Notes: Known from Burdur, Antalya, Ankara, Mersin, Kayseri, Adana and Erzurum in Turkey (Ekiz et al, 2013). It was a rare species in the park, represented with only four specimens in April, June and November.

Longitarsus kutscherae **(Rye, 1872)**

Material examined: 15.04.2017 1♂, 4♀♀; 29.04.2017, 2♂♂, 2♀♀; 13.05.2017, 1♀; 07.06.2017, 2♂♂; 01.07.2017, 1♂, 2♀♀; 21.10.2017, 2♂♂.

Notes: A species with restricted distribution in Turkey and known only from Isparta and Erzurum (Ekiz et al, 2013). It was found in small numbers in the park during April-October and collected on leaves of *Plantago* sp.

Longitarsus longipennis **Kutschera, 1863**

Material examined: 29.04.2017, 2♂♂; 07.06.2017, 3♂♂, 3♀♀; 30.09.2017, 1♂, 1♀; 25.11.2017, 2♂♂, 4♀♀.

Notes: A species with restricted distribution in Turkey and known only from Isparta, Antalya and Erzurum (Ekiz et al, 2013). It was found in small numbers in the park from April to November. Specimens were collected on various herbaceous vegetation.

Longitarsus lycopi **(Foudras, 1860)**

Material examined: 15.04.2017, 3♂♂, 2♀♀; 29.04.2017, 9♂♂, 14♀♀.

Notes: A common species in Turkey (Ekiz et al, 2013). It was found in large numbers in the park only in April and collected on *Amaranthus* sp.

Longitarsus pellucidus **(Foudras, 1860)**

Material examined: 07.06.2017, 2♂♂; 06.08.2017, 4♀♀; 27.08.2017, 2♂♂, 4♀♀; 30.09.2017, 1♀; 21.10.2017, 10♂♂, 2♀♀; 11.11.2017, 2♂♂, 2♀♀; 25.11.2017, 2♂♂, 6♀♀.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was a common species in the park, found in large numbers on herbaceous plants from April to November.

Neocrepidodera ferruginea **(Scopoli, 1763)**

Material examined: 21.06.2017, 1♂.

Notes: Known from İstanbul, Sakarya, Isparta, Antalya, Ankara, Konya, Kayseri, Artvin and Erzurum in Turkey (Ekiz et al, 2013). It was a very rare species represented with only one male specimen in the study area in June.

Phyllotreta atra **(Fabricius, 1775)**

Material examined: 15.04.2017, 2♂♂, 2♀♀; 29.04.2017, 1♂; 21.06.2017, 1♂.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was found in small number in the park during April-June. It was collected on herbaceous vegetation of Brassicaceae, the main host family of the genus.

Phyllotreta corrugata Reiche & Saulcy, 1858

Material examined: 15.04.2017, 5♂♂, 2♀♀; 29.04.2017, 3♂♂, 1♀; 27.08.2017, 1♂, 1♀.

Notes: A widespread species in Turkey (Ekiz et al, 2013) but not common in the park. It was found in small number in April and August only, and collected on *Cardaria draba*, one of the main host plants.

Phyllotreta nemorum (Linnaeus, 1758)

Material examined: 15.04.2017, 3♂♂, 6♀♀; 29.04.2017, 4♂♂, 5♀♀; 13.05.2017, 2♀♀.

Notes: Generally, a species occurring in central and eastern Turkey (Ekiz et al, 2013). It was a rare species in the park, found in small number during spring season. The specimens were collected on herbaceous vegetation of Brassicaceae, the main host family of the genus.

Phyllotreta ochripes (Curtis, 1837)

Material examined: 15.04.2017, 1♂, 1♀; 29.04.2017, 7♂♂, 4♀♀; 13.05.2017, 1♂, 2♀♀; 07.06.2017, 7♂♂, 1♀; 25.11.2017, 1♀.

Notes: Known only from İstanbul, Niğde and Giresun, with a restricted distribution in Turkey (Ekiz et al, 2013). It was found in relatively large numbers in the park from April to November. The specimens were collected on herbaceous vegetation of Brassicaceae, the main host family of the genus.

Phyllotreta procera (Redtenbacher, 1849)

Material examined: 15.04.2017, 1♂.

Notes: Generally, a species occurring in central and east parts of Turkey (Ekiz et al, 2013). It was a very rare species in the park, represented with only one male specimen in April. The specimen was collected on herbaceous vegetation of Brassicaceae, the main host family of the genus.

Phyllotreta vittula (Redtenbacher, 1849)

Material examined: 15.04.2017, 1♂, 1♀.

Notes: Known from Edirne, İzmir, Aydın, Isparta, Antalya, Ankara, Sivas and Erzurum in Turkey (Ekiz et al, 2013). It was a very rare species in the park, represented with only two specimens in April. The specimens were collected on herbaceous vegetation of Brassicaceae, the main host family of the genus.

Psylliodes circumdata (Redtenbacher, 1842)

Material examined: 01.07.2017, 1♀.

Notes: Known from İzmir, Aydın, Eskişehir, Antalya, Ankara, Konya, Amasya, Erzincan and Erzurum in Turkey (Ekiz et al, 2013). It was a very rare species in the park, represented with only one female specimen in July. It was collected on herbaceous vegetations.

Psylliodes napi **(Fabricius, 1792)**

Material examined: 01.07.2017, 1♂.

Notes: Known from Manisa, Isparta, Antalya, Ankara, Mersin, Kayseri, Adana, Ordu, Artvin and Erzurum in Turkey (Ekiz et al, 2013). It was a very rare species in the park, represented with only one male specimen in July. It was collected on herbaceous vegetations.

Psylliodes tricolor **Weise, 1888**

Material examined: 29.04.2017, 2♀♀; 07.06.2017, 1♂, 2♀♀; 21.06.2017, 4♂♂, 2♀♀; 01.07.2017, 6♂♂; 25.11.2017, 2♂♂, 1♀.

Notes: A very common and widely distributed species throughout Turkey (Ekiz et al, 2013). It was a relatively common species in the park, found in large numbers from April to November. The specimens were collected on herbaceous vegetations belonging to Brassicaceae.

Tribe: Galerucini Latreille, 1802

Galeruca interrupta **Illiger, 1802**

Material examined: 29.04.2017, 1♂.

Notes: Generally a common species occurring in central and eastern Turkey (Ekiz et al, 2013). It was a very rare species in the park, represented with only one male specimen in April.

Tribe: Luperini Gistel, 1848

Luperus xanthopoda **(Schrank, 1781)**

Material examined: 15.04.2017, 4♂♂, 1♀; 29.04.2017, 4♂♂; 13.05.2017, 2♂♂, 1♀; 07.06.2017, 4♂♂, 2♀♀; 21.06.2017, 1♂, 1♀; 01.07.2017, 5♂♂, 1♀; 21.10.2017, 3♂♂.

Notes: Generally, a very common species occurring in central and eastern Turkey (Ekiz et al, 2013). It was relatively common in the park, found in large numbers from April to October. The specimens were collected on *Prunus* sp., *Betula* sp. and *Salix* sp.

Subfamily: Cryptocephalinae Gyllenhal, 1813

Tribe: Clytrini Kirby, 1837

Labidostomis cyanicornis **(Germar, 1822)**

Material examined: 01.07.2017, 1♂.

Notes: Only known from Düzce, Konya and Adana in Turkey (Ekiz et al, 2013). It is a rare species represented with only one male specimen in the park in July.

Labidostomis oertzeni Weise, 1889

Material examined: 07.06.2017, 4♂♂, 3♂♂.

Notes: Widely distributed species in Turkey (Ekiz et al, 2013). It was found in small numbers in the park in June.

Tribe: Cryptocephalini Gyllenhal, 1813

Cryptocephalus connexus Olivier, 1807

Material examined: 01.07.2017, 1♂, 4♀♀; 23.07.2017, 4♂♂, 1♀; 06.08.2017, 5♂♂, 2♀♀; 27.08.2017, 3♂♂.

Notes: A very common and widely distributed species in Turkey (Ekiz et al, 2013). It was found in large numbers in the park in July-August.

Cryptocephalus duplicatus Suffrian, 1847

Material examined: 21.06.2017, 1♂; 01.07.2017, 1♂.

Notes: A common and widespread species in Turkey (Ekiz et al, 2013); however, it was represented with only two male specimens in the park during June-July.

Cryptocephalus moraei (Linnaeus, 1758)

Material examined: 21.06.2017, 1♂.

Notes: A very common and widely distributed species in Turkey (Ekiz et al, 2013); however, it was a rare species represented with only one male specimen in the park in June.

Pachybrachis fimbriolatus (Suffrian, 1848)

Material examined: 13.05.2017, 3♂♂; 07.06.2017, 5♂♂, 6♀♀; 21.06.2017, 3♂♂; 01.07.2017, 1♂.

Notes: A common and widely distributed species in Turkey (Ekiz et al, 2013) and found in large numbers in the park between May-July.

Subfamily: Eumolpinae Hope, 1840

Tribe: Bromiini Baly, 1865

Pachnephorus villosus (Duftschmid, 1825)

Material examined: 15.04.2017, 1♂; 13.05.2017, 1♂; 07.06.2017, 1♂; 21.06.2017, 1♀; 01.07.2017, 1♂.

Notes: A widely distributed species in Turkey except eastern regions (Ekiz et al, 2013). We found a total of only five specimens in the park between April-July.

Faunistic and taxonomic evaluations and abundance

During the studies conducted in 2017, a total of 1060 specimens belonging to 46 species in 23 genera from 6 subfamilies were collected. All species were recorded for the first time in 26 Ağustos Nature Park.

The distribution and percentage ratios according to subfamilies of 46 species identified in the study were given in Fig. 1. Galerucinae, with 27 species, was the most abundant subfamily comprising about 59% of all species collected from 26 Ağustos Nature Park. It was followed by Chrysomelinae and Cryptocephalinae with 6 species each (13%), Cassidinae with 5 species (11%), Criocerinae and Eumolpinae with 1 species each (2%).

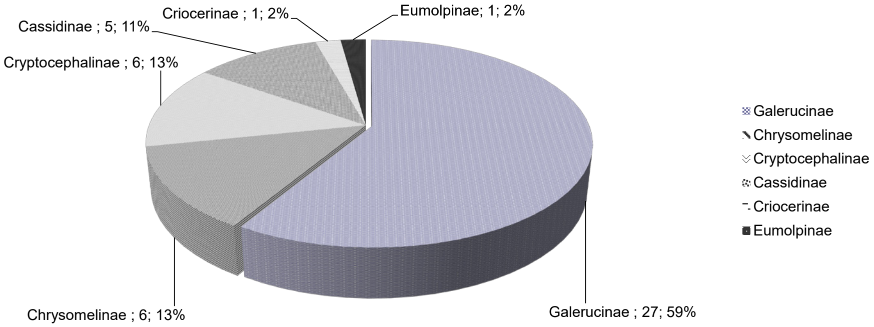


Fig. 1. The distribution and percentage ratios of the species according to subfamilies.

Chaetocnema concinna (17.92%), *Altica carduorum* (13.01%), and *Chaetocnema mannerheimi* (11.22%) were the three most dominant species in the park. The rarest species in the study area were *Labidostomis cyanicornis*, *Cryptocephalus moraei*, *Leptinotarsa decemlineata*, *Aphthona pygmaea*, *Neocrepidodera ferruginea*, *Phyllotreta procera*, *Psylliodes circumdata*, *Ps. napi*, *Galeruca interrupta* and *Cassida seraphina* (0.09%).

Alticini is one of the most diverse groups in Chrysomelidae (Nadein, 2012; Korotyaev, Konstantinov, & Volkovitsh, 2017). The most dominant and abundant three species were members of the tribe Alticini (Galerucinae). Considering the species richness of the group, it was an expected result that the members of this tribe were most dominant and abundant in the field.

Thirty-nine species in the study area were very common and widely distributed species throughout Turkey. Afyonkarahisar is located at the intersection point of Aegean, Central Anatolian and Mediterranean regions and both temperate and cold continental climate types are seen in there. The geographical position and climatic characteristics of the area created a suitable distribution area for these common species.

Two species should be re-evaluated in terms of taxonomy and zoogeography throughout Turkey. *Oulema rufocyanea* is morphologically very similar to *O. melanopus*, and these two species are generally confused with each other. It is highly probable that *O. rufocyanea* is distributed in some of the provinces where *O. melanopus* is distributed in the checklist published by Ekiz et al (2013). Therefore, taxonomic and zoogeographical situation of these two species in Turkey should be re-evaluated. Accordingly, *Hypocassida cornea* is very similar to *H. subferruginea*, and the situations of these two species in Turkey are needed to be reconsidered in taxonomic and zoogeographic terms.

Host-plant evaluation

Leaf beetles are one of the most important phytophagous beetle families in terms of species diversity and relationships with their host plants (Jolivet & Verma, 2002; Clark et al, 2004). The high species diversity of this group is associated with the preference of various plant families as hosts (Fuss, Geiser & Patzner, 2005). It is not always easy to establish host plant preferences of leaf beetles except some species. Therefore, leaf beetles and host plant preferences require a special effort in field studies.

Host plant preferences of the leaf beetles in the study area, and host plant families and preference percentages by leaf beetles are presented in Fig. 2. The host plant of 23 species (50%) out of 46 leaf beetles in 26 Ağustos Nature Park was identified at genus and species level (Table 1). It was observed that most of the leaf beetles in the study area preferred members of the Brassicaceae (27%). Other preferred plant families are Salicaceae (19%), Asteraceae (11%), Amaranthaceae (8%), Poaceae (7%), Rosaceae (4%), Betulaceae (4%), Plantaginaceae (4%), Lamiaceae (4%), Euphorbiaceae (4%), Polygonaceae (4%), and Papaveraceae (4%) (Fig. 2). Most of the identified host plants were compatible with the host plant preferences given in the literature (Kısmalı & Sassi, 1994; Fuss et al, 2005; D'Alessandro & Biondi, 2008; Rozner & Rozner, 2008; Aslan, Gök, Gürbüz & Ayvaz, 2009; Gavrilović & Ćurčić, 2013; Gavrilović, Gavrilović, Ćurčić, Stojanović, & Savić 2014; Baviera & Biondi, 2015; Bezděk & Baselga, 2015; Petitpierre, Sacares, & Jurado-Rivera, 2017). However, *Longitarsus kutschera* was collected while feeding on *Plantago* species. This data is interesting because there are some signs that this species has been feeding on *Plantago* species recently (Rheinheimer & Hassler, 2018). So this data confirms the literature of Rheinheimer & Hassler (2018). It was observed that *Labidostomis oertzeni*, *Chrysomela populi*, *Plagiodera versicolora*, *Crepidodera aurata* and *Luperus xanthopoda* were related with the ligneous vegetations in the area. These species prefer mainly species belonging to Salicaceae, Betulaceae and Rosaceae.

Phenological evaluation

The highest species numbers of leaf beetles were observed in the late spring and at the beginning of summer, while the least species numbers were registered in autumn (Fig.3). The most active period of the leaf beetles was generally the spring season because the increase in diversity of the host plants with rich nutrient content in this season leads to an increase in leaf beetle species diversity (Aslan et al, 2009). The results obtained from the fieldwork confirm these data.

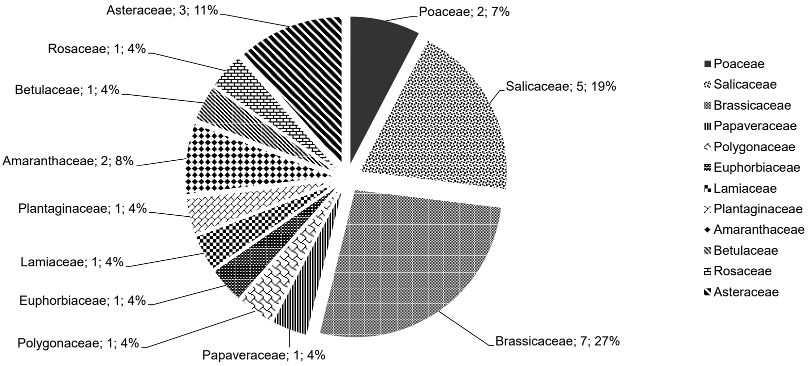


Fig. 2. Host plant preferences of the leaf beetles and their families and preference percentages.

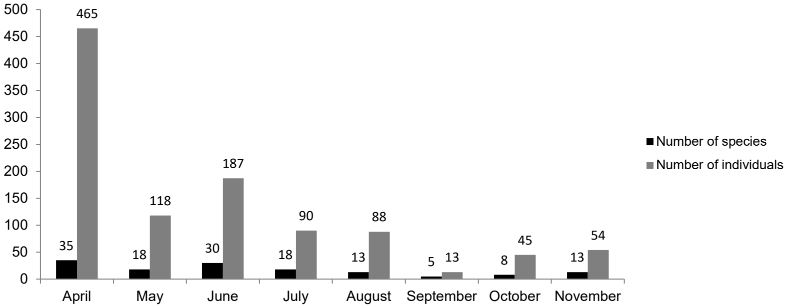


Fig. 3. Species and individual numbers according to months.

CONCLUSION

Consequently, the present study is important as it is the first faunistic study performed in the field. As a result, a total of 46 species were recorded; however, this number is low for a nature park. Possible reasons may be the lack of rich plant diversity, the cutting of the existing vegetation occasionally, the arrangement of the majority of the park as a picnic area and intensive human activities in the area. In addition to these, another reason is that the sampling includes a one-year period. The species number can rise with further collections trips. But the lack of rich plant biodiversity, especially of plants which occur on nutrient-poor grassland is the main factor for the restriction of the species number there. These are serious pressures for leaf beetles and other species. As a conclusion, this study will contribute to the understanding of leaf beetle fauna in a small Nature Park. Also, this study could be used as a first step to make suggestion for landscape management.

ACKNOWLEDGEMENTS

This study was supported by Scientific Research Project Management of Süleyman Demirel University (SDÜBAP) with project number 4906-YL1-17.

REFERENCES

Aslan, E.G., Gök, A., Gürbüz, M.F., & Ayvaz, Y. (2009). Species composition of Chrysomelidae (Coleoptera) in Saklıkent vicinity (Antalya, Turkey) with observations on potential host plants. *Journal of the Entomological Research Society*, 11(3), 7-18.

Baviera, C. & Biondi, M. (2015). The Alticini (Coleoptera: Chrysomelidae, Galerucinae) of Sicily: recent records and updated checklist. *AAPP Physical, Mathematical, and Natural Sciences*, 93(2), 1-50. DOI: 10.1478/AAPP.932A2

Bezděk, J. & Baselga, A. (2015). Revision of western Palaearctic species of the *Oulema melanopus* group, with description of two new species from Europe (Coleoptera: Chrysomelidae: Criocerinae). *Acta Entomologica Musei Nationalis Pragae*, 55(1), 273-304.

Čížek, P. & Doguet, S. (2008). *Key to the determination of flea beetles (Coleoptera: Chrysomelidae: Alticinae) in the Czech Republic and Slovakia* (in Czeh). VH Print Nové Město nad Metují, Česko Czechia, 232 pp.

Clark, S., LeDoux, D.G., Seeno, T.N., Riley, E.G., Gilbert, A.J., & Sullivan, J.M. (2004). Host Plants of Leaf Beetle Species Occurring in the United States and Canada (Coleoptera: Orsodacnidae, Megalopodidae, Chrysomelidae exclusive of Bruchinae). *The Coleopterists Society, Special Publication 2*, Canada., 601 pp.

D'Alessandro, P. & Biondi, M. (2008). Contribution to the knowledge of the Chrysomelidae of Vincheto di Celarda (Coleoptera). In Hardersen, S., Mason, F., Viola, F., Campedel, D., Lasen, C. & Cassol, M. (Ed.). *Research on the natural heritage of the reserves Vincheto di Celarda and Val Tovanella (Belluno province, Italy). Conservation of two protected areas in the context of a Life Project* (153-179). Verona:.Quaderni Conservazione Habitat, 5. Arti Grafi che Fiorini.

Ekiz, A.N., Şen, İ., Aslan, E.G., & Gök, A. (2013). Checklist of leaf beetles (Coleoptera: Chrysomelidae) of Turkey, excluding Bruchinae. *Journal of Natural History*, 47(33-34), 2213-2287.

Fuss, G., Geiser, E., & Patzner, R. (2005). On the host plants of several leaf beetles of Central Europe the problem of fame and evidence (Coleoptera: Chrysomelidae). *Koleopterologische Rundschau*, 75, 359-371.

Gavrilović, B. & Ćurčić, S. (2013). The Diversity of the Family Chrysomelidae (Insecta: Coleoptera) of the Obedska Bara Special Nature Reserve (Vojvodina Province, Serbia), with Special Reference to the Host Plants. *Acta Zoologica Bulgarica*, 65(1), 37-44.

Gavrilović, B., Gavrilović, B., Ćurčić, S., Stojanović, D., & Savić, D. (2014). Leaf Beetles (Coleoptera: Chrysomelidae) of MT. Fruška Gora (Vojvodina Province, Northern Serbia), with an Overview of Host Plants. *Šumarski List*, 1(2), 29-41.

Gök, A. & Turantepe, E. (2019). Additions to the fauna of Chrysomelidae (Coleoptera) from Hatila Valley National Park (Artvin, Turkey), with notes on host plant preferences and zoogeographic evaluations. *Caucasian Entomological Bulletin*, 15(1), 135-146.

Gruev, B.A. & Tomov, V. (1986). *Fauna Bulgarica. 16 Coleoptera, Chrysomelidae Part II Chrysomelinae, Galerucinae, Alticinae, Hispinae, Cassidinae*. Aedibus Academie Scientiarium Bulgaricae, Sofia, 388 pp.

Jolivet, P. & Verma, K.K. (2002). *Biology of Leaf Beetles*. Intercept Publisher, United Kingdom, 335 pp.

Kervankıran, İ. (2013). *Afyonkarahisar İlinin Sürdürülebilir Turizm Açısından Değerlendirilmesi*. Kocatepe Akademi Yayınları, Afyonkarahisar, 284 pp.

Kısmalı, Ş. & Sassi, D. (1994). Preliminary list of Chrysomelidae with notes on distribution and importance of species in Turkey. II. Subfamily Cassidinae Spaeth. *Türkiye Entomoloji Dergisi*, 18(3), 141-156.

Konstantinov, A.S., Baselga, A., Grebennikov, V.V., Prena, J., & Lingafelter, S.W. (2011). *Revision of the Palearctic Chaetocnema Species (Coleoptera: Chrysomelidae: Galerucinae: Alticini)*. Pensoft Publishers, Bulgaria, 334 pp.

Korotyaev, B.A., Konstantinov, A.S., & Volkovitsh, M.G. (2017). Insect Biodiversity in the Palearctic Region. In R.G. Foottit & P.H. Adler, (Eds.). *Insect Biodiversity: Science and Society, Volume I*, (2nd ed.). Chichester: John Wiley & Sons Ltd, 632 pp.

Löbl, I. & Smetana, A. (2010). *Catalogue of Palaearctic Coleoptera: Chrysomeloidea*, Volume 6. Apollo Books. Stenstrup, 924 pp.

Nadein, K.S. (2012, June 18). *Catalogue of Alticini genera of the World (Coleoptera: Chrysomelidae)*. Retrieved from http://www.zin.ru/Animalia/Coleoptera/eng/alticinw.htm

Petitpierre, E., Sacares, A., & Jurado-Rivera, J.A. (2017). Updated checklist of Balearic leaf beetles (Coleoptera: Chrysomelidae). *Zootaxa*, 4272(2), 151-177.

Rheinheimer, J. & Hassler, M. (2018). *Die Blattkäfer Baden-Württembergs*. Kleinsteuber Books, Karlsruhe, 928 pp.

Rozner, I. & Rozner, G. (2008). Data to the leaf-beetle fauna of Macedonia (Coleoptera, Chrysomelidae). *Natura Somogyiensis*, 12, 111-131.

Sekerka, L. (2010). *Icones Insectorum Europae Centralis: Coleoptera: Chrysomelidae Cassidinae, No: 13*. Folia Heyrovskyana, Czech Republic, 24 pp.

Warchałowski, A. (2003). *Chrysomelidae: The Leaf-beetles of Europe and the Mediterranean Area*. Natura Optima Dux Foundation, Warszawa, 600 pp.

Warchałowski, A. (2010). *The Palaearctic Chrysomelidae. Identification Keys. Vol. 1-2*. Natura Optima Dux Foundation, Warszawa, 1212 pp.

Winkelman, J. & Debreuil M. (2008). *Les Chrysomelinae de France (Coleoptera, Chrysomelidae)*. Rutilans, France, 188 pp.

Received: June 19, 2019 *Accepted: February 19, 2020*

J. Entomol. Res. Soc., 22(1): 101-105, 2020
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

New Species and Additional Records of the Genus *Leptobium* Casey (Coleoptera: Staphylinidae: Paederinae) from Central Anatolia

Sinan ANLAŞ[1] Semih ÖRGEL[2]

[1]Alaşehir Vocational School, Manisa Celal Bayar University, TR-45600, Alaşehir, Manisa, TURKEY
[2]Biology Department, Ege University, TR-35400, Bornova, İzmir, TURKEY
e-mails: sinan.anlas@gmail.com orgelsemih@gmail.com;
ORCID IDs: [1]0000-0001-7059-5677, [2]0000-0002-6362-3091

ABSTRACT

In this paper, *Leptobium angoranum* from Ankara (Beypazarı) province in central northern Anatolia is described. Its diagnostic characters are illustrated and distinguished from related congeners. Additional records of three species of *Leptobium* from Turkey are reported. The genus is now represented in Turkey by 18 species, 13 of them are endemic.

Key words: Coleoptera, Staphylinidae, Paederinae, Leptobium, new species, Turkey.

Anlaş, S. & Örgel, S. (2020). New species and additional records of the genus Leptobium Casey (Coleoptera: Staphylinidae: Paederinae) from Central Anatolia. *Journal of the Entomological Research Society*, 22(1), 101-105.

INTRODUCTION

In the Palaearctic region, the genus *Leptobium* Casey is represented by 70 species, 17 of which occur in Turkey (Schülke & Smetana, 2015; Assing, 2017; Anlaş, 2017), and that represents nearly 25% of *Leptobium* of the Palaearctic fauna. Most species of the genus in Turkey are brachypterous and more or less endemic. 12 of 17 species are restricted to Anatolia and represent 70% of the Turkish *Leptobium* fauna (Anlaş, 2017).

The fauna of the subfamily Paederinae is known to be rich in the central Anatolia region, but is poorly studied. To overcome this information deficiency, a research project on the diversity and biogeography of the Paederinae of the central Anatolia was carried out. This paper continues the series of descriptions of new *Leptobium* species collected in central Anatolia within this project. A new species are described here: *Leptobium angoranum* sp. n. A total of 18 *Leptobium* species is now known from Turkey with 13 of them found only in Turkey.

MATERIAL AND METHODS

The morphological studies were conducted using a Stemi 508 microscope (ZeissGermany). Photographs of the habitus, forebody and aedeagus of the new species were taken with a digital camera (Zeiss Axiocam ERC5s). All photographs were edited with the Helicon Focus v. 6, and Corel Draw v. X5 software. The map was made using the software Google Earth Pro (2019).

Nomenclature of the terminalia and the style of the description follow Assing (2005). Head length was measured from the anterior margin of the frons to the posterior margin of the head, length of the pronotum was measured along the median line, elytral length was measured at the suture from the apex of the scutellum to the posterior margin of the elytra. The length of the median lobe of the aedeagus was measured from the apex of the ventral process to the base of the capsule.

The material referred to in this study is stored in Alaşehir Zoological Museum, Manisa, Turkey (AZMM).

RESULTS

Faunistic records

Leptobium ilgazicum Assing, 2010 (Fig. 1)

Material examined: Çankırı: 5♂♂, 4 ♀♀ , 19.05.2018, Atkaracalar, Ilıpınar 5 km SE, 1750 m, 40°46’10”N, 33°09’32”E, leg. Örgel & Yaman (AZMM, NHMO). 3♂♂, 3♀♀, 19.05.2018, Kurşunlu 15 km N, Bayramören 3 km E, 1648 m, 40°55’42”N, 33°15’50”E, leg. Örgel & Yaman (AZMM). 2♂♂, 1♀, 20.05.2018, Kurşunlu 5 km E, Taşkaracalar, 1647 m, 40°42’11”N, 33°19’43”E, leg. Örgel & Yaman (AZMM). 5♂♂, 4♀♀, 21.V.2018, Ilgaz, Kuyupınar 2 km E, 1414 m, 40°51’06”N, 33°37’37”E, leg. Örgel & Yaman (AZMM). 6♂♂, 4♀♀, 21.05.2018, Ilgaz, Ilgaz Dağları, 1926 m, 41°02’49”N, 33°42’46”E, leg. Örgel & Yaman (AZMM).

Distribution: This species is only known from the surroundings of Ilgaz Mountains in Çankırı province of central northern Anatolia (Assing, 2010; Anlaş, 2017).

Leptobium yagmuri Anlaş, 2017 (Fig. 1)

Material examined: Ankara: 4♂♂, 3♀♀, 10.03.2018, Mamak, Kutludüğün Plateau, 39°52’11”N, 33°06’04”E, 1436 m, leg. Örgel & Yaman (AZMM).

Distribution: The recently described species was only known from Ankara province (Anlaş, 2017).

Leptobium ponticum Assing, 2005 (Fig. 1)

Material examined: Sinop: 1♂, Hamsiler village, 42°03’44”N, 35°01’55”E, leg. Koç (AZMM).

Distribution: This species endemic to Sinop province of central northern Anatolia (Assing, 2005).

Description of new species

Leptobium angoranum Anlaş, Örgel, 2020 sp. n. (Figs. 1-7)

Type material. Holotype: TURKEY: ♂, “TR. Ankara, Beypazarı, Üreğil, 40°17’07”N, 32°04’11”E, 1375 m, 11.04.2017, leg. Örgel & Yaman. / Holotypus ♂, *Leptobium angoranum* sp. n. det. S. Anlaş & S. Örgel 2019” (AZMM). Paratypes: 11♂♂, 4♀♀, same data as holotype (AZMM); 6♂♂, 3♀♀, same data as holotype but 23.09.2017, leg. Örgel & Yaman. (AZMM).

Description: Habitus as in Fig. 1. Species of small size, 4.7-5.1 mm long. Coloration: head and abdominal segments III-VI black to blackish brown, pronotum reddish to reddish brown, elytra reddish, with the anterior margin infuscate, abdominal segments VII-X reddish, antennae and legs reddish yellow.

Head oblong approximately 1.15-1.20 times as long as wide (Figs. 2-3); eyes average size (Fig. 3), projecting from lateral outline of head, slightly more than half the length of postocular region in dorsal view; puncturation coarse and sparse, irregularly spaced, larger in median dorsal area; interstices on dorsal surface on average about 2.5 times as wide as punctures, microsculpture absent; pubescence black and sparse. Antennae approximately 1.3-1.4 mm long; antennomere III slightly longer than II; antennomeres IV-VI longer than the width, antennomeres VII-X about as wide as long; antennomere XI almost twice as long as wide (Fig. 2).

Pronotum distinctly oblong, approximately 1.3 times as long as wide and as wide as head (Figs. 2-3), lateral margins subparallel in dorsal view; puncturation similar to that of head, but sparser; microsculpture absent; pubescence yellowish and sparse.

Elytra slightly wider than pronotum, approximately 1.05-1.10 times as wide as pronotum (Figs. 2-3) and shorter than pronotum, at suture about 0.70 times as long as pronotum; punctation weakly granulose, finer and denser than that of pronotum and head; microsculpture absent; pubescence yellowish or reddish yellow, more distinct than that of head and pronotum. Hind wings reduced. Tarsi relatively long (Fig. 2).

Abdomen wider than elytra (Fig. 2); approximately 1.10 times as wide as elytra; puncturation fine and moderately dense; microsculpture visible, composed of dense and fine transverse meshes and striae; pubescence brown and moderately dense; posterior margin of tergite VII without palisade fringe.

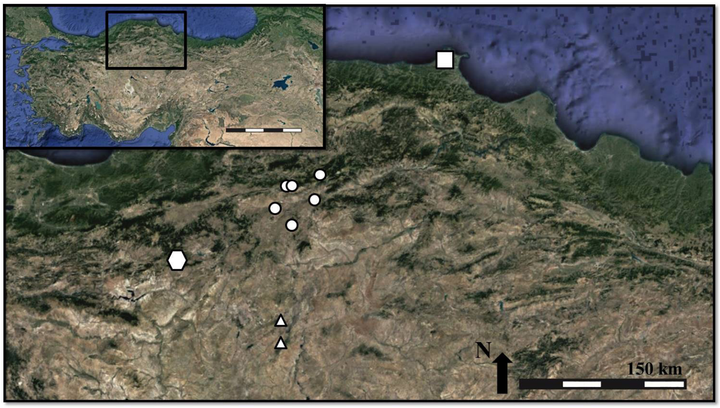


Fig 1. Distribution of endemic *Leptobium* species in central northern Anatolia: *L. ilgazicum* Assing (circles); *L. ponticum* Assing (square); *L. yagmuri* Anlaş (triangles), *L. angoranum* sp. n. (hexagon).

♂: sternite VII with concave posterior margin, but without modified pubescence and median impression (Fig. 4); sternite 8 with posterior incision not reaching middle of the sternite, little more than 1/3 the length of the sternite (Fig. 5); aedeagus small and slender, with ventral process of highly distinctive shape, 1.0-1.1 mm long (Figs. 5-6).

Comparative notes. The species is distinguished from all its congeners by the different morphology of the aedeagus. From other species occurring in central and northern Anatolia, *L. ilgazicum* Assing (Çankırı, Ankara), *L. ponticum* Assing (Sinop), *L. yagmuri* Anlaş (Ankara) and in southern Anatolia, *L. assingi* Bordoni (Antalya, Gaziantep, Hatay, Kahramanmaraş, Osmaniye) it is additionally separated as follows:

From *L. ilgazicum* and *L. ponticum* by the different coloration of forebody (*L. ilgazicum* and *L. ponticum*: head and pronotum blackish; elytra uniformly reddish), the more oblong head and pronotum; by the male sternite VII without modified setae (*L. ilgazicum* and *L. ponticum*: male sternite VII with extensive cluster of longer and stouter black setae) and by the somewhat different shape of the dorsal plate, and by the differently shaped ventral process of the aedeagus.

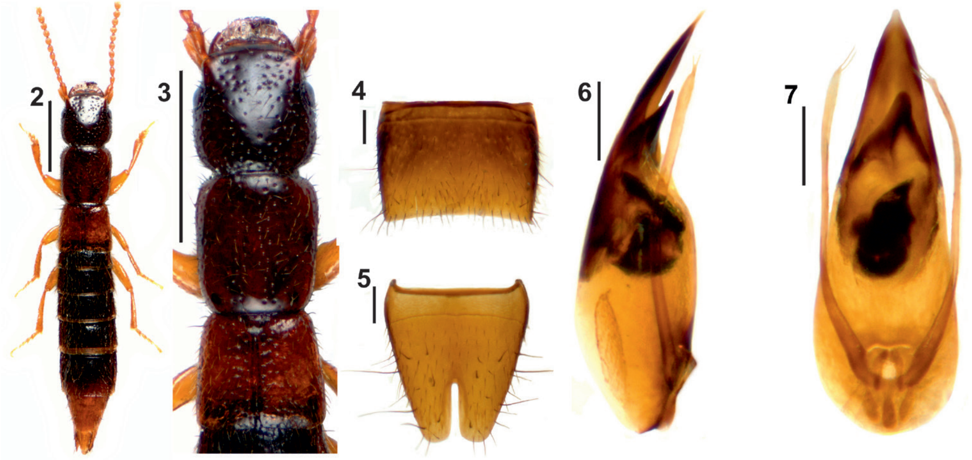
From *L. yagmuri* by the different coloration of forebody (*L. yagmuri*: head and pronotum blackish; elytra reddish), by the male sternite VII without modified setae (*L. yagmuri*: sternite VII with weakly modified pubescence) and by the different morphology of the aedeagus, especially the differently shaped ventral process.

From *L. assingi* by the different coloration of forebody forebody (*L. assingi*: pronotum blackish; elytra uniformly reddish), by smaller eyes, by the male sternite VII without modified setae (*L. assingi*: sternite VII with weakly modified pubescence, in posterior median area with two more or less distinct clusters of rather sparse, slightly darker and stouter setae), and by the differently shaped ventral process of the aedeagus.

For more illustrations of *L. ponticum, L. ilgazicum*, *L. yagmuri* and *L. assingi* see Assing (2005, 2010), Anlaş (2017).

Etymology. The name is derived from ancient name of Ankara (=Angora) where the type locality is situated.

Distribution and bionomics. The species was collected only one locality in Ankara province of central northern Anatolia. The type specimens were collected under stones in grassland and sifted from leaf litter in a small lakeside at altitudes of 1375 m.



Figs. 2-7. *Leptobium angoranum* sp. n. 2-habitus; 3-forebody; 4-Male sternite 7; 5-Male sternite 8; 6-Aedeagus in lateral view; 7-Aedeagus in ventral view. Scale bars: 1.0 mm (Figs. 2-3); 0.2 mm (Fig. 4-7).

ACKNOWLEDGMENTS

We are most grateful to our colleagues for making their staphylinid collections available to me. This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK, project number 215Z80).

REFERENCES

Anlaş, S. (2017). A new species and new country records for the genus *Leptobium* Casey in the Palaearctic Region (Coleoptera: Staphylinidae: Paederinae). *Zootaxa*, 4338, 173-181

Assing, V. (2005). A revision of the genus *Leptobium* Casey (Coleoptera: Staphylinidae: Paederinae). *Stuttgarter Beiträge zur Naturkunde Serie A* (*Biologie*), 673, 1-182.

Assing, V. (2010). A revision of *Leptobium* Casey VII. Two new species from Turkey and Spain, and additional records (Coleoptera: Staphylinidae: Paederinae). *Linzer Biologische Beiträge,* 42*,* 1037-1043.

Assing, V. (2017). A revision of *Leptobium* Casey VIII. A new species from Iran, a new synonymy, and additional records (Coleoptera: Staphylinidae: Paederinae). *Linzer biologische Beiträge,* 49*,* 275-284.

Schülke, M. & Smetana, A. (2015). Staphylinidae, In I. Löbl & D. Löbl (Eds.). *Catalogue of Palaearctic Coleoptera.* (2: pp. 304-1134.) *Hydrophiloidea-Staphylinoidea*. Revised and updated edition. *Leiden: Brill:* xxvi + 1702.

Received: July 13, 2019 *Accepted: February 11, 2020*

AUTHOR GUIDELINES

Journal of the Entomological Research Society (J. Entomol. Res. Soc.) accepts and publishes original research articles in the all fields of entomology. The journal publishes regular research papers and review articles. Brief and timely reports may be submitted as short communications, where articles with less detailed results and evaluations sections can be accepted as short communication. The Editors first evaluate all manuscripts. At this stage, manuscripts that fail to be original, have serious scientific flaws, have poor grammar or English language, or are outside the aims and scope of the Journal will be rejected. Those that meet the minimum criteria are passed onto at least 2 experts for review. Authors should suggest four reviewers with their names, addresses and e-mail addresses who would review their manuscript. Information on the reviewers should also be uploaded as an appendix to the manuscript. Of these four reviewers, at most two should be in the author's native country and the others will be in other countries. Two reviewers are selected from these four suggested reviewers or editors may assign other reviewers. A final decision to accept or reject the manuscript will be sent to the author along with any recommendations made by the reviewers. Reviewers make recommendations to the Editor whether to accept or reject the manuscript for publishing. The Editor reaches a final decision based on the reviewers' recommendations, as well as his/her own evaluation of the manuscript.

The manuscripts should be written in Arial with 12 type size with double spacing in Microsoft Office Word. The paragraphs should not be indented. The Manuscripts in general should not exceed 30 pages.

Heading: The title of the manuscript should be informative, but preferably not exceed twenty words. Just under the heading, please provide the title, full name(s) of author(s) (The name(s) of all authors should be start with capital letter, and surname(s) should be typed in upper case), with full address and e-mails of each author on a separate line. If a genus or species name is included in the manuscript heading, these should be written in full with no abbreviations, including the author name and date; e.g. Aphodius lividus (Olivier, 1789)

Abstract: An abstract provided at the beginning of the manuscript should indicate the main aspects of the subject, not exceed 200 words, and should be followed by 5-7 key words.

Text: The standard order of sections for original manuscripts is as follows: Introduction, Material and Methods, Results, Conclusions and Discussion, Acknowledgements, References. Sub-titles should be up to the third level and Italic format should be avoided except for species names. . The scientific names (e.g. genus- and species-group names) are the only words to be italicized. References should be cited in the text by the last name(s) of the author(s) and year of publication. Atribution in main text must be given like that (Surname, 1900a; 1900b; 1991; Surname, et al, 2000, Surname1 & Surname2, 2001). Two Authors: The surname of both authors is stated with either 'and' or an ampersand (&) between. For example: Surname1 & Surname2 (2017) state… Or …(Surname1 & Surname2, 2017). Three, Four or Five Authors: For the first cite, all names should be listed: Surname1, Surname2, & Surname3 (2017) state… Or …(Surname1, Surname2, & Surname3, 2017). Further cites can be shorted to the first author's name followed by et al: Surname1 et al (2017) state… Or …(Surname1 et al, 2017). Six or more authors: Only the first author's surname should be stated followed by et al: (Surname1 et al, 2017). Works should be cited with a, b, c etc following the date. For example: (Surname1, 2017a) or (Surname1, 2017b). If these works are by the same author, the surname is stated once followed by the dates in order chronologically. For instance: Surname (2007, 2013, 2017) Or (Surname, 2007, 2013, 2017). If a page number will be given for a quote, the page number must be given after the date, for example (Surname, 2017, p.104). This rule holds for all of the variations listed. Groups of references should be listed chronologically.
For faunistic research follow this order, Distribution:.., Material examined:…, Host plant:….etc.

Example:
Sphex oxianus Gussakovskij, 1928
Distribution: Central and South West Asia, Afghanistan, Iran, Israel, Turkey (Bohart and Menke, 1976; Menke and Pulawski, 2000; Kazenas, 2001), Turkey: Artvin (De Beaumont, 1967).
Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29.06.1998, 1 ♂; Kalecik, 600 m, 24. 07. 2001, 2 ♀♀, Kalecik, 800 m, 25. 07. 2001, 3 ♀♀
Host plant: Echinophora sp.
Please use ♀, ♂ symbols. Please write upper genus categories with capital letters.

Illustrations: Illustrations, graphs, their caption or legends should form a separate, and a self-explanatory unit. Abbreviations in the legends should be explained but if there are too many, they should be included into a separate list. The original drawing and photographs should not be more than twice as large as when printed.

Morphological illustrations should include a scale bar. Photographs and electron micrographs should be in high-resolution JPEG file format (300 dpi). Drawings (black and white type) should be in TIFF format and their size should be no more than 10 MB. Graphs should also be in black and white and submitted in excel file format. Tables should include headings and explanations, and should be numbered consecutively. Their approximate position in the text should be indicated in the margin. Legends and titles of the graphs and tables should be in Arial with 12 type size. Please do not embed the figures, graphs and table into the text, and send them as supplementary files. In the text attribution to the figures should be given in parenthesis and must be abbreviate like this; (Fig.1).Figs. 1-10. A. marriotti sp. n.. 1. Male (holotype), dorsal. 2. Female (paratype)

References:

Titles of manuscripts published in languages other than the major ones (English, German, French, Spanish, Portuguese, Turkish) should be an English translation (in parentheses) with an explanatory note at end, e.g. (in Russian). The list of references should be given at the end of the article and listed alphabetically, according to the following examples. All periodical names should be unabbreviated and italicized. In references, journal titles must be written in full (not abbreviated).

Journal Article

Beirne, B.P. & Young, D.A. (1953). The North American species of Cicadula (Homoptera, Cicadellidae). *Canadian Entomologist*, 85(1), 215-226.

Mitchell, J.A. (2017). Citation: Why is it so important. *Mendeley Journal*, 67(2), 81-95. Retrieved from https://www.mendeley.com/reference-management/reference-manager

Book

Steinmann, H.A. & Zombori, L. (1985). *An atlas of insect morphology*. (2nd ed.). Akadèmiai Kiadò, Budapest, Hungary.

Edited Book

Williams, S.T. (Ed.). (2015). *Referencing: A guide to citation rules* (3rd ed.). New York, NY: My Publisher

Edited Book Chapter

Troy, B.N. (2015). APA citation rules. In S.T, Williams (Ed.). *A guide to citation rules* (2nd ed., pp. 50-95). New York, NY: Publishers.

E-Book

Mitchell, J.A., Thomson, M., & Coyne, R.P. (2017). *A guide to citation*. Retrieved from https://www.mendeley.com/reference-management/reference-manager

Author, A. (date). *Title of book*. doi:xxxxxxxxxxxx

E-Book Chapter

Troy, B.N. (2015). APA citation rules. In S.T, Williams (Ed.). *A guide to citation rules* (2nd ed., pp. 50-95). Retrieved from https://www.mendeley.com/reference-management/reference-manager

Author, A. (date). Title of chapter. In E. Editor (Ed.). *Title of book* (pp. xx-xx). doi:xxxxxxxxxx

URLs

Mitchell, J.A. (2017, May 21). *How and when to reference*. Retrieved from https://www.howandwhentoreference.com.

Nomenclature should be in absolute agreement with the current ICZN rules. The only acceptable type concepts are: holotype, paratype, etc. The following abbreviations should be adopted: gen. n., sp. n., stat. n. and comb. n. Journal of the Entomological Research Society uses the Open Journal Systems (OJS) platform, which will enable the journal to accept submissions online. For submitting a manuscript please go to web page http://www.entomol.org and register as author and submit your manuscript online.

Copyright form: You can download JERS copyrigth form in our web site, then sign it with all authors and send us.

URL: http://www.entomol.org

e-mails: jers@entomol.org

Address: Journal of the Entomological Research Society, P.box.110 Bahcelievler P.Isl.Mud. 06502, Ankara/TURKEY

CONTENTS