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Heavy metals in bones from Harbour Porpoises *Phocoena phocoena* from the Western Black Sea Coast

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Abstract. During the last few years, the Western Black Sea coast has documented increase in the number of stranded marine mammals, particularly the harbour porpoise (*Phocoena phocoena*). This species is a subject to threats such as exposure to contaminants, fishery by-catch and introduced new marine species. The aim of this study was to analyse spatial and age trends in bone metal concentration in harbour porpoises from the Western Black Sea Coast. Selected heavy metals (Cu, Pb, Zn, Cd and Ni) were measured in bones of 33 harbour porpoises stranded along the Bulgarian Black Sea Coast from 2017. Spatially, we found higher metal levels in the harbour porpoises stranded in the Northern region compared with those from Southern region. The effect of aging was evident only for Zn content – the levels were higher in juveniles than in adults. The obtained results suggested that heavy metal contamination represent an important threat encountered by harbour porpoises.

Key words: Heavy metals, Harbour Porpoises, Western Black Sea.

Introduction

The harbour porpoise (*Phocoena phocoena relicta* Linnaeus, 1758) is one of the three species of cetaceans found in the Black Sea. It is the second to abundance marine mammal inhabiting the Black Sea and adjacent waters (Evtimova *et al.* 2015, Evtimova *et al.* 2016, Evtimova *et al.* 2018). Other studies on the distribution of cetaceans in the Black Sea revealed that harbour porpoise is widely distributed in coastal areas (Panayotova *at al.* 2017), which are subject to contamination from various pollutants.

Studies on cetacean strandings in this area showed increase in stranding events during the last few years (Evtimova *et al.* 2015, Evtimova *et al.* 2016, Evtimova *et al.* 2018). Due to their role as top predators within the marine food web, marine mammals such as porpoises have been used as indicators for ecosystem changes. Since chemical contaminants may affect the health of harbour porpoises, contamination by particular heavy metals may be associated with the increased stranding of harbour porpoises in the Western Black Sea along the Bulgarian coast. Therefore, the aim of this study was to assess

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for the first time the bone heavy metals concentration in harbour porpoises along the Bulgarian Black Sea Coast.

Material and Methods

Bones were collected from 33 harbour porpoises (15 males and 18 females) stranded in 2017 along the Bulgarian Black Sea coast (Fig. 1). The samples were processed in a laboratory by drying, grinding and mixing with concentrated acids. Metal concentrations (Cu, Pb, Zn, Cd and Ni) were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES) Optima 7000 DV (PerkinElmer, USA). Statistical analysis was performed using SPSS v.21. The harbour porpoises were aged using dentinal Growth Layer Group (GLG) method (Boutiba 2012).

Fig. 1. Map of the Western Black Sea showing the sampling sites along the Bulgarian Black Sea Coast.

Results and Discussion

Mean bone concentrations of metals determined in harbour porpoises from the Southern and Northern Bulgarian Black Sea coast are presented in Table 1. The results show that, Zn had the highest mean levels in bones of porpoises followed by Pb, Cd, Cu and Ni.

For the geographical comparison, Cu, Pb, Zn, Cd and Ni concentrations displayed significantly higher levels in the harbour porpoises stranded in the Northern Black Sea coast compared with those from Southern Black Sea coast ($p<0.05$). In addition, harbour porpoises found in Aheloy (Southern region) and Shabla (Northern region) had the highest levels of Cu (3.75 mg/kg and 3.5 mg/kg, respectively) while animals found in Krapets (Northern region) had significantly higher bone concentrations of Ni (1.87 mg/kg) in comparison with all other sites.

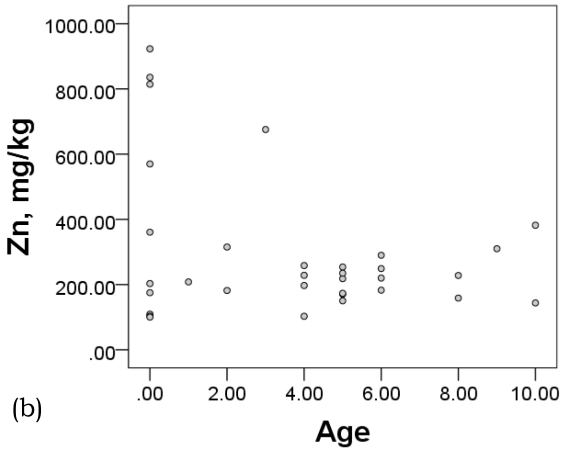
Table 1. Trace metal (mg/kg) concentrations in bones of harbour porpoises stranded between 2006 and 2013 along the Southern and Northern Bulgarian Black Sea Coast. Mean ± SD; range of concentrations (minimum – maximum); n - number of samples.

	Metal concentrations (mg/kg)				
	Cu	**Pb**	**Zn**	**Cd**	**Ni**
Southern region	2.61 ± 0.88	11.45 ± 1.30	275.02 ± 177.86	2.17 ± 0.40	0.76 ± 0.34
n=14	0.97-3.82	9.18-13.82	100.24-814.20	1.57-3.07	0.41-1.45
Northern region	2.09 ± 1.07	15.53 ± 3.55	309.25 ± 247.38	2.66 ± 0.71	1.13 ± 0.60
n=19	(0.21-3.91)	(8.84-25.23)	102.80-922.90	1.44-3.96	0.50-2.56
Total	2.31 ± 1.01	13.80 ± 3.46	294.73 ± 218.11	2.45 ± 0.64	0.98 ± 0.53
n=33	(0.21 - 3.91)	(8.84-25.23)	(100.24-922.90)	(1.44-3.96)	(0.41-2.56)

For harbour porpoises stranded in the Bulgarian Black Sea coast in 2017, correlations in bones between metals are presented in Figure 2. There was a significant correlation between Cd and Pb concentrations (Spearman correlation value R=0.576, $p<0.05$) (Fig. 2a). A significant negative correlation was detected between Zn concentration and the age of the harbour porpoise (R=-0.318, $p<0.05$) (Fig. 2b).

	Cu	**Pb**	**Zn**	**Cd**	**Ni**
Cu	1				
Pb	.113	1			
Zn	-.061	-.324	1		
Cd	.078	**.576**	-.035	1	
Ni	-.268	.231	-.122	-.120	1
Age	.183	.023	**-.318**	-.027	.078

(a)



(b)

Fig. 2. (a) Correlation matrix between heavy metals and age in bone tissue of harbour porpoises (*Phocoena phocoena*) beached at Black Sea, Bulgaria (significant correlations highlighted in bold). (b) Zink (Zn) concentration in the bones of common harbour porpoises (mg/kg) as a function of age (years).

Heavy metals, such as Cu and Zn are essential elements, and thus they are homeostatically regulated and their concentration can significantly change for a particular tissue in different specimens (Marcovecchio *et al.* 1990). Cu concentration in bone harbour porpoises tissues from Shabla and Aheloy were the highest registered. This result should be related with possible contamination in these regions. Our study also revealed a significant negative correlation of Zn bone concentration in harbour porpoises with age. Similarly, Agusa *et al.* (2008) found a negative correlation in striped dolphins. In addition, Cd concentration was related with Pb levels. We did not found an expected significant correlation between Cu and Zn. Neither the Cd-Cu nor the Cd-Zn relationships of this study were significant. A possible explanation for this result could be that Cd concentrations found in harbour porpoises are probably not enough to induce Cu or Zn ion displacement, leading to co-accumulation with Cd. Similar results were found by Lahaye *et al.* (2007).

Present evidence suggests a possible enrichment of the studied heavy metals in the Northern Black Sea Coast, where farming activities are present. These novel results fulfill the information gap existing about heavy metals pollutants presence in the Western Black Sea waters. Nevertheless, further studies are necessary in order to clarify the contamination and bioaccumulation process of heavy metals in the marine mammals from this region.

Several authors reported different feeding habits and diet related to age in harbour porpoises (Das *et al.* 2004). Some prey species are more important in the diet of young porpoises, such as gobies and shrimps, compared to adult ones (Santos *et al.* 2004). Santos and Pierce suggested that juveniles cannot dive as deep as adults and could be limited by their small size from catching big prey. Our data suggest that the different Zn levels between juveniles and adults may also be related to the maturity status.

In the present study a passive monitoring of stranded animals was presented, which can provide insight into environmental impacts on marine mammals. Our findings indicate that we cannot reject the hypothesis that metallic contaminants may influence the health of harbour porpoises and contribute to the increased stranding events.

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Histochemical alterations in liver of Common Carp *Cyprinus carpio* (Linnaeus, 1785) after glyphosate exposure: Preliminary study

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Abstract. The present study was designed to provide some preliminary data on the toxic effects of 96 h exposure to glyphosate on the liver of Common Carp (*Cyprinus carpio* L.) under *ex situ* conditions. For this purpose we used Sudan III staining which could be suggested as fast and low-cost histochemical biomarker for pesticide contamination effects.

Key words: fish, pesticides, liver, histochemistry.

Introduction

Aquatic environments are inevitably the ultimate receptors for many different contaminants, including pesticides. Throughout the world, 40% of the applied pesticides are herbicides, 33% are insecticides, while 10% are fungicides and 17% are classified as others (Glinski *et al.* 2018). Glyphosate (N-phosphonomethyl glycine) is a post-emergent and broad-spectrum herbicide that belongs to the glycine group, and it is the most widely used non-selective herbicide to control plant growth worldwide because it has a powerful herbicidal action and interferes with 5-enolpyruvyl-shikimate-3-phosphate synthase, an important enzyme for the synthesis of essential aromatic amino acids in plants (Sergiev *et al.* 2006). It is currently the best-selling herbicide in the world, used in agricultural and non-agricultural areas and the use of glyphosate for crop production is world-wide spread, both in industrialized and developing countries (Benbrook 2016). According to WHO (2005) the acute toxicity of glyphosate to animals is considered low. However, given its dramatic increase in usage over the last 20 years, there have been growing concerns regarding chronic low-dose exposure to glyphosate (Myers *et al.* 2016). Moreover, the major problem with the continuous and uncontrolled use of this herbicide is its effect on non-target organisms. Thus, at present glyphosate is receiving increased attention as potential toxicant to aquatic organisms and ecosystems (Van Bruggen *et al.* 2018). In fish, it has been shown that glyphosate-based herbicides cause biochemical alterations and morphological lesions in tissues such as gills and liver (Stoyanova *et al.* 2015). Use of sentinel organisms for environmental quality monitoring by biological tools provide a sensitive and reliable approach to estimate the possible negative effects of pollutants. Fish, among them, are

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recognized as an excellent experimental model for toxicological studies because of their importance as protein source. Furthermore, fish are concerned as a proper indicator for the assessment of contamination in aquatic ecosystems, as they receive the toxicants both directly through water and indirectly through food, thereby resulting in bioaccumulation in their tissues and biomagnification in the food web.

Based on the literature above we find that it is essential to obtain information on the toxic effects of glyphosate on non-target organisms such as fish. Therefore, the objective of the present work is to study the toxic effects of glyphosate (commercial product NASA 360) on the liver of Common Carp, *Cyprinus carpio* (Linnaeus, 1785) which is an important species for aquaculture and aquatic toxicology by applying histochemical methods. We also aimed to see if Sudan III staining could be proposed as a sensitive, rapid and low-cost biomarker for the negative effects of pesticides.

Material and Methods

The fish were purchased from the Institute of Aquaculture and Fisheries, Plovdiv, Bulgaria where they are reared in strictly controlled conditions. The Common Carps were of the same size-age group without external pathological changes. Their average weight and length were as follows: 47.8 g ± 15.2 and 16.3 cm ± 2.7. After transportation, they were placed into 100 L glass tanks with dechlorinated water to acclimate for a week. During the acclimation period a constant temperature was maintained within 23°C ± 1.5. A twelve-hour light period was provided for the fish. Thereafter, the Common Carps were randomly divided (n=15) in each glass tank and treated with glyphosate once for 96 h (short-term exposure). The concentrations of glyphosate (NASA 360) were determined by dilution of a stock solution prepared according to the manufacturer's guidelines for recommended crop-specific quantities as previously described (see Stoyanova *et al.* (2015)). The basic physical parameters of water (pH, oxygen level, temperature and conductivity) were monitored three times a day with a combined field meter (WTW, Germany). The fish were dissected and the requirements of Directive 2010/63/EU on the protection of animals used for scientific purposes were met. The liver was immediately frozen at -25°C for further histochemical analysis. The histochemical study was conducted on a freezing microtome (Leica, Jung Frigocut 2800 N) and cryosections of 6 μm were prepared from the liver. They were then stained for lipid determination by Sudan III staining (Sigma, USA) as described by Daddi (1896). By this colouring method, the lipids in the hepatocytes are stained in orange and the nuclei of the cells in pale blue. In addition, the histochemical changes in the liver were evaluated according to a standardized assessment tool by using a modified version of the protocol described by Bernet *et al.* (1999). The Sudan III staining evaluation was scored as follows: (0) - Negative histochemical staining reaction; (1) - Very weak positive histochemical reaction with discreet yellow coloration; (2) - Slightly positive histochemical reaction with yellow-orange staining; (3) - Moderately positive reaction of histochemical staining with intense yellow-orange staining; (4) - Highly positive histochemical reaction in hepatocytes with intense orange coloration.

The statistical analysis was performed using Graph Pad Prism 7 for Windows. The raw data on basic physical properties and histochemical scores were distributed normally and analyzed using Graph Pad Prism 7 for Windows (USA). The differences between the variables were tested using Student's t-test at significance level of 95% ($p<0.05$). The results were reported as mean±SD.

Results and Discussion

The values of water properties were constant during the experiment, without significant differences ($p<0.05$) and they are not presented in the manuscript. Thus, we consider that the histochemical alterations that we observed in the liver of Common Carp are not due to changes in the abiotic factors.

The results of the histochemical alterations are presented on Fig. 1 and the average scores in Table 1, respectively. Overall, from the obtained results on histochemical alterations in the liver of Common Carp after glyphosate exposure changes we observed a tendency towards an increase of the lipid content in the hepatocytes along with the increase in the concentration of glyphosate. The scores were evaluated as (1) - Very weak positive histochemical reaction with discreet yellow coloration; (2) - Slightly positive histochemical reaction with yellow-orange staining; (3) - Moderately positive reaction of histochemical staining with intense yellow-orange staining for 20, 40 and 72 mg/L concentrations, respectively.

Table 1. Average results for Sudan III staining intensity in liver of Common Carp after 96 h exposure to glyphosate (n=10 for each concentrations). † - statistically significant than the others (p<0.05).

Common Carp liver	**Nasa 360/glyphosate concentration, mg/L**			
	control	20	40	72
Sudan III staining intensity score	0	1	2	3†

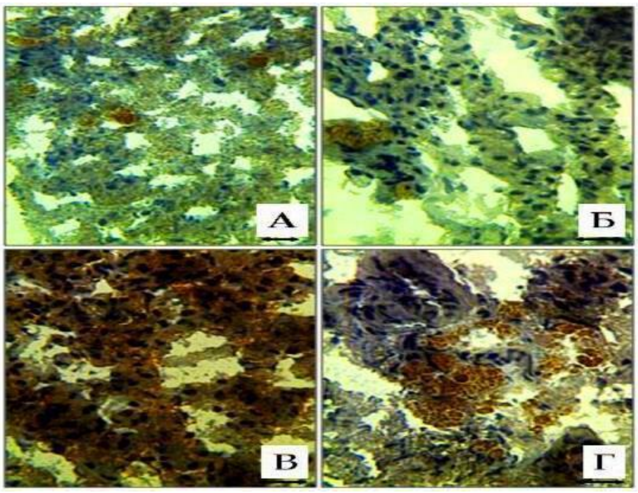

Fig. 1. Sudan III staining intensity in liver of Common Carp after 96 h exposure to glyphosate: A – control, x200; Б – 20 mg/L glyphosate, x400; В – 40 mg/L glyphosate, x400; Г – 72 mg/L glyphosate, x400.

The negative effects of pesticides, along with changes in glycogen levels in the liver, may cause other degenerative changes such as fat degeneration, expressed as accumulation of lipid droplets in hepatocytes which may affect lipid metabolism negatively.

The large amount of lipid droplets accumulated in the cytoplasm of hepatocytes is due to fatty tissue degeneration in the liver cells which is confirmed in our previous study (see Stoyanova *et al.* 2015). This is probably due to increased amounts of pyruvate in the liver, and hence through the pyruvate dehydrogenase complex of increased amounts of Acetyl-CoA which is used for the synthesis of fatty acids and cholesterol. The increased fatty acid synthesis leads to increased triglyceride synthesis and hyperlipidemia associated with fat infiltration in hepatocytes. Along our findings, other authors similarly detected changes in the lipid content of hepatocytes after pesticide exposure. Gultekin *et al.* (2000) observed fat degeneration in the liver due to lipid metabolism disorders after insecticidal exposure.

Similarly to us, Ayoola (2008) found fatty degeneration in the African catfish, *Clarias gariepinus* (Burchell, 1822) hepatocytes under the action of glyphosate for 96 h. We agree with the authors that variations associated with changes in the amount of lipids (or glycogen) in the liver of exposed animals generally may be due to changes in the glycolysis processes which in turn depend on the toxicant concentrations or its toxic character, as well as exposure period.

In sum, we can conclude that glyphosate has a negative effect on the liver of Common Carp and Sudan III staining could be recommended as a sensitive, rapid and low-cost biomarker for the effects of pesticide contamination on freshwater fish.

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Research on nesting birds on the territory of Kaliakra Wind Farm

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Abstract. The study presents data about the status of the nesting ornithofauna in the region of Kaliakra wind farm (Kaliakra Cape, northeast Bulgaria). The data shows the number and the density of the birds observed in this region in 2005, before the construction of the wind farm, and in 2009 after it's commissioning. Transect method was applied to establish the species composition, numbers and density of the nesting birds. A total amount of 24 nesting species was established. In 2005 the recorded nesting bird species were 17, and in 2009 - 20. Although the number of nesting species in 2009 is slightly higher as compared to 2005, they were established in lower numbers and density. In May the density was lower compared to this in April. The data collected in May show about the same density for the two compared years.

Key words: nesting birds, wind farm, Kaliakra.

Introduction

Through the last years the alternative, renewable sources of energy are being the main aim in the economy of many countries. On the Black Sea coast the winds are relatively strong, with constant direction and are present through the whole year. The area of the study is located in one of the easternmost territories of Bulgaria. It encompasses the steppe territories from Balgarevo village to Kaliakra Cape. In this area is located the "Kaliakra" wind farm (Fig. 1).

The study presents data about the status of the nesting ornithofauna in the region of Kaliakra wind farm. The data shows the number and the density of the birds observed in this region in 2005, before the construction of the wind farm, and in 2009 after it's commissioning.

The publication summarizes the results, collected during the two month periods, from the years 2005-2009 in order to represents data about the birds, nesting in the area. The aim of the study is to establish the species composition and to characterize the nesting communities over the territory of "Kaliakra" wind farm

Another aim of the study was to clarify the effect of the wind farms in the area of Black Sea coast over the nesting birds.

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Fig. 1. Location of “Kaliakra” wind farm.

Material and Methods

The method used during the research is based on methods in the “Atlas of European Breeding Birds” (Ward et al. 1997) and “Bird Census Techniques” (Bibby *et al.* 1992).

Transect method was applied to establish the species composition, numbers and density of the nesting birds. The transects were 2150 m in length and their total area was 43 ha. The observations were made in April and May during the breeding season in 2005 and then in 2009.

The habitat is steppe phytocenoses along the Northern Black Sea Coast. An open low-grass steppe area with bushes or single bushes, with significant anthropogenic influence.

Results and Discussion

A total amount of 24 nesting species was established. In 2005 the recorded nesting bird species were 17, and in 2009 – 20. During April 2005 thirteen species were established and identified, during May 2005 the number of species recorded is fourteen.
During April 2009 the species were 15 and during May the same year the species recorded were 14. During May in both 2005 and 2009 the Red-backed Shrike (*Lanius collurio* Linnaeus, 1758) was not recorded in the previous month.

Although the number of nesting species in 2009 is slightly higher, they were established in lower numbers. The average density through 2005 was 26,98 p/10ha and during 2009 it drops to 19,54 p/10ha. Highest density rate was recorded during April 2005 – 40,93 p/10ha (Table 1 and Table 2).

Table 1. Density and dominant structure of birds during April. Legend: p - density of pairs per 10 ha; d - dominance.

№	Species	April 2005		April 2009	
		p/10 ha.	d	p/10 ha.	d
1	*Alauda arvensis* Linnaeus, 1758	11,63	28,4	1,86	7,72
2	*Melanocorypha calandra* (Linnaeus, 1766)	9,53	23,3	7,44	31,68
3	*Sturnus vulgaris* Linnaeus, 1758	6,74	16,48	4,88	20,79
4	*Calandrella brachydactyla* (Leisler, 1814)	5,35	13,07	0,23	0,99
5	*Oenanthe oenanthe* (Linnaeus, 1758)	2,09	5,11	1,16	4,95
6	*Passer domesticus* (Linnaeus, 1758)	1,86	4,54	0	0
7	*Burhinus oedicnemus* (Linnaeus, 1758)	1,4	3,41	0	0
8	*Cuculus canorus* Linnaeus, 1758	0,7	1,7	0	0
9	*Corvus monedula* Linnaeus, 1758	0,46	1,14	0	0
10	*Saxicola rubetra* (Linnaeus, 1758)	0,46	1,14	1,16	4,95
11	*Miliaria calandra* (Linnaeus, 1758)	0,23	0,57	2,56	10,89
12	*Upupa epops* Linnaeus, 1758	0,23	0,57	0,93	3,96
13	*Anthus campestris* (Linnaeus, 1758)	0,23	0,57	1,63	6,93
14	*Streptopelia turtur* (Linnaeus, 1758)	0	0	0,46	1,98
15	*Oenanthe isabellina* (Temminck, 1829)	0	0	0,23	0,99
16	*Pica pica* (Linnaeus, 1758)	0	0	0,23	0,99
17	*Luscinia megarhynchos* Brehm, 1831	0	0	0,23	0,99
18	*Carduelis carduelis* (Linnaeus, 1758)	0	0	0,23	0,99
19	*Passer montanus* (Linnaeus, 1758)	0	0	0,23	0,99
	Total:	**40,93**	**100%**	**23,49**	**100%**

Conservation status. From the established bird species in the area of the "Kaliakra" wind farm 7 are object of conservation of the wild birds in the territory of protection area "Kaliakra" BG0002051 - Calandra Lark (*Melanocorypha calandra*), Greater Short-toed Lark (*Calandrella brachydactyla*), Eurasian Thick-knee (*Burhinus oedicnemus*), Tawny Pipit (*Anthus campestris*), Red-backed Shrike (*Lanius collurio*), Lesser Grey Shrike (*Lanius minor*) and the Woodlark (*Lulula arborea*). Among them with highest density is the Calandra Lark with average numbers for the both years is 7,15 p/10ha.

Tree bird species are included in the Red Book of Bulgaria and two species are with protected status of "Vulnerable" - Eurasian Thick-knee (*Burhinus oedicnemus*) and Greater Short-toed Lark (*Calandrella brachydactyla*). One of the species is with status of "Endangered" - Calandra Lark (*Melanocorypha calandra*).

The species composition and the numbers of the birds in the territory of "Kaliakra" wind farm are close and identical to those of the ornitocomplexes in the similar habitats (Karaivanov, 2015; Karaivanov et all, 2006; Ivanov et all, 1998; and also Zehtindjiev, 2009: Monitoring of breeding birds on the territory of the wind farm "St. Nikola", Kavarna in 2009. Report AES Geo Energy, unpublished report).

The density of the nesting birds in 2009 was also much lower than in 2005, although the species composition is a little bit richer

In May the density was lower compared to this in April. The data collected in May show about the same density for the two compared years.

The species whose numbers have decreased the most are the Skylark (*Alauda arvensis*), the Short-toed lark (*Calandrella brachydactyla*) and the Calandra lark (*Melanocorypha calandra*). As opposed to 2005, in 2009 the Stone curlew (*Burhinus oedicnemus*) was not found to breed on the territory of the wind farm.

Table 2. Density and dominant structure of birds during May. Legend: p - density of pairs per 10 ha; d - dominance.

№	Species	**May 2005 г.**		**May2009 г.**	
		p/10ha.	d	p/10ha.	d
1	*Melanocorypha calandra* (Linnaeus, 1766)	7,21	55,37	4,42	28,36
2	*Alauda arvensis* Linnaeus, 1758	1,16	8,93	1,16	7,46
3	*Upupa epops* Linnaeus, 1758	0,46	3,58	0,46	2,98
4	*Sturnus vulgaris* Linnaeus, 1758	0,93	7,14	2,32	14,92
5	*Lanius collurio* Linnaeus, 1758	0,7	5,36	0,93	5,97
6	*Miliaria calandra* (Linnaeus, 1758)	0,23	1,78	1,16	7,46
7	*Oenanthe oenanthe* (Linnaeus, 1758)	0,23	1,78	0,7	4,48
8	*Streptopelia turtur* (Linnaeus, 1758)	0,46	3,58	0,23	1,49
9	*Pica pica* (Linnaeus, 1758)	0,23	1,78	0	0
10	Turdus merula Linnaeus, 1758	0,23	1,78	0	0
11	*Cuculus canorus* Linnaeus, 1758	0,23	1,78	0	0
12	*Burhinus oedicnemus* (Linnaeus, 1758)	0,46	3,58	0	0
13	*Anthus campestris* (Linnaeus, 1758)	0,23	1,78	0,46	2,98
14	*Calandrella brachydactyla* (Leisler, 1814)	0,23	1,78	1,63	10,45
15	*Oenanthe isabellina* (Temminck, 1829)	0	0	0,46	2,98
16	*Lanius minor* Gmelin, 1788	0	0	0,46	2,98
17	*Passer domesticus* (Linnaeus, 1758)	0	0	0,46	2,98
18	Emberiza melanocephala Scopoli, 1769	0	0	0,46	2,98
19	*Lulula arborea* (Linnaeus, 1758)	0	0	0,23	1,49
	Total:	**13,02**	**100%**	**15,58**	**100%**

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Lipid accumulation in *Cyprinus carpio* (Linnaeus, 1785) liver induced by thiamethoxam

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Abstract. The aim of the present study is to investigate the effects of a thiamethoxam based insecticide on the expression of lipid droplets in Common Carp, *Cyprinus Carpio* (Linnaeus, 1785) liver. The selected concentrations of the test pesticide were 6.6 mg/L, 10 mg/L and 20 mg/L under laboratory conditions for an acute period of 96 h. The Sudan III staining method was applied for detection of fatty degeneration in the fish hepatocytes. Overall, we found that the fat storage in the liver cells increased proportionally with the increased pesticide concentrations. The results demonstrated fat accumulation in the fish liver which in addition, could be used as an easy to perform and relatively inexpensive biological tool for studying the effects of pesticide contamination on fish.

Key words: histochemistry, fish, liver, lipid accumulation, pesticide.

Introduction

Aquatic ecosystems receive a complex mixture of contaminants from anthropogenic sources, including agricultural practices (Zhang & Zhao 2017). One of these chemicals are the neonicotinoids which are the most widely used class of insecticides nowadays and thiamethoxam is one of them (Morrissey *et al.* 2015). In addition, the average residue levels of thiamethoxam in water ecosystems have increased over the past 15 years and its physical-chemical characteristics increase the chances of environmental contamination via surface-runoff or drainage into areas adjacent to the crops reflecting the worldwide trend in usage of this compound (Sánchez-Bayo *et al.* 2016; Iturburu *et al.* 2018).

Fish biomarkers represent an useful tool in order to evaluate the risk assessment process: effect, exposure and hazard assessment, risk characterization or classification, and monitoring the environmental quality of aquatic ecosystems (van der Oost *et al.* 2003). Thus, histopathological studies are conducted to establish fundamental relationships of contaminant exposure and its biological responses (Pathan *et al.* 2010). Moreover, according to Rajini *et al.* (2015) the histopathological evaluation is an important part of the assessment of the adverse effects of xenobiotics on the whole organism.

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Based on the above, the main objective of the present work was to study the lipid accumulation in Common Carp liver in order to evaluate the negative effects of a thiamethoxam based insecticide.

Material and Methods

The experiment was conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purpose. Three groups of fish (n=15) were exposed to the insecticide at test concentrations of 6.6 mg/L, 10 mg/L and 20 mg/L which were prepared by dilution of the stock solution of the commercial product purchased from a certified agricultural pharmacy. Common Carps were obtained from the Institute of Fisheries and Aquaculture, located in the city of Plovdiv, Bulgaria where fish are reared under strict conditions. They were of the same size-group. The exposure was carried out in static conditions according to APHA (2005). The experiment last for 96 h and no lethal outcome was reported. Cryosections of each specimen were prepared according to standard methodology and the samples were stained with Sudan III according to Pearse (1972). Evaluation of the histochemical changes was carried out using the scale according to Mishra & Mohanty (2008) which we slightly modified: (0) – negative reaction of histochemical staining; (1) – very weak positive reaction of histochemical staining; (2) – weak positive reaction of histochemical staining; (3) – moderate positive reaction of histochemical staining; (4) – strong positive reaction of histochemical staining in the hepatocytes. The results are presented as average. Statistical analysis was performed using Graph Pad Prism 7 for Windows and significant level was set at 0.05.

Results and Discussion

The results of the control group showed that the intensity of Sudan III staining was very weak. It was expressed in a discreet yellow staining in the hepatocytes. At the lowest concentration of 6.6 mg/L, a mild positive histochemical reaction was found and it was expressed in yellow-orange staining. This showed a slight accumulation of lipid droplets in the hepatocytes cytoplasm. At the higher insecticide concentration of 10 mg/L an increase in the intensity of Sudan III staining was observed and according to the proposed semi-quantitative scale it was evaluated as a moderate positive histochemical reaction. At the highest concentration of 20 mg/L strong positive Sudan III staining was found which indicated the largest amount of lipid droplets accumulated in the liver, and it was expressed as intense orange staining (Table 1, Fig. 1).

Table 1. Intensity of Sudan III staining in Common Carp liver, n=15 for each group. (0) – negative reaction of histochemical staining; (1) – very weak positive reaction of histochemical staining; (2) – weak positive reaction of histochemical staining; (3) – moderate positive reaction of histochemical staining; (4) – strong positive reaction of histochemical staining in the hepatocytes. * - significantly different (p<0.05).

Insecticide concentration, mg/L	**Control group**	**6.6**	**10**	**20**
Intensity of Sudan III staining	1*	2	3	4*

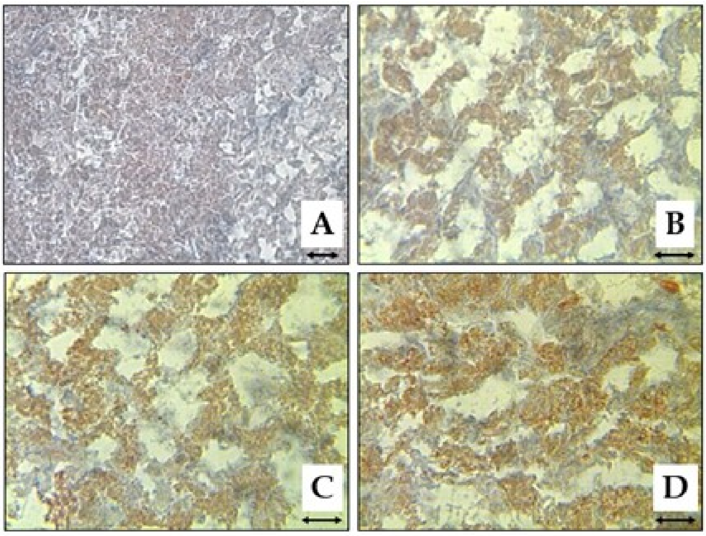

Fig. 1. Intensity of Sudan III staining in Common Carp liver: A – control group, x200; B – 6.6 mg/L insecticide, x400; C – 10 mg/L insecticide, x400; D – 20 mg/L insecticide, x400.

In regards to our previous study (see Stoyanova et al. 2012) we found that thiamethoxam induces also accumulation of glycogen which formed conglomerates in single hepatocytes. Hence, we determined induced gluconeogenesis in Common Carp. In addition, we found that the tested insecticide affected liver lactate dehydrogenase (LDH), enzymatic activity, as well as, aminotransferases aspartate (ASAT) and alanine (ALAT) activity (unpublished data). In this study, we found fatty degeneration in the fish liver along with an increase in the accumulation of glycogen levels in the liver cells. Similarly to us, Namita *et al.* (2007), Rajini *et al.* (2015) and Javed *et al.* (2016) also found fatty degeneration in the fish liver due to different toxicants, such as pesticides and heavy metals. Therefore, we consider that the observed changes in the lipid metabolism could be used as a biomarker for a non-specific response of the fish organism to various toxicants but not only pesticides. Bechmann et al. (2012) stated that hepatic lipogenesis includes de novo synthesis of fatty acids from acetyl-CoA or malonyl-CoA and further processing to triglycerides. According to the authors, these changes showed that the impaired carbohydrate and lipid metabolism could be due the toxicant activity and its chemical characteristics. We think that the observed fatty infiltration in the liver cells could be associated with the absence of the enzyme glucose-6-phosphatase and the inability to release glucose in the blood which leads to hypoglycemia in the organism. On the other hand, this could be due to increased amounts of pyruvate in the liver, and hence by the pyruvate dehydrogenase complex of excess amounts of acetyl-CoA which is used for the synthesis of fatty acids and cholesterol. Therefore, the increased fatty acid synthesis results in increased triglyceride synthesis and hyperlipidemia associated with fatty infiltration of hepatocytes.

We consider that the observed histochemical alterations could be proposed as a biological tool for the effects of water contamination in monitoring programs and assessment of aquatic pollution.

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New records of *Pygopleurus* Motschulsky, 1860 species (Scarabaeoidea: Glaphyridae) from Bulgaria

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Abstract. New chorological data of three *Pygopleurus* Motschulsky, 1860 (Scarabaeoidea: Glaphyridae) (i.e. *P. apicalis* (Brullé, 1832), *P. diffusus* (Petrovitz, 1958) and *P. humeralis* (Brullé, 1832)) are reported for Bulgaria. The records of *P. apicalis* (Brullé, 1832) and *P. diffusus* (Petrovitz, 1958) are new for the country.

Key words: Glaphyridae, Pygopleurus, Bulgaria.

Introduction

Up to now, based on available references (Minkova 1959, Nikodým & Bezděk 2016) only four species of genus *Pygopleurus* were known for Bulgaria: *Pygopleurus foina* (Reitter, 1890), *Pygopleurus hirsutus* (Brullé, 1832), *Pygopleurus humeralis* (Brullé, 1832) and *Pygopleurus vulpes* (Fabricius, 1781). In the present work, we report new data on the distribution of three species from Bulgaria. Nearly all examined specimens are deposited in the Zoological Collection of Sofia University "St. Kliment Ohridski", Faculty of Biology (BFUS).

Results and Discussion

***Pygopleurus apicalis* (Brullé, 1832)** (Fig. 2: A, B, C)

Material examined: Bulgaria: Belasitsa Mts., Klyuch Vill., 500 m., 30.iv.2008, 1 ♂, D. Gradinarov leg.; Belasitsa Mts., Belasitsa Vill., 41°22.100'N 23°07.835'E, 369 m., 30.iv.2018, roadside meadows, 7 ♂♂, 2 ♀♀, Y. Petrova leg.; Sandanski-Petrich Valley, 2 km NE Kolarovo Vill., 41°22.570'N 23°08.017'E, 274 m., 30.iv.2018, roadside verges next to agricultural fields, 1 ♂, in flower of *Papaver* sp., Y. Petrova leg.

Even if the species is reported from Bulgaria, Greece and Macedonia (Nikodým & Bezděk 2016), no verified Bulgarian specimens were available, so the specimens herein reported are the first verified data for the Country.

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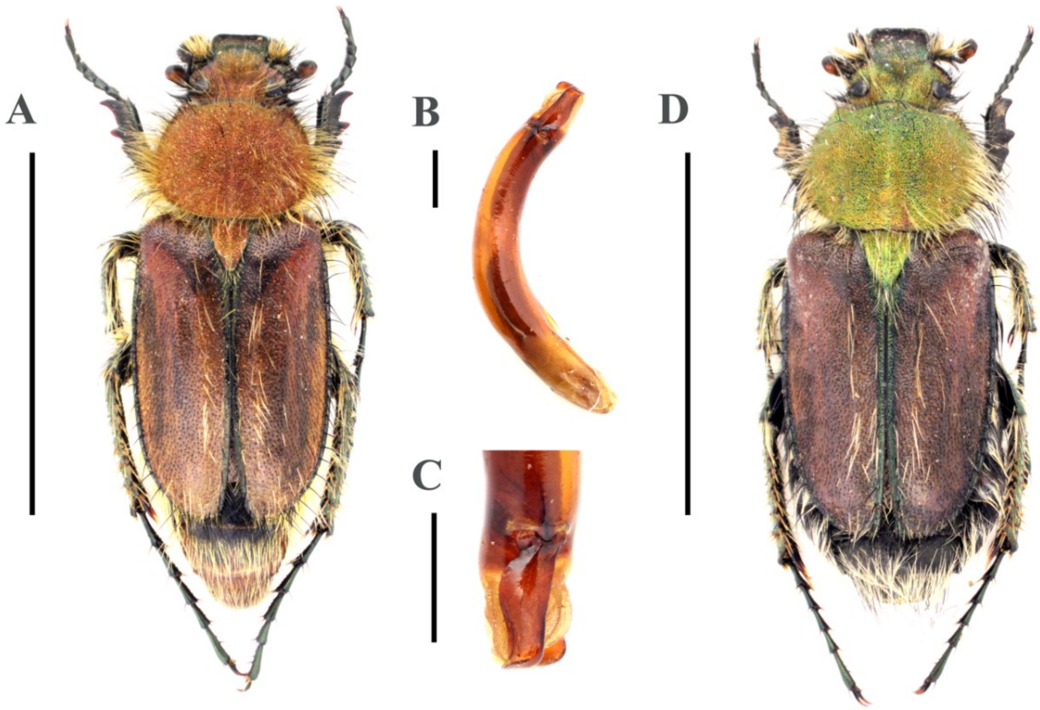

Fig. 1. *Pygopleurus diffusus* (Petrovitz, 1958), Ograzhden Mts., 30.iv.2018. A: male; B: aedeagus, lateral; C: parameres; D: female. Scale bars: A, D: 10 mm.; B, C: 1 mm.

***Pygopleurus diffusus* (Petrovitz, 1958)** (Fig. 1: A - D)

Material examined: Bulgaria: Varna, Kalimanci, 4.vi.1982, A. Podlussány leg., 3 specimens (in coll. Szaloki); Sandanski-Petrich Valley, Rupite Place, 41°27.145'N 23°16.040'E 90 m., 06.v.2010, 1 ♂, net-sweeping, O. Sivilov & G. Hristov leg. (BFUS); Ograzhden Mts., 1 km SE Borovichene Vill., 41°24.860'N 23°01.449'E, 453 m., 30.iv.2018, roadside meadows, 2 ♂♂, 2 ♀♀, inflowers of *Papaver* sp., Yana Petrova & Denis Gradinarov leg. (BFUS).

The species is distributed in Albania, Greece, Macedonia, while the report for Turkey (Nikodým & Bezděk 2016) is highly questionable. New record for Bulgaria.

***Pygopleurus humeralis* (Brullé, 1832)** (Fig. 2: D, E, F)

Material examined: Bulgaria: Kresnenski Prolom Gorge, 200 m., 04.v.2008, 1 ♂, on Asteraceae, D. Gradinarov leg.; Pirin Mts., slope above Railway station P. Yavorov, 41°45.124'N 23°09.291'E, 220 m., 04.v.2008, 1 ♂, 2 ♀♀, D. Gradinarov leg.; Ograzhden Mts., 2 km NW Strumeshnitsa Vill., 41°24.708'N 23°02.147'E, 375 m., 30.iv.2018, pine forest, 1 ♂, on *Ranunculus* sp., Y. Petrova & D. Gradinarov leg.; Sandanski-Petrich Valley, Kozhuh Hill., 100 m., 02.v.2008, 1 ♂, 3 ♀♀, D. Gradinarov leg.; Sandanski-Petrich Valley, Kozhuh Hill.,41°27.648'N 23°15.309'E, 181 m., 02.iv.2016, 1♂, on *Anemone* sp., D. Gradinarov leg.; the same locality and date, 1 ♂, on *Iris* sp., D. Gradinarov leg.; Sandanski-Petrich Valley, N Kresna town, left bank of Struma Riv., 41°44.110'N 23°09.459'E, 182 m., 28.iv.2018, riverside meadows, 1 ♂, on *Papaver* sp., Y. Petrova & D. Gradinarov leg.; the same locality, 01.v.2018, riverside meadows, 1 ♂, on *Ranunculus* sp., Y. Petrova leg.

The species is distributed in Bulgaria, Greece, Macedonia and Turkey (Nikodým & Bezděk 2016). In Bulgaria it is reported from Plovdiv, Malko Tarnovo, Burgas, Primorsko, Haskovo and Sakar Mountain (Minkova 1959, as *Amphicoma vulpes* ab. *viridisuturata* Reitt.), Sandanski-Petrich Valley and Kresna gorge (Zaharieva – Stoilova & Dimova 1980; Sakalian *et al.* 1993; Kalushkov & Dimova 1997 as *Amphicoma petrovitzi* Miks.).

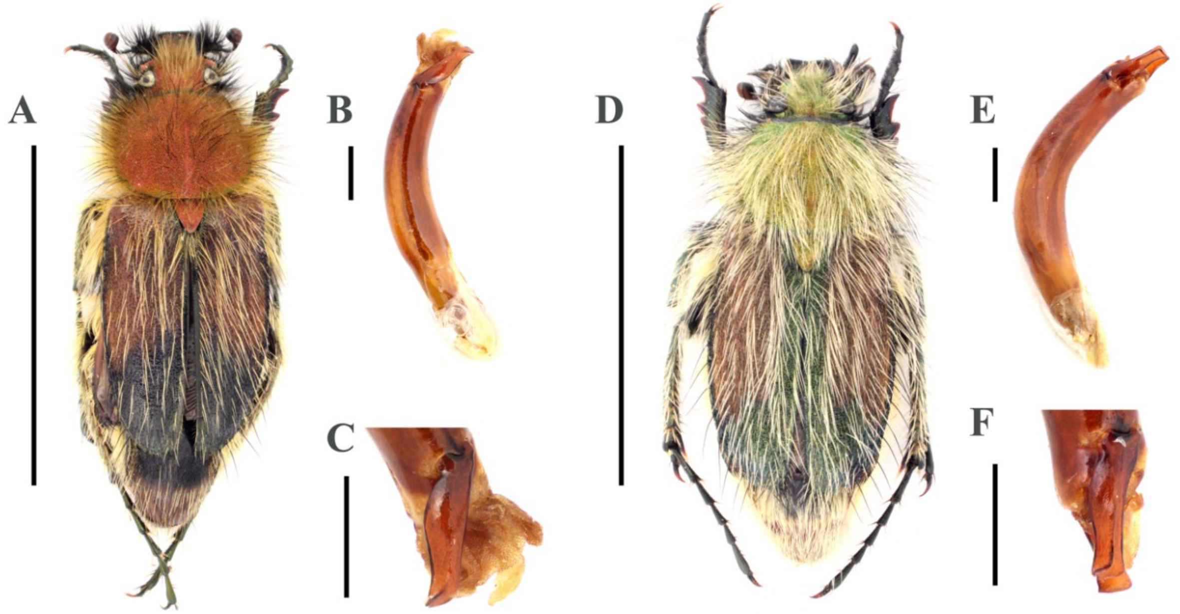

Fig. 2. *Pygopleurus apicalis* (Brullé, 1832), Kolarovo Vill., 30.iv.2018 (A, B, C); *Pygopleurus humeralis* (Brullé, 1832), Kresna town, 28.iv.2018 (D, E, F); A, D: males; B, E: aedeagus, lateral; C, F: parameres. Scale bars: A, D: 10 mm.; B, C, E, F: 1 mm.

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The Eurasian Collared Dove (*Streptopelia decaocto* Frivaldszky, 1838) – a subrecent invasive species of the avifauna of Bulgaria (subfossil records)

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Abstract. Although the Collared Dove appeared in Bulgaria at 17-18th century, the subfossil record of the species in the country is rather scant. Only five Late Holocene sites contain bone remains (88 bones of 9 individuals), but all of them remained imprecisely dated. Some of the sites could be of older (possibly: Early Medieval Ages) age. All sites are situated in the NE Bulgaria and represent former feeding places of Eagle owls (*Bubo bubo*).

Key words: Eurasian Collared Dove, Invasive, Holocene.

Introduction

The Eurasian Collared Dove (*Streptopelia decaocto* Frivaldszky, 1838) is a unique species in respect to two facts: (1) It is one of the only a few species of birds that carried out a massive trans-continental invasion before the eyes of humans, but without their participation (Harrison 1982). Over the past 300-350 years, in the Middle Ages the collared dove has moved to the northwest, the eastern Mediterranean islands, the Balkans, and Europe, and the last third of the 20th century it invaded even the North America. (2) The collared dove is the only species of the recent avifauna, described for the world science from Bulgaria (from Plovdiv /former Philipopolis/ in 1837 by the Hungarian naturalist Emerich von Frivald (Imre Frivaldszky; 1799-1870).

Results and Discussion

Appearance on the Balkans and other parts of Europe and North America

The species appeared in Bulgaria at the end of 17th - early 18th century, although the first record in Europe was registered in Crete in the 2nd half of the 16th century (Boev 1963). It is supposed to have originally been introduced to a number of Ottoman Empire Muslim countries in the continent. It has penetrated in the Balkan Peninsula from Asia Minor. In Bulgaria resettlement took place along the bigger towns in the Thracian Plain. In 1890, the bird was nesting in today's Sofia district of Knyazhevo, and in 1893 in the town of Stara Zagora. According to Boev (1963), in the end of the 19th century the collared dove reached our most northern boundaries of the Balkan Peninsula and passed beyond the Danube. Then it was already nesting in the Danube towns from Ruse to Vidin. It penetrated in Dobrudzha around 1926 and in the town of Varna in 1943 (Boev 1963).

The invasion towards northwest continued uninterruptedly and in 1912 the species reached Belgrade, Bucharest in 1938, the Netherlands in 1947, the United Kingdom in 1952, and the Faeroe Islands in 1970. In 1909 and in 1921, it was already observed in Los

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Angeles, but it was supposed that these were escaped cage birds. But since 1970, *S. decaocto* was spread all over California. Today, it has conquered all the United States (except Alaska) and South Canada (del Hoyo & Collar 2014).

In nowadays its number in Europe is estimated at around 7 million breeding pairs (Hagemeijer & Blair 1997). In Bulgaria it is a resident species and it is almost ubiquitous up to 1100 m. *S. decaocto* is a protected species, its capture and killing are pursued by the law. The Bulgarian population numbers 90 000 - 200 000 pairs (Mitev 2007).

Present study provides all available data on the former species' distribution throughout the Bulgarian lands and try to trace the colonization process in the past according to its subfossil record.

Subfossil record of *S. decaocto* in Bulgaria

1. Topchii

UTM:MJ 53. Rock nishe in the canyon of the Topchiyska River, in a rock of 20 m high at about 1 km NW of the Topchii village. 350 m a.s.l. Former feeding place of the Eurasian Eagle Owl (*Bubo bubo*) (Linnaeus, 1758)). Late Holocene. Two bones of 1 ind. (Boev 1999; Mitev 2016).

2. Madara – 1

UTM:NH 09. Rock niche of a 60 m high rock massive, 1 km N of Madara village (Shumen Region). 500 m a.s.l. Former feeding place of *Bubo bubo*. Late Holocene. Six bones of 1 ind. (Mitev 2016). Three bones of 1 ind. (Boev 1999).

3. Madara – 2

UTM:NH 09. Cave in the rock massive north of the Madara village. 300 m. a.s.l. Former feeding place of *Bubo bubo*. Late Holocene (Late Medieval Ages). One bone of 1 ind. (Mitev 2016).

4. Shirokovo

UTM:MJ 12. Cave in the canyon of the Cherni Lom River in a rock massive 20 m high, 2 km north of Shirokovo village. 150 m a.s.l. Former feeding place of *Bubo bubo*. Early to Late Holocene (Mitev 2006). 82 bones of 5 ind. (Mitev 2016).

5. Isperih

UTM:MJ 84. Rock niche in a rock massive 15 m high in the valley of Chernodlanitsa River in the Sboryanovo locality, 7 km NW of town of Isperich. 150 m a.s.l. Former feeding place of *Bubo bubo*. Late Holocene. Two bones of 1 ind. (Boev 1999; Mitev 2004).

Bone remains of the collared dove in Bulgaria are scanty. Although over 120 sites of fossil and subfossil record of birds are explored so far throughout all over the country (Boev, 1999; unpubl. data), only five localities yielded osteological finds proving former occurrence of that species in the past.

As seen all five localities have non-human accumulation agent (Eagle Owl) and were dated imprecisely. All they are situated in the NE Bulgaria. All subfossil finds (88 bones of 9 individuals) of the collared dove came from Late Holocene deposits, but unfortunately the exact date could not be determined. Two localities (Madara – 1 and Shirokovo) contain mixed deposits of late and possibly an early ("middle") Holocene (Boev 1999; Mitev 2004).

Thus, at present stage, they could not be used for tracing of the colonisation process of the species invasion in Europe. On the other hand they mark species' subrecent occurrence in this part of the country.

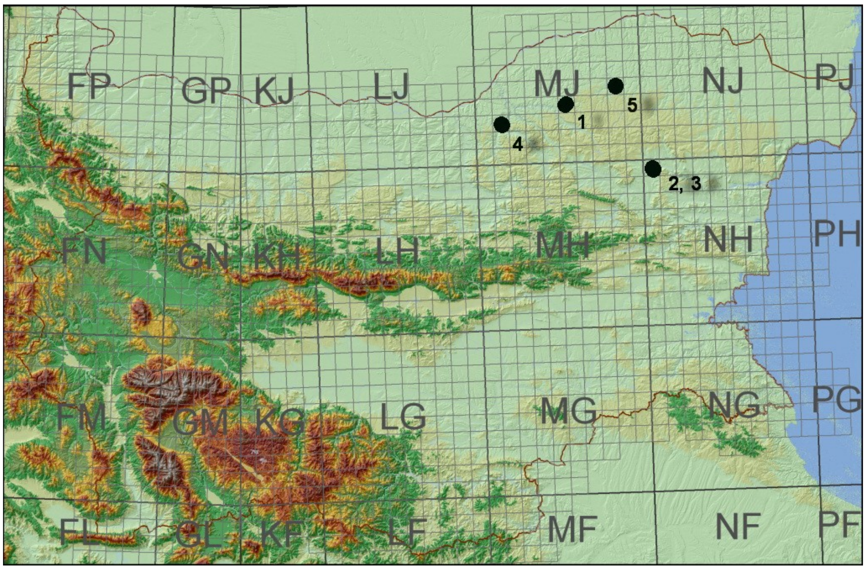

Fig. 1. Late Holocene subfossil record of the Eurasian Collared Dove (*Streptopelia decaocto*) in Bulgaria: Topchii (1); Madara - 1 (2); Madara - 2 (3); Shirokovo (4); Isperih (5).

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Preliminary data on the defensive behavior and vocalization of the Lesser blind mole rat, *Nannospalax leucodon* (Nordmann, 1840)

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Abstract. The defensive behavior of two individuals of the Lesser blind mole rat, *Nannospalax* (superspecies) *leucodon* (Nordmann, 1840), was investigated. A characteristic defensive posture and two types of vocalization were recorded: teeth grinding and grunting, expressed by consecutive series of complex harsh calls, consisting of sequences of single very short (1.7 – 2.6 ms) phases (with maximum energy at about 9.8 KHz), accompanied by strong noise component. More research is needed to reveal the full vocal repertoire and behavioral characteristics of the species.

Key words: *Nannospalax leucodon*, Lesser blind mole rat, vocalization, defensive behavior.

Introduction

The Lesser blind mole rat, *Nannospalax* (superspecies) *leucodon* (Nordmann, 1840) is a subterranean rodent species inhabiting open habitats (mostly grasslands and agricultural lands) in Southeast Europe. The species is threatened by habitat loss due to agricultural intensification and urbanization (Kryštufek & Amori 2017). In Bulgaria it is included in the National System for Environmental Monitoring.

Although the blind mole rats are known as strictly subterranean animals, a surface activity caused by a variety of factors, is reported also. However, their narrow specialization to underground lifestyle makes mole rats very vulnerable out of their tunnel systems. They are captured by predators most often during these emergences on the surface. Thus, being small sized and slow moving, mole rats need some kind of defensive strategy when encounter predators above ground. Warning postures and facial expression, as well as vocalizations that are meant to confuse the enemy, are among the most common behavioral responses in cases where the physical features of the animal do not allow escape.

While there are relatively many available data for the vocalization and behaviour of subterranean rodents, including some species of mole rats (mostly *Nannospalax ehrenbergi* (Nehring, 1898), *Fukomys mechowii* (Peters, 1881), *Cryptomys* sp.) (Heth *et al.* 1986; Rado *et al.* 1987; Credner *et al.* 1997; Hrouzková 2012; Bednářová *et al.* 2013; Bednov *et al.* 2013), no information is found about *Nannospalax leucodon*.

The vocalization in subterranean rodents is characterized by low frequencies which are better transmitted by the soil (Credner *et al.* 1997; Rickye *et al.* 1992; Heth *et al.* 1986). Seismic signals resulted from head thumping against the tunnel ceiling are also typical for the mole rats (Heth *et al.* 1987; Rado *et al.* 1987).

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Material and Methods

Two male Lesser blind mole rats (1 adult and 1 subadult) were captured in a sunflower field near Ravno pole village in the vicinity of Sofia, Bulgaria (42.6737 N 23.5221 E, 533 m a. s. l.) in May 2019. The animals were caught by opening the tunnels and blocking the way back after they appeared at the opening. The defensive behavior of the mole rats was observed in the field first and later in the laboratory. It was provoked by stressful situation - removing the animal from the cage and touching its back. Vocalization was recorded by M500 USB Ultrasound Microphone and laptop using BatSound Touch recording program that saves the recordings as 16 bit wav files. The microphone was held at approximately 20–30 cm from the animals. Totally 10 series of grunting sounds were analyzed. The computer analysis was performed using BatSound 3.1 software for Windows. The frequency components of the calls were measured from the Fast Fourie Transform (FFT) power spectrum, size 1024, Hamming window. The main call parameters are considered: total call duration (ms), frequency with the most energy (KHz) and shape of spectrograms.

Results

Both in the field and in the laboratory the disturbance by the observer made the mole rats to take a defensive posture. The animal raised head upward and widely opened its mouth demonstrating its long incisors (Fig. 1). At the same time, it produced warning calls and occasionally gnashed its teeth. All the time it tried to localize the threat and to remain faced with the intruder, but it did not try to escape. When the mole rat was given the opportunity to hide itself in a plastic tube imitating its natural tunnel, the animal preferred to go into it in reverse, remaining all the time facing the “enemy”.

Fig. 1. Defensive posture of the Lesser mole rat, *Nannospalax leucodon* (Nordmann, 1840).

Two types of sounds produced by the threatened mole rats were recorded: grunting and teeth grinding. The grunting was expressed by consecutive series of complex harsh calls, consisting of sequences of single very short (1.7 – 2.6 ms) phases (with maximum energy at about 9.8 KHz), accompanied by strong noise component (from 2 – 3 KHz and dying to 50 KHz). The number of separate phases of the calls varies between 18 and 25 (Fig. 2 and 3).

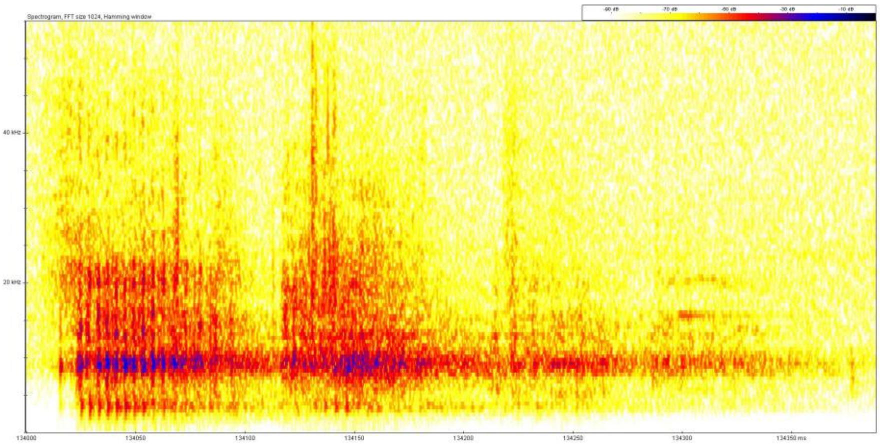

Fig 2. Harsh calls consisting of sequences of single very short phases.

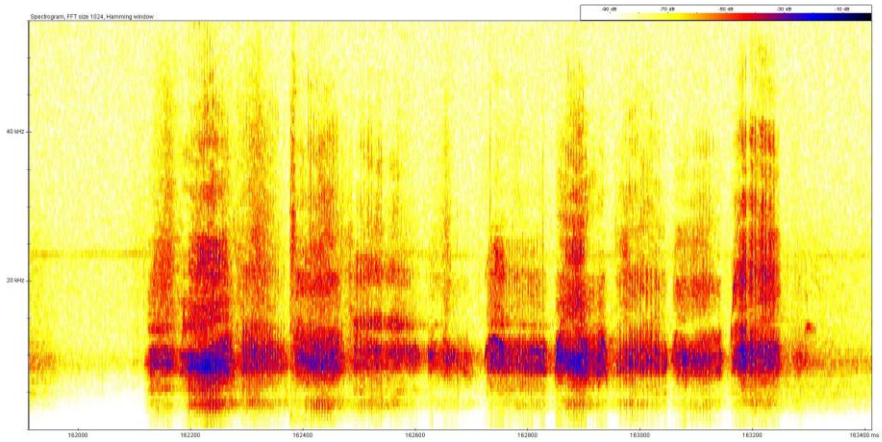

Fig 3. Consecutive series of 10 harsh calls.

Discussion

The observed posture in response to disturbance by the researcher may be associated with defensive anti-predator behavior. Perhaps it could be observed also during intraspecific agonistic interactions. The mole rat's refusal to enter the tube head-on could be explained by its vulnerability to such a situation - it can defend itself from attack only through its large incisors. So, returning to the tunnel backwards is probably inborn behavior in mole rats. When out of their burrows, escape is not a solution - the animal is too slow to have a chance to save itself.

Although previous studies show that teeth grinding is a typical mechanically produced sound in subterranean rodents, this sound hasn't been reported for *N. leucodon* so far. In mole rats' teeth grinding is associated with aggressive behaviour (as in the present study), but interestingly, this sound is more often produced while the animals are resting and sleeping (Credner *et al.* 1997; Bednářová *et al.* 2013).

Individual, age, sex, population, and geographical differences in the vocalization of *Nannospalax leucodon* could be expected in view of the fact that some kind of variability has been observed in other mole rat species. For example, different vocal dialects are reported for the chromosomal species of *Nannospalax ehrenbergi* superspecies (Nevo *et al.* 1987). Dvorakova *et al.* (2017) found individual differences in mating calls of Mashona mole rat - *Fukomys darlingi* (Thomas, 1895).

Some researches reveal comparatively rich repertoire of vocalisation in rodent species (Simeonovska-Nikolova & Dekov 2013; Credner *et al.* 1997; Bednářová *et al.* 2013).

In our study the individuals from *N. leucodon* superspecies demonstrated uniform and slightly variable sounds accompanying their defensive behavior. Recorded sounds certainly do not deplete the set of acoustic communication signals, produced by the Lesser blind mole rat. In-depth researches on a large number of individuals from different localities are needed to shed light on the complete repertoire of this species, its variability and the significance of individual sounds in the intra-species and between species communication.

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A few notes on the diet and the copulation of *Theba pisana* (Müller, 1774) snails: observations on terrarium kept animals

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Abstract. The specimens used to eat 17 grass species from all 44 species offered (38.6% from all). They refused to eat all 3 liana and 21 tree species and eat only leafs of *Paliurus spina-christi* from the offered bushes. Contrary they eat most of the fruits, vegetables and animal food. Hanging on the tips of the soles from the top of terrarium was observed during copulation.

Key words: diet, snail, invasive species.

Introduction

The terrestrial snail species, *Theba pisana* (Müller, 1774) (Gastropoda: Helicidae), usually occur in coastlands, in or near sandy habitats, in hot climates estivating, often directly exposed to the sun, attached to grasses, shrubs or succulent plants. In dunes it can live on nearly bare sand, poorly fixed by grasses. In the north the snails do not estivate but they climb on plants in dry weather. This species cannot survive serious winter frosts (Welter-Schultes 2012). The native range of *Th. pisana* is Mediterranean region and adjacent Atlantic coasts from central Morocco to Belgium, South-West England, South Wales, South-East Ireland and central Atlantic islands. It has been introduced in many areas with proper climate condition all over the world (Däumer *et al*., 2012). This species is considered to be an invasive pest, damaging many types of crops, native wild plants (for example fynbos vegetation in South Africa) and animal species (Odendaal *et al*. 2008). However, the studies of the species diet and food preferences are scarce. This short note is a small piece of contribution to the knowledge of its feeding and copulation, data revealed from some terrarium kept animals.

Material and Methods

Five *Theba pisana* specimens were occasionally brought with ornamental plants from Malta Island, and were kept a small terrarium for about two months (21.09.2013 – 06.11.2013) (till the end of their life). In the same tank also one specimen of *Otala punctata* (O. F. Müller, 1774) from Morocco was living. Some feeding experiments were made, as offering a variety of different types of potential food and following direct observations. A total of 113 potential food items were provided. They were divided into following groups: leafs of grass and low vegetation, lianas, bush, trees, fruits; vegetables, nuts and seeds, other food with higher plant origin; lichens; mushrooms; animal food. Also, some observations on copulation behaviour were made and time and air temperature were noted.

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Results

Notes on the diet. The specimens used to eat 17 grass species from all 44 species offered (38.6% from all). They refused to eat all 3 liana and 21 tree species and eat only leafs of *Paliurus spina-christi* from the bushes offered. Contrary they eat most of the fruits, vegetables and animal food (Table 1).

Copulation. Date: 04.11.2013, air temperature: 18.6°C; observed duration: 3 hours, from 16:10 to 19:10; behaviour: hanging on the tips of the soles from the top of terrarium was observed during copulation (Fig. 1).

Fig. 1. A pair of copulating *Theba pisana* hanging from the glass cover of the terrarium.

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Table 1. Different types of food offered to the *Theba pisana* specimens during the study period (in gray is the food which was accepted by the animals) (next page).

Date	Food type	Consumption
	Plant food	
	Leaves	
	Grass and low vegetation	
21.9.2013	*Portulaca grandiflora*	-
22.9.2013	*Petunia* sp.	+
22.9.2013	*Apium graveolens*	-
22.9.2013	*Melissa* sp.	-
23.9.2013	*Chrysanthemum* sp.	+
23.9.2013	*Convonvulus* sp.	+
23.9.2013	*Trifoilum* sp.	+
23.9.2013	*Lamium* sp.	+
23.9.2013	*Amaranthus* sp.	+
23.9.2013	*Taraxacum* sp.	+
23.9.2013	*Cynodon* sp.	-
23.9.2013	*Polygonum* sp.	-
26.9.2013	unidentified moss	-
26.9.2013	*Saponaria* sp.	-
26.9.2013	*Teucrium* sp.	-
26.9.2013	*Geranium* sp.	-
26.9.2013	*Urtica* sp.	+
26.9.2013	*Mentha* sp. - not cultivated species	-
26.9.2013	*Mentha spicata*	-
26.9.2013	*Antirrhinum* sp.	-
26.9.2013	*Portulaca* sp.	-
26.9.2013	*Armoracia rusticana*	+
26.9.2013	*Sambucus* sp.	+
28.9.2013	*Clinopodium* sp.	-
28.9.2013	*Agrimonia* sp.	-
28.9.2013	*Potentilla* cf *reptans*	-
28.9.2013	*Galium aparine*	-
28.9.2013	*Achillea millefolium*	-
29.9.2013	*Capsicum annuum*	+
4.10.2013	*Rumex* sp.	+
4.10.2013	*Verbascum* sp.	+
4.10.2013	*Atriplex* sp.	-
4.10.2013	*Artemisia* sp.	-
4.10.2013	*Tribulus* sp.	-
4.10.2013	*Iris* sp.	-
4.10.2013	*Onopordum* sp.	-
4.10.2013	*Lactuca* sp.	+
4.10.2013	*Solanum lycopersicum*	+
4.10.2013	*Medicago sativa*	+
4.10.2013	*Xantium* sp.	-
4.10.2013	*Foeniculum vulgare*	-
14.10.2013	*Sedum* cf *album*	-
14.10.2013	*Fragaria* sp.	-
7.11.2013	*Hordeum murinum*	+
	Lianas	
28.9.2013	*Clemathis* sp.	-
4.10.2013	*Humulus* sp.	-
14.10.2013	*Vitis vinifera*	-
	Bush vegetation	
21.9.2013	*Crassula ovata*	-
23.9.2013	*Syringa* sp.	-
14.10.2013	*Paliurus spina-christi*	+
26.9.2013	*Rosa* sp.	-
28.9.2013	*Rubus* sp.	-
14.10.2013	*Prunus spinosa*	-
	Tree vegetation	
23.9.2013	*Prunus cerasifera*	-
23.9.2013	*Carpinus* sp.	-
26.9.2013	*Cercis siliquastrum*	-
26.9.2013	*Amygdalus* sp.	-
26.9.2013	*Fraxinus* sp.	-
27.9.2013	*Quercus* sp.	-
28.9.2013	*Cerasus sativa*	-
28.9.2013	*Juglans regia*	-
28.9.2013	*Acer campestre*	-
28.9.2013	*Crataegus* sp.	-
4.10.2013	*Ailanthus*	-
4.10.2013	*Gleditchia*	-
4.10.2013	*Ficus carica*	-
4.10.2013	*Robinia pseudoacacia*	-
4.10.2013	*Pyrus* sp.	-
4.10.2013	*Acer negundo*	-
4.10.2013	*Morus* sp.	-
4.10.2013	*Koelreuteria paniculata*	-
14.10.2013	*Celtis australis*	-
14.10.2013	*Ulmus* sp.	-
14.10.2013	*Acer tataricum*	-
	Fruits	
19.9.2013	*Vitis vinifera*	+
20.9.2013	*Prunus domestica*	+
24.9.2013	*Prunus persica*	+
26.9.2013	*Rosa* sp.	-
27.9.2013	*Cydonia oblonga*	+
28.9.2013	*Malus* sp.	+
28.9.2013	*Crataegus* sp.	+
28.9.2013	*Rubus* sp.	-
14.10.2013	*Prunus spinosa*	+
12.10.2013	*Rubus idaeus*	+
16.10.2013	*Malus domestica*	+
5.11.2013	*Musa* sp.	+
	Vegetables	
20.9.2013	*Solanum lycopersicum*	+
21.9.2013	*Capsicum annuum*	+
22.9.2013	*Capsicum annuum*	+
21.9.2013	*Solanum tuberosum*	+
22.9.2013	*Daucus carota*	+
22.9.2013	*Beta vulgaris*	+
17.10.2013	*Brassica oleracea* var. *capitata*	+
19.10.2013	*Cucumis sativus*	+
20.10.2013	*Brassica oleracea* var. *botrytis*	+
	Nuts and seeds	
22.9.2013	*Cicer arietinum*	-
27.9.2013	*Juglans regia*	+
29.9.2013	*Helianthus anuus*	+
	Other	
22.9.2013	bread	+
26.9.2013	print paper	+
6.11.2013	fine bark of dead, dry *Fraxinus*	+
	Lichens	
	unidentified lichens	-
	Mushrooms	
23.10.2013	*Agaricus* sp.	-
23.10.2013	*Macrolepiota* sp.	-
23.10.2013	*Calvatia* sp.	+
	Animal food	
18.9.2013	aquarium fish food - vitamines	+
24.9.2013	aquarium fish food - dry liver	+
24.9.2013	aquarium fish food - dry *Daphnia*	+
19.9.2013	cat food - granules	+
26.9.2013	*Columba livia* - excrement	+
20.10.2013	dog food - granules	+
7.11.2013	sausage	+
	Predation or mucus eating /?/	
6.11.2013	live, contracted *Otala punctata*	+

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A White Stork (*Ciconia ciconia* (Linnaeus, 1758)) nest – an unique case of multiple nesting commensalism of five species from Dragoman (W Bulgaria)

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Abstract. A rare case of contemporaneous nesting of five species of birds in a single nest is described.

Key words: Nesting commensalism, White Stork, Urban birds.

Introduction

In Southern Europe it is common phenomenon to observe nesting sparrows (g. *Passer*) among the sticks and twigs of the White Stork (*Ciconia ciconia* (Linnaeus, 1758)) nest. In most regions the House Sparrow (Linnaeus, 1758) is the most common commensal nesting species in the stork nests. Such examples of nesting commensalism were registered in many parts of Bulgaria too.

We accept commensalism as case of two interacting populations/species, where one of them has a positive effect, and the other is not susceptible to a noticeable action of the first (Shishkin, 1982).

Results

Dragoman "collective" nest

While sparrows' nesting in the White Stork nests is well known, the nesting of the Eurasian Collared Dove in these nests is extremely rare. As an example of rare case of nesting of Collared Dove in the nest of the White Stork, Boev (1963) mention an observation of Harrison (1933) in the town of Petrich (SW Bulgaria).

On 19.05.2019 between 09:15 and 11:30 h we observed an old occupied nest of White Stork (42°55'54.87"N; 22°55'51.23"E) with two juveniles in it in the town of Dragoman (W Bulgaria). Surprisingly we observed tree species of sparrow – Eurasian Tree Sparrow (*Passer montanus* (Linnaeus, 1758), Fig. 1), House Sparrow (*P. domesticus* (Linnaeus, 1758) Fig. 2) and Spanish Sparrow (*P. hispaniolensis* (Temminck, 1820 Fig. 3)) nesting in the cavities among the sticks and twigs of the stork nest in its lower part.
The nest was built on an artificial substrate, a ferro-concrete electric pole along the street, ca. 7 m high.

The Spanish Sparrows were the most numerous (?4-6 nests), followed by the Tree Sparrows (?3-4 nests), and the House Sparrows (?2-3 nests). In addition in the central part of the nest bottom by the tip of the concrete pole, a pair of Eurasian Collared Dove (*Streptopelia decaocto* Frivaldszky, 1838) was occupying their single nest (Fig. 4). The species is a common urbanist throughout all the country, but as Harrison (1982) notes, it

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occurs in "… drier open areas … and elsewhere has spread through cultivated country in association with grain cultivation and human habitation" (p. 159). After Dr. Petar Yankov (Bulgarian Society for the Protection of Birds) all mentioned sparrows and the Collared Dove were registered separately in the nests of White Storks, but never so far they had been observed to nest together in a single nest of storks.

Conclusion

The observed case of multiple nesting commensalism of five species of birds in the town of Dragoman is a rare example of the multiple interspecies interaction. A total of 5 species of 3 orders (Ciconiiformes, Columbiformes and Passeriformes) and 3 avian families (Ciconiidae, Columbidae and Passeridae) are presented in the nest.
Such a nest is an indication of both abundant food resources in its vicinities, as well as lacking of enough suitable nesting places. It also reveals the diversity of the species nesting substrates/habitats and forced us to be more careful in treating even the abandoned nests of storks.

Fig. 1. Eurasian Tree Sparrows nesting in a nest of White Stork, Dragoman, 19.05.2019. Photo: Z. Boev.

Fig. 2. House sparrows nesting in a nest of White Stork, Dragoman, 19.05.2019. Photo: Z. Boev.

Fig. 3. Spanish sparrows nesting in a nest of White Stork, Dragoman, 19.05.2019. Photo: Z. Boev.

Fig. 4. Eurasian Collared Dove nesting in a nest of White Stork, Dragoman, 19.05.2019. Photo: Z. Boev.

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Late Antiquity animal remains of the military settlement near Barkach village (Pleven Region, CN Bulgaria)

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Abstract. A total of 86 bones and bone fragments of wild and domestic mammals collected in the Late Antiquity military settlement near Barkach village (Pleven Region, CN Bulgaria) have been identified as 9 species of 4 orders. Most numerous are the remains of Donkey and the Red deer. The record of the Fallow deer proves the species occurrence in the Late Antiquity in the North Bulgaria.

Key words: Subfossil mammals, Late Antiquity, Fallow deer.

Introduction

Region of the Barkach village (Central Northern Bulgaria) lies 7 km from the Vit River and provided an important subfossil fauna. A military settlement was built in the vicinities of the village. It is believed that the settlement was inhabited for a short period and soon after it was abandoned because of the invasion of the Goths.

Material and Methods

The animal material (bones, bone splinters and teeth) was collected in the spring of 2019 during the archaeological excavations leaded by the archaeologist Dr. Katya Melamed, who dated it Late Antiquity (4th century A. D.). Location of the site: 43°17'N, 24°26'E. The material was handed for examination in August 2019. It originates from 11 samples of the excavations of 1.89 to 2.33 m depth.

Results and Discussion

Remains of domestic mammals number 19 finds, while those of wild mammals are 17 (Table 1). The majority of bones are heavily damaged. So, the unidentifiable bone splinters represent 55.8 percent. Three bones of *C. elaphus*, *Capra hircus* and *Ovis*/ *Capra* are burnt. No other traces on bones are found. Two human bones (partial radius and thoracal vertebrae) are identified too. The share of deer (*C. elaphus*, *D. dama* /Fig. 1 – A, B/) in the bone material is surprisingly high. Both species represent up to 28.9 percent of the identified remains. Wild and domestic mammals are almost equally represented.

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Table 1. Animal representation in the collected archaeozoological material from the Late Antiquity military settlement (4th century A. D.) near Barkach village, Pleven Region (CN Bulgaria).

No	Taxa	English Name	Number of bone finds
Artiodactyla			
1.	*Bos taurus* Linnaeus, 1758	Domestic cattle	2
	Bos cf. *taurus*	Domestic cattle	4
2.	*Cervus elaphus* Linnaeus, 1758	Red deer	8
	Cervus cf. *elaphus*	Red deer	1
3.	*Dama dama* (Linnaeus, 1758)	Fallow deer	2
4.	*Sus scrofa scrofa* Linnaeus, 1758	Wild boar	4
	Sus scrofa scrofa/ domestica	Wild boar / Domestic pig	1
5.	*Capra hircus* (Linnaeus, 1758)	Domestic goat	1
	Ovis / Capra	Sheep/ Goat	1
Perissodactyla			
6.	*Equus ferus caballus* Linnaeus, 1758	Domestic horse	1
7.	*Equus africanus asinus* Linnaeus, 1758	Donkey	9
Carnivora			
8.	*Canis lupus* Linnaeus, 1758	Gray wolf	2
Primates			
9.	*Homo sapiens* Linnaeus, 1758	Wise man	2
	Unidentifiable bone splinters		48
	Total		***86***

The relative abundance of the remains of deer, wild boar (and gray wolf), representing 44.7 percent of all identified bone remains, suggests vast woodland landscape in the settlement's surrounding. Even more, all these species are the only wild animals found in the studied material. All other animals are domestic forms. Donkey (Fig. 1 – C) was the most numerous among all species/domestic forms.

At present the largest oak (*Quercus* spp.) forests in the Dolni Dabnik Municipality survived in the region of the Barkach and Sadovets villages (Nikiforov 2017).

As human and animal bone remains were deposited in the same place, we could conclude that the excavations uncovered a former dump, where debris of any kind were dumped.

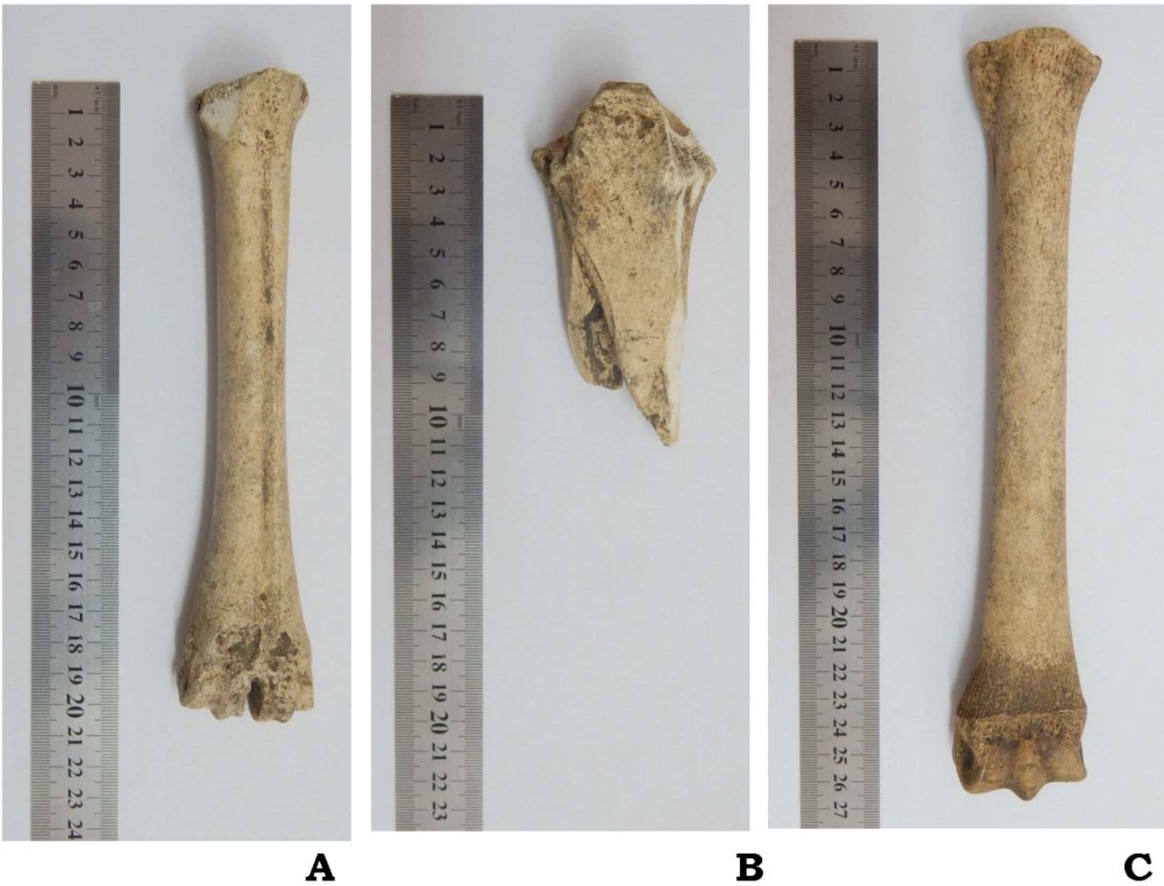

Fig. 1. Some bone finds from the Late Antiquity military settlement near Barkach village (Pleven Region): *Dama dama* – metacarpus dex. (A), ulna sin. dist. (B); *Equus africanus asinus* – metatarsus sin. juv. (C). Photo: Z. Boev.

Acknowledgements. The author thanks Dr. Katya Melamed for the handed animal subfossil material for examination.

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Scythris sinensis (Felder & Rogenhofer, 1875), a new species for Bulgaria and the Balkan Peninsula (Insecta: Lepidoptera: Scythrididae)

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Abstract. *Scythris sinensis* (Felder & Rogenhofer, 1875) is reported for the first time from Bulgaria. The species has never been recorded from the Balkan Peninsula and the present locality is one of the few southern points in its range in Europe.

Key words: faunistics, Microlepidoptera, new record.

Introduction

Similarly to many Microlepidoptera families, the fauna of the family Scythrididae is poorly known in Bulgaria. According to the Fauna Europaea web site (Bengtsson 2013), 33 species are listed for this country and 201 for Europe. Many species have been recorded from neighbouring countries, but not from Bulgaria, therefore many new species awaiting discovery may be expected. One of them is *Scythris sinensis* (Felder & Rogenhofer, 1875), recently photographed in the most north-western corner of Bulgaria, near Vidin. This part of the country is rarely explored by entomologists, both amateur and professional.

Material and Methods

A single moth was observed and photographed by the first author in a private yard in Vinarovo Village, Vidin Province, 44.0988° N, 22.8127° E, 147 m a.s.l., on 20 July 2019, with Canon EOS 1200D digital camera. No specimen was preserved.

Results and Discussion

The present record is the first known from Bulgaria and the Balkans as well. The moth was easily recognised by its characteristic habitus with basal and terminal yellow spots on the forewings (Fig. 1A). The yellow wing markings are characteristic for the second generation and absent in the first one, i.e. the observed specimen apparently belongs to the second generation (Takács & Szabóky 2009, Malkiewicz & Dobrzański 2011). Another species of the same genus, *S. flabella* (Mann, 1861), is somewhat similar to *S. sinensis* and also occurs in Bulgaria (Fig. 1B). It is distinguished by more basally displaced terminal yellow spots on the forewings.

Scythris sinensis is known from central Europe since 1970's (Sattler 1971) and then spread its range to many European countries. Now it is known from Eurasia and North America as well: Great Britain, Germany, Hungary, Estonia, Latvia, Lithuania, Belarus, Ukraine, Moldova, Russia (north-western and central part of the European Russia, Western

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Caucasus, Middle Volga region, Southern Ural, Southern Siberia, Transbaikal, Amur Region, Southern Primorie, Sakhalin), China, Japan, Korea, Taiwan; introduced in Eastern North America (Bidzilya et al. 2017 and references therein, Fazekas 2008). Lepiforum e.V. (2019) illustrates specimens from Slovakia, Austria and Italy as well. Though widely distributed, the species appear to be very local in Europe. This contradicts the wide distribution of its larval host plant, *Chenopodium album* L., which is a common ruderal weed in Europe.

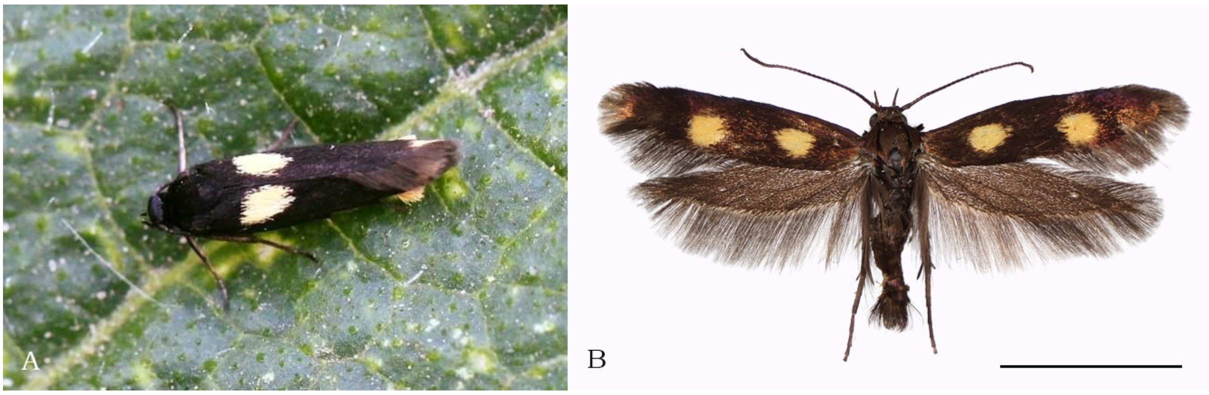

Fig. 1. Adults of *Scythris* spp. A - *S. sinensis*, Vinarovo, Vidin Province. B - *S. flabella*, Kresna Gorge, Blagoevgrad Province. Scale bar = 5 mm.

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First Psocoptera records from Northwest Bulgaria: a case study

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Abstract. The study was carried out on the following dates - 15.6.2019 and 17.6.2019 in NW Bulgaria (Danube River Valley) from three localities: villages of Vrav and Simeonovo, and town of Kozloduy. Five widely distributed species of Psocoptera were registered: *Valenzuela flavidus* (Stephens, 1836), *Graphopsocus cruciatus* (Linnaeus, 1768), *Lachesilla pedicularia* (Linnaeus, 1758), *Ectopsocus briggsi* McLachlan, 1899 and *Ectopsocus meridionalis* Ribaga, 1904.

Key words: Northwest Bulgaria, Insecta, new records

Introduction

The region of Northwest Bulgaria was never studied according to the Psocoptera fauna. In this short note we provide the first data for this area.

Material and Methods

The study was carried out on 15.6.2019 and 17.6.2019 in NW Bulgaria from three localities. The barkflies were collected by beating the vegetation above white plastic container and sweep netting of vegetation. Specimens were then stored in ethanol and after processing, deposited in the collection of the first author. After identification they were preserved in 96% ethanol. All specimens were determined by D. Georgiev. Species identifications are based on Lienhard (1998), taxonomical order and nomenclature follows Lienhard & Smithers (2002). As a supporting source, Saville (2008) was also used.

Results and Discussion

Five widely distributed species of Psocoptera were registered:

Caeciliusidae

***Valenzuela flavidus* (Stephens, 1836)**

Material examined: 17.6.2019, Danube River Valley, Near village of Simeonovo, mixed broad leaf forest, N43 50 24.2 E22 51 25.2, 37 m a.s.l., 1♀, collected by beating the vegetation, from *Clemathis vitalba.*

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Stenopsocidae

***Graphopsocus cruciatus* (Linnaeus, 1768)**

Material examined: 17.6.2019, Danube River Valley, Near village of Simeonovo, mixed broad leaf forest, N43 50 24.2 E22 51 25.2, 37 m a.s.l., 2♀, collected by beating the vegetation, from various bushes.

Lachesiliidae

***Lachesilla pedicularia* (Linnaeus, 1758)**

Material examined: 17.6.2019, Danube River Valley, town of Kozloduy town, Danube River Bank, a small park near the "Radetski" ship, N43 47 55 E23 40 37.1, 37 m a.s.l., 1 ♀, collected by beating the vegetation, from *Cupressus* sp.

Ectopsocidae

***Ectopsocus briggsi* McLachlan, 1899**

Material examined: 15.6.2019, Danube River Valley, a small park near the "Port of Vrav village", N44 11 39.0 E22 44 11.3, 46 m a.s.l., 2 ♀, collected by beating the vegetation, from *Cupressus* sp.

***Ectopsocus meridionalis* Ribaga, 1904**

Material examined: 17.6.2019, Danube River Valley, town of Kozloduy town, Danube River Bank, a small park near the "Radetski" ship, N43 47 55 E23 40 37.1, 37 m a.s.l., 2 ♀, collected by beating the vegetation, from *Cupressus* sp.

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Animal remains of the medieval settlement near Brankovtsi village (Vidin Region, NW Bulgaria)

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Abstract. A total of 9 taxa of 6 domestic forms and 3 wild species of animals have been identified, among them wild boar, red deer and roe deer. All finds are dated Middle Ages (10^{th} – 13^{th} century A. D.).

Key words: mammals, birds, Medieval fauna.

Introduction

Archaeozoological data from the settlements of Northwestern Bulgaria are extremely scant. We have only fragmentary information for 12 archaeological sites, most of them prehistoric: (1) Kozarnika (Suhi Pech) Cave near town of Belogradchik (Vidin Region), late Pleistocene (MNQ 18-26, end of Saalian, Eemian and Weischelian) (Fernandez 2009); (2) Mishin Kamik Cave near Gorna Luka village (Montana Region), late Pleistocene (135 000 – 85 000 BC) (Gurova *et al.* 2016, 2017, 2018); (3) Magurata Cave near Belogradchik (Vidin Region), late Pleistocene (39 280 BC) (Ivanova *et al.* 2016); (4) Ohoden mear Ohoden village (Vratsa Pegion), early Neolithic (N. Spassov – unpubl. data) (5) Gradeshnitsa - Malo Pole near Gradeshnitsa village (Vratsa Region), early Neolithic (Spassov *et al.* 2015); (6) Temnata Dupka Cave near Targovishte village (Vidin Region), Chalcolithic (Nikolov 1977, 1983); (7) Lepenitsa Cave near Belogradchik (Vidin Region); late Chalcolithic – early Bronze Age (3000-2000 BC) (Boev & Iliev 1991); (8) Baley near Baley village (Vidin Region); early Bronze Age (N. Spassov – unpubl. data); (9) Bagachina between Brankovtsi and Rasovo villages (Vidin Region), early Iron Age (1300-1000 BC) (Boev 1996); (10) Ratiaria near Archar village (Vidin Region), Roman period (2^{nd}-4^{th} century AD) (Iliev *et al.* 1993; Boev 1996); (11) Baba Vida fortress (Vidin), Middle Ages (10-13^{th} century AD) (Boev 1995, 1996); and (12) Smardan Dupka Cave near Krachimir village (Vidin Region), late Holocene (before 17th c. A.D.) (Boev 2013, Boev & Spassov, in press).

In the region of the site there was probably also a fortification during the Roman period - a castle or larger military site and settlement.

Material and Methods

Recently (October, 2019) a small sample (164 pieces) of animal finds have been handed for examination by the archaeologist Dr. Katya Melamed (National Archaeological Institute and Museum – BAS). They originated from the archaeological excavations (April-August 2019; 13 samples of 1.84-1.88 m depth), dated Middle Ages (10^{th} – 13^{th} century A. D.). Location of the site: 43°49'N, 22°34'E.

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Results and Discussion

A total of 9 taxa of 6 domestic forms and 3 wild species of animals have been identified (Table 1). All wild animals are highly valued hunting mammals even until recent time. One of them, the red deer now is both a hunting and protected species in Bulgaria. One of the four roe deer finds, a proximal (basal) part of an antler (Fig. 1-a, b), bear traces of processing. It was carved at the base (Fig. 1-b).

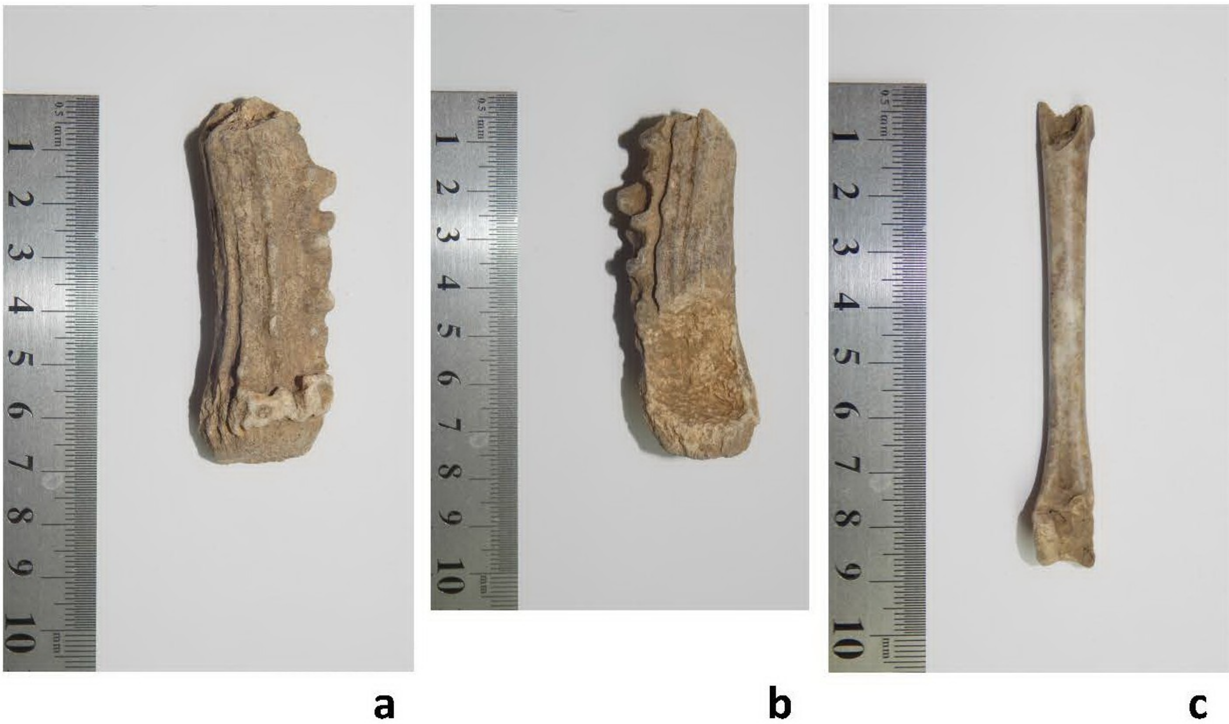

Fig. 1. Some animal remains from the medieval settlement near Brankovtsi village: *Capreolus capreolus*, proximal right antler (a, b), *Gallus gallus domestica*, tibiotarsus sin. (c). Photo: Z. Boev

Table 1. Composition and representation of the examined animal remains from the medieval settlement (10th – 13th century A. D.) near Brankovtsi village, Vidin Region, NW Bulgaria

No	Taxa	English Name	Number of bone finds
1.	*Bos taurus* Linnaeus, 1758	Domestic cattle	36
2.	*Cervus elaphus* Linnaeus, 1758	Red deer	2
3.	*Capreolus capreolus* (Linnaeus, 1758)	Roe deer	4
4.	*Sus scrofa scrofa* Linnaeus, 1758	Wild boar	6
5.	*Sus scrofa domestica* Linnaeus, 1758	Wild boar/Domestic pig	20
6.	*Capra hircus* (Linnaeus, 1758)	Domestic goat	3
7.	*Ovis aries* Linnaeus, 1758	Domestic sheep	8
	Ovis / Capra	Sheep/ Goat	3
8.	*Equus ferus caballus* Linnaeus, 1758	Domestic horse	8
9.	*Gallus g. domesticus* (Linnaeus, 1758)	Chicken	1
	Unidentifiable bone splinters		75
	Total		***164***

45.7 percent of the finds represent unidentifiable bone splinters. The majority of the remains belonged to domestic cattle and domestic pig. Only one bone of domestic birds has been found. It belonged to a medium/ small-sized domestic chicken (Fig. 1-c).

Wild (hunted) animals represent ca. 13 percent (i. e. one eighth) of identified animal remains. Hunting was only a supplementary mean of supply of meat resources for the inhabitants of the settlement. All hunted mammals are forest dwellers and indicate the former presence of woodland habitats in the surroundings of the medieval settlement.

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Earthworms (Annelida: Lumbricidae) biodiversity affected by pyrogenic carbon emissions at the "Maritsa-Iztok" basin (Bulgaria)

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Abstract. The paper provides the first exploration of earthworm populations from region of thermal power plant Maritsa East 2 (Bulgaria). During the investigation was observed low biodiversity, count and biomass of lumbricid earthworms in reclaimed soils from coal mining. Five earthworm species were registered from the studied area: *Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), *Aporrectodea trapezoides* (Dugès, 1828), *Aporrectodea jassyensis* (Michaelsen, 1891) and *Cernosvitovia rebeli* (Rosa, 1897). First record of endemic species *Cernosvitovia rebeli* (Rosa, 1897) from Thracian Lowland.

Key words: earthworms, soil reclamation, coal mining.

Introduction

Maritsa Iztok-2 is the largest thermal power plant in the Balkans. It is located both at the Thracian Lowland and the Sakar Mountain, South Bulgaria. It consists of three lignite-fired thermal power stations. The complex is located in a large lignite coal basin, which includes several mines. Open cast coal mining is inevitably accompanied by a significant interference in the environment. This requires the due restoration of the disrupted balance and the forming of a new environment consistent.

This part of Thracian Lowland, which surrounds Maritsa Iztok basin wasn't investigated for earthworm biodiversity yet. The first study of earthworm fauna in the Thracian Lowland from Bulgaria was published by Mihailova (1964; 1966). Since then her work was continued by Šapkarev (1986). Recently Valchovski & Szederjesi (2016), Valchovski & Misirlioglu (2017) added new records of lumbricid biodiversity of the region. According to these studies 17 lumbricid species were recorded from Thracian Lowland.

Material and Methods

The field investigations were carried out during the autumn of 2018. Earthworms were collected with digging and hand-sorting. All the specimens were killed in 96% ethanol, fixed in 4% formalin solution and in 96% ethanol, then transfered into 75% ethanol. The

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materialis deposited in the Institute of Soil Science, Agrotechnologies and Plant Protection "N. Poushkarov", Sofia, Bulgaria. Identification of species was done in accordance to Mršić (1991).

Collecting sites:
1. Scalitsa village, meadow, 152 m a.s.l., 42° 16'28N 26° 15' 10E.
2. Scalitsa, cultivated land, 159 m a.s.l., 42° 16' 24N 26° 15' 40E.
3. Kovachevo village, meadow, 128 m a.s.l., 42° 13' 41N 26° 04' 00E.
4. Maritsa Iztok basin, reclaimed land, 154 m a.s.l., 42° 14'53N 26° 07' 23E.°
5. Maritsa Iztok basin, reclaimed land, 173 m a.s.l., 42° 14'37N 26° 06' 42E.
6. Maritsa Iztok basin, reclaimed land, 137 m a.s.l., 42° 14'00N 26° 05' 34E.

Results and Discussion

During the investigation of studied area five earthworm species were collected altogether, belonging to two genera: *Aporrectodea caliginosa* (Savigny, 1826), *Aporrectodea rosea* (Savigny, 1826), *Aporrectodea trapezoides* (Dugès, 1828), *Aporrectodea jassyensis* (Michaelsen, 1891) and *Cernosvitovia rebeli* (Rosa, 1897). The collected specimens are presented in Tab. 1.

Tab. 1. Earthworm species, count and biomass from collected sites.

Locality	Species	Average count (n/m^2)	Average biomass (g/m^2)
1	*Aporrectodea rosea* (Savigny, 1826) *Aporrectodea trapezoides* (Dugès, 1828) *Aporrectodea jassyensis* (Michaelsen, 1891)	74	24
2	*Aporrectodea rosea* (Savigny, 1826) *Aporrectodea trapezoides* (Dugès, 1828)	42	17
3	*Cernosvitovia rebeli* (Rosa, 1897) *Aporrectodea caliginosa* (Savigny, 1826) *Aporrectodea rosea* (Savigny, 1826)	57	19
4	*Aporrectodea caliginosa* (Savigny, 1826) *Aporrectodea rosea* (Savigny, 1826)	13	7
5	*Aporrectodea caliginosa* (Savigny, 1826) *Aporrectodea rosea* (Savigny, 1826)	24	10
6	*Aporrectodea caliginosa* (Savigny, 1826) *Aporrectodea rosea* (Savigny, 1826)	15	8

During the investigation observed lower biodiversity in the reclaimed soils. Only the most adaptable peregrine species survives in that area: *Aporrectodea caliginosa* and *Aporrectodea rosea*. Biodiversity in control sample sites was twice bigger - four taxa: *Aporrectodea trapezoides*, *Aporrectodea jassyensis*, *Aporrectodea rosea* and *Cernosvitovia rebeli*. This is the first finding of endemic species *Cernosvitovia rebeli* in Thracian Lowland.

Abundance in control sample sites ranged from 42 to 74 exemplars per m^2 with a general average of 57.66 exemplars per m^2. In reclaimed soils near the thermal plant earthworm density ranged from 13 to 24 exemplars per m^2 with a general average of 17.33 exemplars per m^2. Biomass although was significantly higher in undisturbed plots (from 17 to 24 g/m^2), than that in reclaimed plots (from 7 to 10 g/m^2).

Conclusion

In reclaimed soils (Spolic Technosols), are registered only peregrine species. Near the thermal power plant survives only the most adaptive and ecologically plastic earthworm species. In genetically old soils (Pellic Vertisols), the Balkan endemic *Cernosvitovia rebeli* is found, which is the first record from Thracian Lowland.

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The effect of pingers on cetaceans bycatch and target catch in the turbot gillnets in Bulgarian Black Sea

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Abstract. The present study is the first experiment to determine whether acoustic deterrent devices (pingers) reduce cetaceans' bycatch in the turbot gillnets and catch rates of the target fish in the Bulgarian Black Sea territorial waters. During the study period 2017–2019, 12.4 km of turbot gillnets were included in the experiment. They were equipped with 10 kHz Porpoise Pingers "Future Oceans". Observations were carried out on regular bases on active (with pingers) and on control nets (without pingers). The results showed that the pingers used were very effective in reducing cetaceans bycatch in turbot gillnets without affecting the target catch.

Key words: cetaceans, pingers, gillnets.

Introduction

The cetaceans' mortality is a major conservation and welfare issue over the last two decades (Kuiken *et al.* 1994, Read *et al.* 2012, Desportes 2014). In Europe incidental catches are of concern for a number of cetacean species (ICES 2010), especially harbour porpoise which is particularly vulnerable to bycatch in gillnets (Birkun 2002, Read et al. 2012). This global problem also occurs in the Black Sea with the three species of cetaceans found there - Black Sea harbour porpoise (*Phocoena phocoena* ssp. *relicta* Abel, 1905), Black Sea common dolphin (*Delphinus delphis* ssp. *ponticus* Barabash, 1935), and Black Sea bottlenose dolphin (*Tursiops truncatus* ssp. *ponticus* Barabash-Nikiforov, 1935). Turbot gillnet is the most dangerous fishing gear for the dolphins and porpoises in the Black Sea (Radu et al. 2003).

One of the possible solutions to reduce cetacean' bycatch in gillnets is the use of pingers (Kraus *et al.* 1997, Gearin *et al.* 2000, Burke 2004, Gönener & Bilgin 2009). This study presents the first efforts to test pingers effectiveness on the turbot fishing in the Bulgarian Black Sea.

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Material and Methods

The experiment was carried out during the spring seasons (March - April) of 2017 to 2019 years in the Northern and Southern Bulgarian Black Sea Coast. A total of 12.4 km gillnets (124 pieces of nets) with 40 mm mesh sizes were included in the survey. Half of the nets – 6.2 km, were equipped with pingers (active) and the other half – 6.2 km – were without pingers (control). The distance between active and control nets was around 500 meters. The active nets were equipped with Porpoise Pingers ("Future Oceans"). The pingers frequency was 10 kHz and with source level of 132 dB. Pingers were installed according to producer's recommendations and in compliance with the specifics of the gillnets (from 100 to 150 m distance between pingers). Bycatch rates - number of specimens per km of net and number of specimens per soak time (days) were calculated.

Results and Discussion

During the study period a total 12.4 km gillnets were observed. The average soaking depth of the monitored gillnets was 40 meters and the total soak time for the study period was 89 days. A total of 14 *Ph. phocoena*, were bycaught in the control nets (8 in 2017, 3 in 2018 and 3 in 2019), and zero in the active nets. The harbour porpoise was the only cetacean species entangled and all bycaught individuals were dead. The bycatch rate of the harbour porpoise (individuals per km of net) for the control nets was 2.3 and zero for active nets for the whole study period. The bycatch rate in control nets was highest in 2017 (8 individuals) and similar in 2018 and 2019 – 1.2 and 1.11 respectively (Fig. 1).

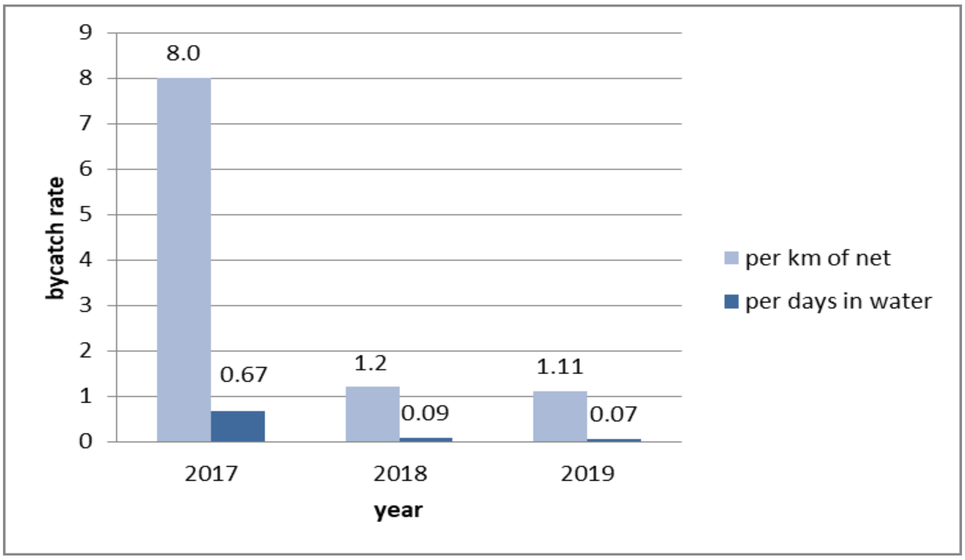

Fig. 1. *Ph.phocoena* bycatch rate (individuals per km of net and per soak time (days) by years in the control nets.

The catch rate (size) of the target species of turbot (*Scophthalmus maeoticus* Pallas, 1814) was 205 kg in the control nets and 479 kg in the active ones for the whole study period. In order to make turbot catch data comparable to cetaceans' bycatch data, kilograms were recalculated as individuals per km of net, as it is assumed that one turbot weighs an average of 3 kg (Fig. 2).

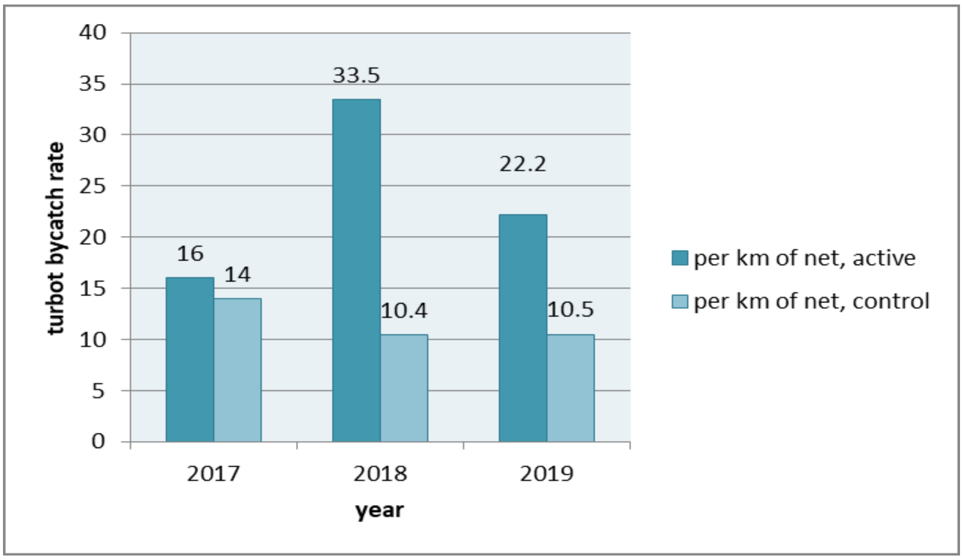

Fig. 2. *S. maeoticus* bycatch rate (individuals per km of net) by years in the active and control nets.

According to a number of studies, the harbour porpoise bycatch almost always represents the major part of the bycatch of cetaceans recorded in different places in the Black Sea (Birkun 2002). Most often, the annual part of *Ph. phocoena* make up 90-100%, while those of *D. delphis* and *T. truncatus* tend to zero (BLASDOL 1999). The results of the current study showed that 10 kHz Porpoise Pingers "Future Oceans" could have significant effect in reducing *Ph. phocoena* bycatch in turbot gillnets without affecting target fish – the turbot. Several other researches with the use of pingers also demonstrated that this method could be effective in reducing the bycatch of small cetaceans in different areas at least in a short term (Kraus *et al.* 1997, Burke 2004). One experiment in the Turkish part of the Black Sea demonstrated that the pingers caused a significant reduction of the bycatch of *Ph. phocoena*, in a turbot gillnets, using pingers with similar technical characteristics. This study showed that in the control nets bycatch rate is 46 times higher than in the active nets and the harbour porpoise bycatch rate is 0.01 for the active nets (Gönener & Bilgin 2009).

No addictive effect was observed throughout the three years study period, because there were no cetaceans' bycatch in the active nets. Probably, the fact that gillnets do not stay in the water for a long time is a reason for not observing this effect, therefore more long-term studies are needed to establish this. The results showed that pingers have no negative effect on the target catch. This result is consistent and with findings in other studies using pingers (Gearin *et al.* 2000, Gönener & Bilgin 2009). In 2017 the bycatch rate of harbour porpoise is very high in the control nets and there is a very small difference in the catch rate of turbot in active and passive nets comparing to 2018 and 2019 but further research is need to find out if this a random event or there is some relation.

The present study shows 100 % success rate of pingers as a means to reduce the incidental catch of cetaceans in the turbot gillnets in the Bulgarian part of Black Sea.

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New records of *Trox* Fabricius, 1775 species (Scarabaeoidea: Trogidae) from Bulgaria

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Abstract. New data on the distribution of four species of genus *Trox* Fabricius, 1775 (Scarabaeoidea: Trogidae): *Trox niger* P. Rossi, 1792, *T. perrisii* Fairmaire, 1868, *T. sabulosus sabulosus* (Linnaeus, 1758) and *T. scaber* (Linnaeus, 1767) are reported from Bulgaria.

Key words: Trogidae, *Trox*, Bulgaria.

Introduction

Five species of genus *Trox* Fabricius, 1775 are known for Bulgaria: *Trox niger* P. Rossi, 1792, *T. perrisii* Fairmaire, 1868, *T. sabulosus sabulosus* (Linnaeus, 1758), *T. scaber* (Linnaeus, 1767) and *T. sordidatus* Balthasar, 1936 (Guéorguiev & Bunalski 2004). The species *T. eversmannii* Krynicki, 1832 is reported from Sofia (Joakimov 1904), but according to Guéorguiev & Bunalski (2004) this record is doubtful and needs further confirmation. Findings of *Trox* species in Bulgaria are scarce (Guéorguiev & Bunalski 2004) and rarely published. In the present work, we provide new data on the distribution of four species of the genus in the country. The material was collected in the period 2004 – 2019 in different regions of Bulgaria, mainly by light trapping. The examined specimens are preserved in the Zoological Collection of Sofia University, Faculty of Biology (BFUS) and in the collection of the first author (YP). Abbreviations for collectors names: BZ: B. Zlatkov; DC: Dragan Chobanov; DG: D. Gradinarov; DK: D. Kaynarov; EC: E. Chehlarov; GH: G. Hristov; OK: O. Karsholt; OS: O. Sivilov; PM: P. Mitov; RK: R. Kostova; SB: S. Beshkov; YP: Y. Petrova.

Results and Discussion

***Trox niger* P. Rossi, 1792** (Figs. 1, 2)

Material examined: Strandzha Mts.: SW Slivarovo Vill., 41°57.63'N 27°39.58'E, 220 m, riverine forest, near rocks, 09.vi.-02.vii.2009, 1 ♀, pitfall traps, PM, RK & OS leg. (YP); Pirin Mts.: NE Kalimantsi Vill., 41°27.974'N 23°29.942'E, 340 m, 24.v.2010, 1 ♀, at light, OS, BZ & GH leg. (BFUS); the same data, but 41°27.904'N 23°29.476'E, 327 m, 31.vii.2016, 2 ♂♂, 1 ♀, at light, YP & BZ leg. (BFUS); the same data, but 41°27.995'N 23°30.643'E, 396 m, 03.ix.2016, 1 ♂, at light, YP & DG leg. (BFUS); the same data, but 41°27.902'N 23°29.325'E, 315 m, 28.vi.2019, 1 ♂, at light, OS & BZ leg. (BFUS); E Ilindentsi Vill., 41°39.009'N 23°15.039'E, 455 m, 13.vi.2012, 1 ♂, at night, OS & BZ leg. (BFUS); NE Ilindentsi Vill., 41°39.317'N 23°14.717'E, 500 m, 02.v.2014, 1 ♀, at night, DG leg. (BFUS); NE Kalimantsi Vill., bank of Kalimanska Reka Riv., 41°27.994'N 23°30.964'E, 382 m, 01.viii.2016, 1 ♀, at light, YP & BZ leg. (BFUS); SW Lilyanovo Vill., 41°36.748'N 23°18.715'E,

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470 m, 27.vi.2019, 1 ♀, at light, OS & BZ leg. (BFUS); Black Sea Coast: NE Shkorpilovtsi Vill., 42°59.522'N 27°53.383'E, 1 m, 26.vii.2011, 1 ♂, at light, OS & BZ leg. (YP); Maleshevska Planina Mts.: NW Lebnitsa Vill., 41°31.685'N 23°12.684'E, 185 m, 15.vi.2012, 1 ♂, at light, OS & BZ leg. (BFUS); the same data, but 41°31.634'N 23°12.701'E, 160 m, 02.v.2013, 1 ♂, 1 ♀, at light, OS & BZ leg. (BFUS); NW Mikrevo Vill., 41°38.423'N 23°10.351'E, 163 m, 19.vi.2015, 1 ♀, at light, YP & EC leg. (BFUS); SE Kamenitsa Vill., 41°38.5'N 23°10.0'E, 230 m, 30.vii.2016, 2 ♂♂, 3 ♀♀, at light, YP & BZ leg. (BFUS); the same data, but 14.iv.2018, 2 ♂♂, 1 ♀ at light, BZ & DC leg. (YP); Eastern Rhodopes Mts.: N Trigrad Vill., 41°36.372'N 24°22.800'E, 1300 m, 04.viii.2013, 1 ♂, at light, BZ & OK leg. (YP); Slavyanka Mts.: SE Petrovo Vill., near Izvora Chalet, 41°24.677'N 23°33.582'E, 735 m, 14.vi.2014, 1 ♀, at light, OS & BZ leg. (BFUS); Zemen Gorge: SW Zemen town, 42°28.263'N 22°43.881'E, 593 m, rocky slope, 05.vii.2015, 1 ♀, at light, YP & DG leg. (BFUS); Sveti Iliyski Vazvishenia Hills: SW Boyadzhik Vill., 42°22.738'N 26°15.620'E, 240 m, 17.iv.2016, 8 ♂♂, 4 ♀♀, at light, OS & BZ leg. (YP); E Stara Planina Mts.: Grebenets, SW Sedlarovo Vill., 42°40.617'N 26°33.350'E, 570 m, 31.v.2017, 2 ♂♂, at light, BZ & DK leg. (YP); Aytoska Planina, NW Aytos, 42°43.217'N 27°14.283'E, 265 m, 01.vi.2017, 1 ♂, at light, BZ & DK leg. (YP).

Reported from Sofia (Nedelkov 1905, Guéorguiev & Bunalski 2004), Black Sea Coast (Pittino 1991, Guéorguiev & Bunalski 2004), Eastern Stara Planina Mts. and Pirin Mts. (Pittino 1991), Kraishte Region, Lozenska Planina Mts., Maleshevska Planina Mts., Sandanski-Petrich Valley, Vrachanska Planina Mts. and Western Rhodopes (Guéorguiev & Bunalski 2004). According to Guéorguiev & Bunalski (2004), *T. niger* is the most common species of the genus in Bulgaria, which is also consistent with our data.

Trox perrisii **Fairmaire, 1868** (Figs. 3, 4)

Material examined: Vlahina Mts.: NW Zheleznitsa Vill., 41°55.750'N 23°05.350'E, 530 m, 11.vi.2012, 1 ♀, at light, OS & BZ leg. (BFUS); Pirin Mts.: E Ilindentsi Vill., 41°39.174'N 23°14.738'E, 540 m, 13.vi.2012, 1 ♀, at light, OS & BZ leg. (BFUS); Maleshevska Planina Mts.: NW Lebnitsa Vill., 41°31.634'N 23°12.701'E, 160 m, 02.v.2013, 1 ♂, at light, OS & BZ leg. (BFUS); Slavyanka Mts.: SE Paril Vill., 41°25.960'N 23°42.007'E, 755 m, 17.vi.2013, 1 ♂, at light, OS, BZ & SB leg. (BFUS); Sandanski-Petrich Valley: Rupite Place, SE Ribnik Vill., right bank of Struma Riv., 41°28.039'N 23°16.153'E, 95 m, forest with *Populus* sp., riverine sands, 11.viii.2015, 1 ♂, at light, YP & BZ leg. (BFUS); E Novo Hodzhovo Vill., 41°24.432'N 23°24.460'E, 115 m, near marshes, 30.vi.2019, 1 ♀, at light, OS & BZ leg. (YP).

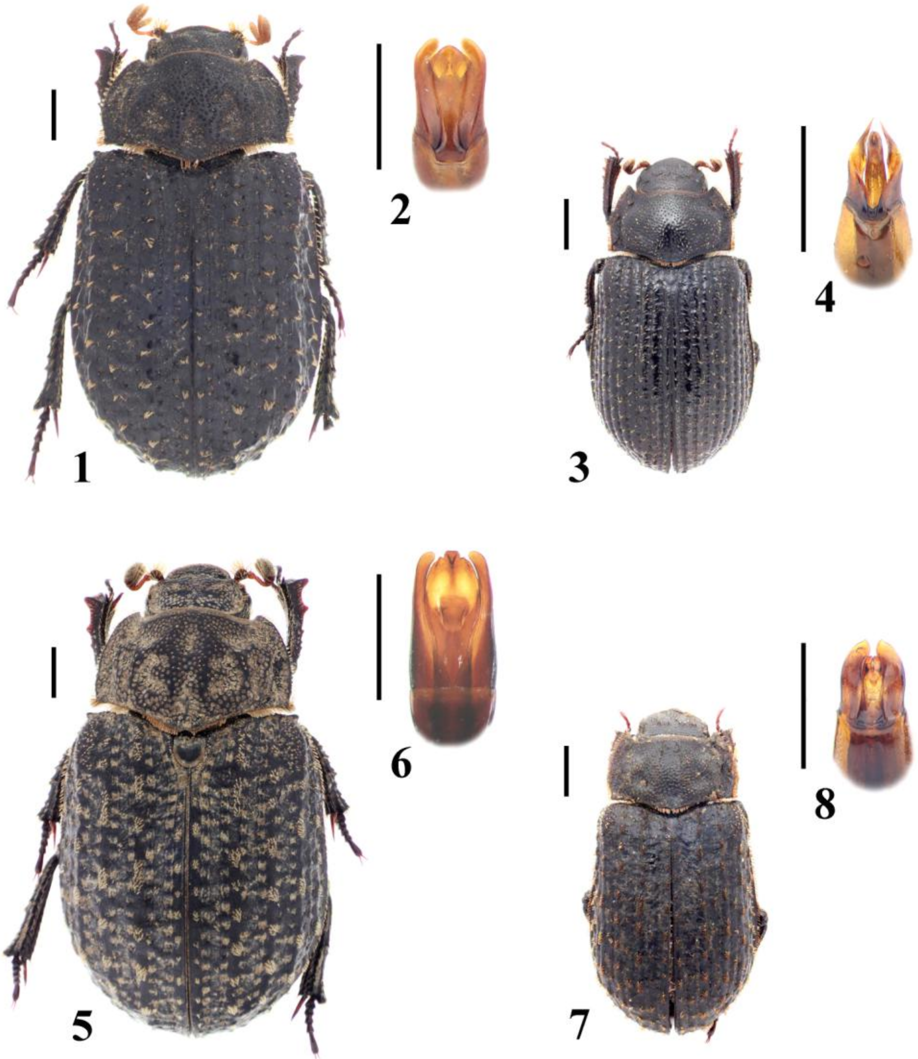
In Bulgaria, the species is reported only from Pirin Mts., Melnik Region (Bunalski 2000) and from Eastern Stara Planina Mts., Natura Site "Ecokoridor Kamchiya-Emine" (Chehlarov *et al.* 2016). The species should not be considered rare as it appears to be widely distributed at least in the SW part of the country.

Trox sabulosus sabulosus **(Linnaeus, 1758)** (Figs. 5, 6)

Material examined: Strandzha Mts.: SW Slivarovo Vill., 41°57.630'N 27°39.580'E, 220 m, riverine forest, near rocks, 15.iv.-08.v.2009, 1 ♂, pitfall traps, PM, RK & OS leg. (YP). Reported from Plovdiv (Angelov 1960), Sofia, Rila Mts. and Strandzha Mts. (Guéorguiev & Bunalski 2004). This species has rather limited distribution in Bulgaria.

Trox scaber **(Linnaeus, 1767)** (Figs. 7, 8)

Material examined: Sofia-city, Lyulin district, 42°43.578'N 23°14.964'E, 565 m, 11.vi.2004, 1 ♀, YP leg. (BFUS); Strandzha Mts.: W Malko Tarnovo, Propada Place, 41°58.827'N 27°29.428'E, 400 m, beech forest, 01.-27.v.2010, 1 ♀, pitfall traps, PM, RK & OS leg. (YP); Pirin Mts.: E Ilindentsi Vill., 41°39.174'N 23°14.738'E, 540 m, 13.vi.2012, 1 ♂,



Figs. 1–8. 1 – *Trox niger*, Sveti Iliyski Vazvishenia Hills, 17.iv.2016, male; 2 – aedeagus of the same specimen; 3 – *T. perrisii*, Lebnitsa Vill., 02.v.2013, male; 4 – aedeagus of the same specimen; 5 – *T. sabulosus sabulosus*, Strandzha Mts., 15.iv. – 08.v.2009, male; 6 – aedeagus of the same specimen; 7 – *T. scaber*, Lebnitsa Vill., 02.v.2013, male; 8 – aedeagus of the same specimen. Scale bars: 1 mm.

at light, OS & BZ leg. (BFUS); NE Kalimantsi Vill., 41°27.917'N 23°29.519'E, 323 m, 31.vii.2016, 1 ♀, at light, YP & BZ leg. (BFUS); the same data, but 41°27.902'N 23°29.325'E, 315 m, 28.vi.2019, 1 ♂, at light, OS & BZ leg. (BFUS); Kresna Gorge, 41°45.635'N 23°09.275'E, 205 m, 01.vii.2019, 1 ♂, at light, OS & BZ leg. (YP); Maleshevska Planina Mts.: NW Lebnitsa Vill., 41°31.634'N 23°12.701'E, 160 m, 02.v.2013, 1 ♂, at light, OS & BZ leg. (BFUS); Slavyanka Mts.: SE Paril Vill., 41°25.960'N 23°42.007'E, 755 m, 17.vi.2013, 1 ♀, at light, OS, BZ & SB leg. (BFUS); Sandanski-Petrich Valley: Kozhuh Hill., 41°27.75'N 23°15.67'E, 108 m, 20.vi.2013, 1 ♀, at light, OS & BZ leg. (BFUS); S Dolna Gradeshnitsa Vill., 41°39.91'N 23°11.49'E, 185 m, 11.vi.2014, 1 ♀, at light, OS & BZ leg. (BFUS); Sveti Iliyski Vazvishenia Hills: SW Boyadzhik Vill., 42°22.738'N 26°15.620'E, 240 m, 17.iv.2016, 2 ♂♂, at light, OS & BZ leg. (YP); E Stara Planina Mts., Grebenets, SW Sedlarovo Vill., 42°40.617'N 26°33.350'E, 570 m, 31.v.2017, 1 ♂, at light, BZ & DK leg. (YP).

Reported from Black Sea Coast (Muche 1964, Bunalski 2000), Eastern Rhodopes Mts. (Bunalski 2000), Sofia and Central Predbalkan (Guéorguiev & Bunalski 2004).

Cosmopolite species (Pittino & Bezděk 2016), in Bulgaria not recorded only from the north parts of the country.

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Dyschiriodes (*Eudyschirius*) *gracilis* (O. Heer, 1837) – new record for the Bulgarian ground beetle fauna (Coleoptera: Carabidae: Scaritinae)

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Abstract. This is the first record of *Dyschiriodes* (*Eudyschirius*) *gracilis gracilis* (O. Heer, 1837) (Coleoptera: Carabidae: Scaritinae) from Bulgaria. It was collected in the Srebarna Reserve in NE Bulgaria via light attraction. Considering its current distributional range, we may suggest that *D. gracilis gracilis* is to be found in Croatia and Romania too.

Key words: Dyschiriini, new record, Srebarna Reserve.

Introduction

Tribe Dyschiriini (Coleoptera: Carabidae: Scaritinae) includes sensitive indicators of the natural river, lake or saltmarsh banks, inhabiting the interstitial space close to the shoreline. All species are hygrophilous and most of them are macropterous (Fedorenko 1993, 1996). They are small digging geobionts (Sharova 1981). In Bulgaria the tribe Dyschiriini is represented by the genera *Dyschirius* Bonelli, 1810 and *Dyschiriodes* Jeannel, 1941. Unlike Balkenohl (2003, 2017), Fedorenko (1996) considers *Dyschiriodes* as an independent genus, not as a subgenus of *Dyschirius*, and we accept his classification.

Wetlands and littoral regions and locations are generally very attractive and common habitats for many ground beetles. According to the literature data, nine species of the tribe Dyschiriini are present in the territory of the Lake Srebarna Nature Reserve (Kodzhabashev & Penev 1998, 2006, Jocque *et al.* 2016).

Dyschiriodes (*Eudyschirius*) *gracilis* (O. Heer, 1837) is a trans-Palaearctic species, widespread from NE Spain to Japan and E China. The subspecies *D. gracilis gracilis* occurs in the entire species range except for its westernmost parts. The other subspecies, *D. gracilis ibericus* Fedorenko, 1996 is present only in NE Spain (Fedorenko 1996, Balkenohl 2017).

Dyschiriodes gracilis is a riparian species, found mainly along rivers in moist loams (Fedorenko 1996). It seems abundant in riparian habitats in NE Italy, where it was captured by light (Allegro & Bulirsch 2012).

The goal of this study is to add a new species to the Carabidae list of Srebarna and to present the first country records of *Dyschiriodes gracilis* in Bulgaria.

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Material and Methods

The specimens were collected in the last week of July 1989 by the second author via light trapping, at the northernmost edge of the village of Srebarna, in the Srebarna wetland Nature Reserve in Northeastern Bulgaria (Figure 1). The shore of the lake is densely vegetated with reed and other hydro- and hygrophytes. A 500W lamp pointing at N/NE direction was mounted on a white wall of a building located on a hill, bordering with the shore of the lake. In the vicinity of the lake there were also many other light sources, which, however, were lying lower, so their light was “blurring”. The collection was at waning moon, from dusk to about 12 p. m. Beetles were killed with ethyl acetate and fixed in a mixture of ethanol, ethyl acetate and glycerol (Hood 1953).

Specimens were identified in 1994 by Dr. Dmitri Fedorenko (A. N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia) and re-examined and confirmed in 2019 by Assoc. Prof. Dr. Borislav Guéorguiev (National Museum of Natural History, Sofia, Bulgaria) and the senior author. The identified specimens are pinned and deposited in the Institute of Biodiversity and Ecosystem Research (BAS, Sofia).

Results and Discussion

The present study gives information about one new species record for the country. Two specimens (Figure 2) of *Dyschiriodes gracilis* were collected and photographed for the first time in Bulgaria. The species was represented by the subsp. *gracilis*.

Family: Carabidae Latreille, 1802

Subfamily: Scaritinae Bonelli, 1810

Tribe: Dyschiriini W. Kolbe, 1880

Species: *Dyschiriodes* (*Eudyschirius*) *gracilis* (O. Heer, 1837);

Synonyms: *Clivina gracilis* Heer, 1837; *Dyschiriodes ibericus* Fedorenko, 1996; *D. lafertei* (Putzeys, 1846); *Dyschirius ibericus* (Fedorenko, 1996); *D. lafertei* Putzeys, 1846; *D. pekinensis* Kult, 1949; *D. tokyoensis* Nakane, 1953.

New data: NE Bulgaria, Srebarna Reserve, Srebarna vill., 44°06'09.36''N 27°03'43.08''E, 53 m a.s.l., 10.VII.1989, at light, 2 ex., leg. N. Kodzhabashev.

So far 30 species from the tribe Dyschiriini are known from Bulgaria (Teofilova & Guéorguiev, in prep.). Our new finding makes that number 31, with 5 *Dyschirius* and 26 *Dyschiriodes* species. According to the last edition of the Catalogue of the Palaearctic Carabidae (Balkenohl 2017) *Dyschiriodes gracilis gracilis* occurs in Austria, Bosnia Herzegovina, Czech Republic, France, Germany, Italy, Moldova, Montenegro, Poland, Serbia, Slovakia, Slovenia, Spain, South European Russia, Switzerland, Turkmenistan, East Siberia, Far East of Russia, Kazakhstan, Japan and China (Hebei, Jiangsu, Xinjiang). Recently it was also reported from Greece (Arndt et al. 2011), Ukraine (Aleksandrowicz *et al.* 2016) and Albania (Paill et al. 2018). Considering its current distributional range, we may suggest that *D. gracilis gracilis* is to be found in Croatia and Romania too (Fedorenko 1996, Balkenohl 2017).

It seems that *D. gracilis gracilis* inhabits different types of riverine biotopes. In Albania it was found on regularly flooded niveaus (coarse-grained (mostly gravel) and fine-grained (mostly silt) sediment bars with and without vegetation); on elevated niveaus (species-poor grassland; habitat within a degradation stage by regular burning and grazing) and in special habitat within the active floodplain (steep erosive embankment with unvegetated, moist, loamy ground) (Paill et al. 2018). In Central-Eastern Poland it was found at clay-pits, loessic xerothermic slopes and loessic stream banks, with association with the staphylinids *Bledius atricapillus* (Germar, 1825), *B. gallicus* (Gravenhorst, 1806), *B. longulus* Erichson, 1839, *B. nanus* Erichson, 1840, *B. tricornis* (Herbst, 1784), apparently used for food (Sienkiewicz & Staniec 2006). The species was also found in suburban areas in Poland (Czechowski 1981).

Almost all Dyschiriini are actively flying, due to their biotope preferences and, in particular, the short-term existence of the near-water habitats. Changes in the hydrological regime of coastal soils, caused by periodic drying or flooding, cause many species to leave their shelters and migrate in search of suitable new habitats (Fedorenko 1993). This explains their relatively high resettlement abilities.

Most probably our specimens were attracted in the vicinity of the village of Srebarna by the light from the lamp. We suggest they flew from the neighbouring lake shore, were this species usually occurs. Light attraction is a common method in entomologic research, but it is rarely used for ground beetle surveys, though many of these beetles are actively flying at light, and the method was proven to be suitable and valuable for investigations of the carabid diversity, especially that of the small, flying, endogeic and other “trap-shy” species (Jocque *et al.* 2016), such as *Dyschiriodes gracilis*.

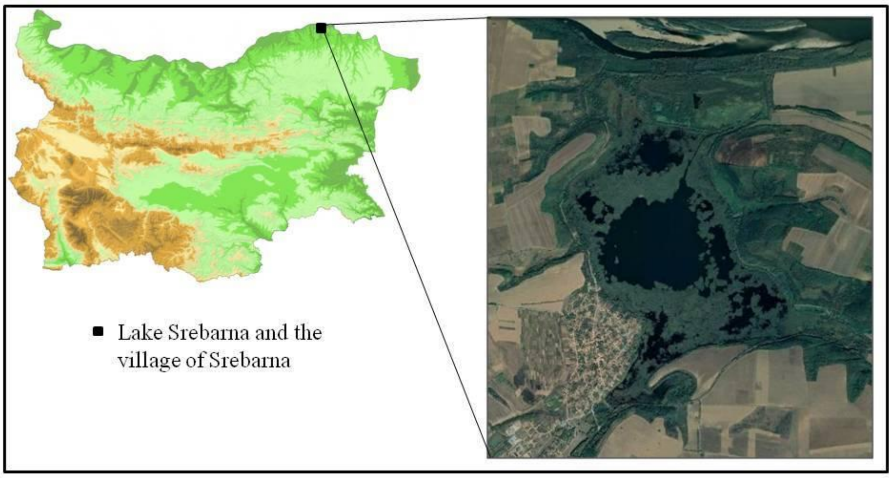

Fig. 1. Locality of the Srebarna wetland Nature Reserve.

Fig. 2. Habitus of the two specimens of *D. gracilis gracilis* from Srebarna.
Scale bar 2 mm.

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