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Ground beetles (Coleoptera: Carabidae) from the region of Cape Emine (Central Bulgarian Black sea coast). Part I. Taxonomic and zoogeographic structure, life forms, habitat and humidity preferences

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Abstract. For the first time the carabid fauna of the area of Cape Emine (the middle of the Bulgarian Black sea coast) was studied. Over the period 2010 – 2012 adult carabid beetles were collected. Investigations were performed at 13 sampling sites and pitfall traps were used. During the study a total of 12618 specimens were captured. They belonged to 134 species, classified into 46 genera, 18 tribes, and 3 subfamilies. *Dyschirius rufipes* Dejean, 1825 and *Laemostenus janthinus* (Duftschmid, 1812) were reported as new species for the carabid fauna of Bulgaria. Three species were new for the fauna of the Bulgarian Black Sea coast. Fifty-four species were defined as new for the area of Cape Emine. Two endemics were found: *Pterostichus merkli* Frivaldszky, 1879 (Bulgarian endemic) and *Cychrus semigranosus balcanicus* Hopffgarten, 1881 (Balkan endemic). Species of ground beetles were characterized and classified according to their zoogeographical belonging, degree of endemism and rarity, habitat and humidity preferences; the life forms they refer to were determined.

Keywords: Carabidae, ground beetles, Bulgarian Black Sea coast, Cape Emine, zoogeography, habitats, life forms.

Introduction

The territory of the zoogeographical region of Bulgarian Black Sea coast (Gruev & Kuzmanov 1994) is one of the most interesting, but not sufficiently explored areas in relation to the carabid fauna. Of particular scientific interest is Cape Emine (the middle of the Bulgarian Black sea coast).

Geo-strategic location of this part of the coast, specific environmental conditions, and the presence of various habitats has led to the creation of unique biocoenoses and original fauna. The climate specificity, coupled with a variety of plant formations have predetermined the forming of a wide range of carabid species and their communities

(Teofilova *et al.* 2012c).

The area of Cape Emine is too outstanding regarding its main priorities. Some clashes between developers and environmentalists have occurred there and are still continuing. Natural resources are the reason for investors' appetites, associated with construction and drastic habitat and landscape changes, directly affecting the spatial distribution of the carabid communities (Pena *et al.* 2003, etc.). In addition, in the selected area comprehensive biological and ecological analyses are missing.

The study aimed at clarifying the species composition and analyzing of the ecological and zoogeographical structure of the carabid fauna and subsequent assessment of the anthropogenic impact in the area.

Material and Methods

In connection with the participation in a project for biological monitoring studies in the area, a series of observations and samplings were carried out in 2010 – 2012. Ground beetles were collected with terrestrial pitfall traps (Barber 1931; Hertz 1927; Dahl 1896). The traps were made of plastic bottles, buried at the level of the substrate. As fixation fluid a 4% solution of formaldehyde was used. The investigations were performed at 13 different types of sampling sites (pine plantation, oak forests, steppe-like and riparian habitats, wheat fields and ecotones).

Specimens were identified according to: Kryzhanovskij (unpublished data), Arndt *et al.* (2011), Lindroth (1974), Hürka (1996), Reitter (2006), Trautner & Geigenmüller (1987). Systematics follows Kryzhanovskij *et al.* (1995).

According to their zoogeographical belonging species were separated in zoogeographical categories and faunal types according to Vigna Taglianti *et al.* (1999) with some changes (Kodzhabashev & Penev 2006). Categorization of the species in respect of their life forms was made according to the classification of Sharova (1981). The most favourable and typical habitats were pointed and species were divided into groups according to their habitat and humidity preferences.

Results

Diversity of species and taxonomic structure

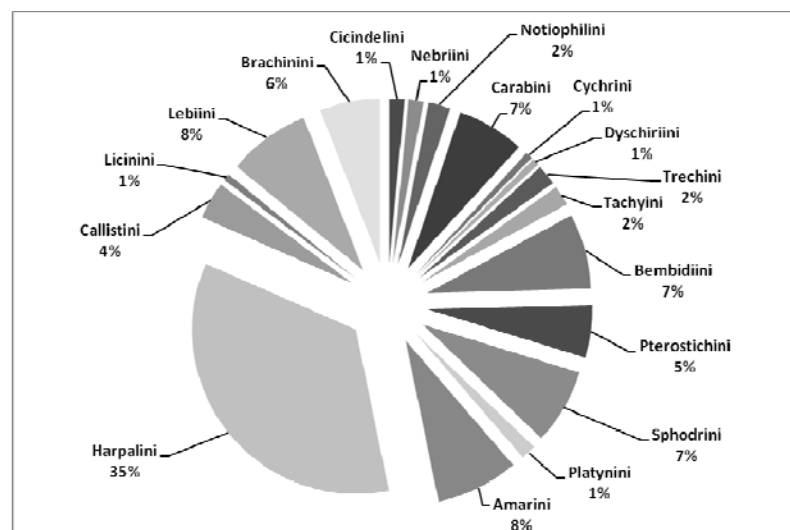
An inventory of the carabid fauna is made for the region of Cape Emine, where similar studies have not been conducted so far. During the study, total of 12618 specimens were captured. Beetles belonged to 134 species, classified into 46 genera, 18 tribes, and 3 subfamilies (Cicindelinae, Carabinae, Brachininae) (see **Appendix**). This figure represents respectively 18% of all established for Bulgarian carabid fauna species, 37% of the genera and 49% of the tribes (Guéorguiev & Guéorguiev 1995). Regarding the known Carabidae taxa from the Bulgarian Black Sea coast (Teofilova *et al.* 2012b), the ones found during the investigation represented respectively 29% of the species, 48% of the genera and 55% of the tribes.

Generally, the least-represented is the subfamily Cicindelinae – with only 2 species from 1 genus and less than 1% of the number of identified specimens. Subfamily Brachininae is represented also with only 1 genus, but there are 8 species and 14.5% of the captured specimens referring to it. Most numerous is the subfamily Carabinae – 16 tribes, 44 genera, 124 species and 85.5% of the specimens (**Table 1**).

Taxonomic structure showed the highest proportion attributable to the representatives of tribe Harpalini (**Fig. 1**). Similar results were obtained by Kodzhabashev & Penev (2006) and Popov & Krusteva (1999) and this group is also characteristic of the Bulgarian Black Sea coast as a zoogeographical region (Teofilova *et al.* 2012b; Teofilova *et al.* 2011).

Table 1. Taxonomic structure of the established carabid complex.

Subfamily	Tribe	Number of genera	%	Number of species	%	Number of specimens	%
Cicindelinae	Cicindelini	1	2,2	2	1,5	8	0,06
Carabinae	Nebriini	2	4,4	2	1,5	1939	15,4
	Notiophilini	1	2,2	3	2,2	83	0,7
	Carabini	2	4,4	9	6,7	1967	15,6
	Cychrini	1	2,2	1	0,7	11	0,09
	Dyschiriini	1	2,2	1	0,7	1	0,01
	Trechini	1	2,2	2	1,5	320	2,5
	Tachyini	2	4,4	2	1,5	3	0,02
	Bembidiini	2	4,4	10	7,5	122	1,0
	Pterostichini	5	10,9	7	5,2	958	7,6
	Sphodrini	2	4,4	10	7,5	1044	8,3
	Platynini	2	4,4	2	1,5	546	4,3
	Amarini	2	4,4	11	8,2	110	0,9
	Harpalini	12	26,0	47	35,1	1468	11,6
	Callistini	2	4,4	5	3,7	1944	15,4
	Licinini	1	2,2	1	0,7	1	0,01
Lebiini	6	13,0	11	8,2	260	2,1	
Brachininae	Brachinini	1	2,2	8	6,0	1832	14,5
	Total:	46	100%	134	100%	12618	100%

**Fig. 1.** Proportions of the species among the tribes.

Based on the number of specimens, the most numerous tribes were Carabini, Callistini and Nebriini (**Fig. 2**).

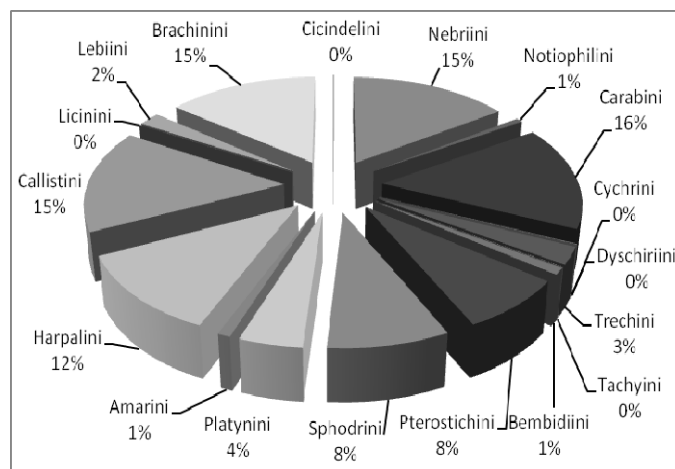


Fig. 2. Proportions of the species among the tribes.

In the open habitats Harpalini and Callistini predominated, and to a lesser extent – Sphodrini and Brachinini. In the transitional habitats, with increasing humidity, we have observed a greater proportion of the tribe Carabini. In less exposed areas a higher proportion of the tribe Pterostichini is present. In the forest habitats normally more representatives of the tribes Pterostichini and Carabini are present. High humidity along the banks of the rivers appeared to be the defining factor for the distribution of the tribes Platynini and Nebriini.

Results of this study showed that the most species rich genus is *Harpalus* Latreille, 1802 (21 species), followed by the genera *Ophonus* Dejean, 1821 (12 species), *Amara* Bonelli, 1810 (10 species), *Bembidion* Latreille, 1802 (9 species), *Brachinus* F. Weber, 1801 (8 species), *Carabus* Linnaeus, 1758 and *Calathus* Bonelli, 1810 (7 species each).

The most numerous genera in regard to the collected specimens are: *Chlaenius* Bonelli, 1810 (1941 indivs.), *Nebria* Latreille, 1825 (1890 indivs.), *Brachinus* (1832 indivs.) and *Carabus* (1776 indivs.), *Calathus* (955 indivs.), *Pterostichus* Bonelli, 1810 and *Harpalus* (885 indivs. each) and *Anchomenus* Bonelli, 1810 (399 indivs.).

The most abundant species are: *Chlaenius nitidulus* (Schrank, 1781) (1927 indivs.), *Nebria brevicollis* (Fabricius, 1792) (1890 indivs.), *Carabus coriaceus* Linnaeus, 1758 (1240 indivs.), *Brachinus crepitans* (Linnaeus, 1758) (1213 indivs.), *Pterostichus melas* (Creutzer, 1799) (880 indivs.), *Calathus fuscipes* (Goeze, 1777) (623 indivs.), *Harpalus dimidiatus* (Rossi, 1790) (555 indivs.), *Brachinus psophia* Serville, 1821 (458 indivs.), *Anchomenus dorsalis* (Pontoppidan, 1763) (399 indivs.), *Trechus quadristriatus* (Schrank, 1781) (319 indivs.), *Carabus convexus* Fabricius, 1775 (288 indivs.).

New, endemic and rare species

During the investigation some species with conservation and biogeographical significance were found, including endemic, new, rare or species with limited distribution:

- ✓ *Pterostichus (Pterostichus) merkli* – Bulgarian endemic;
- ✓ *Cychrus semigranosus balcanicus* – Balkan endemic.

Two species are new to the carabid fauna of Bulgaria:

- ✓ *Dyschirius (Dyschiriodes) rufipes*;
- ✓ *Laemostenus (Laemostenus) janthinus*.

Three species are new to the fauna of the Bulgarian Black Sea coast:

- ✓ *Amara (Amara) communis* Panzer, 1797;

- ✓ *Zabrus (Pelor) graecus* Dejean, 1828;
- ✓ *Harpalus (Harpalus) quadripunctatus* Dejean, 1829.

Fifty-four species are established as new for the area of Cape Emine due to the lack of studies in this region. Thus, the number of the species, which are new to the region, increases to fifty-nine.

Some of the species (e. g. *Carabus scabriusculus* Olivier, 1795) are rare species across their entire area, and others (e. g. *Carabus ullrichi* Germar, 1824, *Carabus intricatus* Linnaeus, 1761, *Stomis pumicatus* (Panzer, 1796) and *Leistus rufomarginatus* (Duftschmid, 1812) have become rare under the action of anthropogenic pressures and changes in their primary habitats. *Carabus cancellatus* Illiger, 1798, *Calosoma inquisitor* (Linnaeus, 1758) and *Calosoma sycophanta* (Linnaeus, 1758) are usually highly sensitive to chemical agents and any intensification of the agriculture leads to severe reductions in their range and numbers (Huusela-Veistola 2000). Typical stenotopic species are some intrazonal psammobionts (*Harpalus melancholicus* Dejean, 1829), halobionts (*Bembidion inoptatum* Schaum, 1857, *Microlestes fulvibasis* Reitter, 1900) and inhabitants of coastal habitats (*Dyschirius rufipes*, *Chlaenius festinus* (Panzer, 1796), *Chlaenius vestitus* (Paykull, 1790), as well as the most of the representatives of the genus *Bembidion*). Inhabitants of the mesophilous forests are: *Leistus rufomarginatus*, *Calosoma sycophanta*, *Carabus intricatus*, *Cychrus semigranosus*, *Stomis pumicatus*, while the occupants of the xerophilous ones are: *Notiophilus rufipes* Curtis, 1829, *Calosoma inquisitor*, *Carabus montivagus* Palliardi, 1825, *Myas chalybaeus* (Palliardi, 1825), *Harpalus politus* Dejean, 1829. *Cicindela germanica* Linnaeus, 1758, *Amara anthobia* Villa, 1833, *Harpalus honestus* (Duftschmid, 1812), *Harpalus hospes* Sturm, 1818, *Lebia cyanocephala* (Linnaeus, 1758) and *Cymindis ornata* Fisher-Waldheim, 1824 are found only in dry, open habitats.

Rare ground beetle species in the Cape Emine area are: *Amara reflexicollis* Motschulsky, 1844, *Harpalus hospes*, *Harpalus politus*, *Harpalus oblitus* Dejean, 1829, *Ophonus oblongus* (Schaum, 1858), *Microlestes maurus* (Sturm, 1827), *Brachinus plagiatus* Reiche, 1868.

Some of the rare species are poorly studied in respect of their way of life, which complements the scientific interest and the need for their protection (e. g. *Brachinus alexandri* F. Battoni, 1984, *Notiophilus interstitialis* Reitter, 1889 and *Notiophilus danieli* Reitter, 1897).

Zoogeographical peculiarities

Carabid species belonged to 25 zoogeographical categories or chorotypes, grouped into 4 major “faunal types” (or complexes). All the zoogeographic complexes show similar percentage of species (**Fig. 3**), but the Mediterranean (s. lato) type prevailed, consisting of 37 (28%) species, distributed in the so-called region of the “Ancient Mediterranean” (Kryzhanovskij 1965; Popov 1927). This complex is closely followed by the European-Asiatic faunal type (species ranges lie between the European-Siberian and Mediterranean zones), which includes 36 species (27%). Representatives of the Northern Holarctic and European-Siberian faunal complex (distributed mainly in the northern regions of the Holarctic, mostly in Europe and Siberia) are 33 species (25%), and of the European complex (species connected to the middle and southern part of Europe) – 28 species (21%).

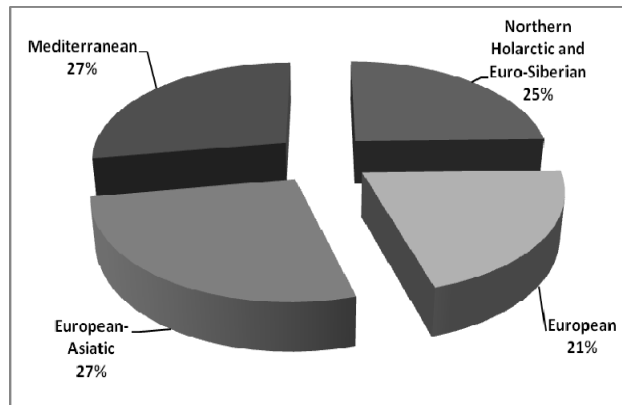


Fig. 3. Proportions of the carabid species by zoogeographical complexes.

We found the highest number of species in the Balkan-Neareastern category – 17 species (13% of all species). The Palearctic and European-Neareastern categories consist of 14 species (about 10%) each of them. In the Mediterranean faunal type the most numerous category in view of the species number is the European-Neareastern-Mediterranean chorotype. It includes 10 species or 7.5% of all species in the region. A similar distribution of categories was also established for the region of the Bulgarian Black sea coast (Teofilova *et al.* 2012b).

Life forms

The 134 ground beetle species established for the area of Cape Emine relate to two classes of life forms of the adult stage, according to the classification proposed by Sharova (1981).

Life forms typification showed predominance of class Zoophaga, represented by 76 species. Mixophytophagous are 58 species (**Fig. 4**). The most numerous are respectively the crevice-dwelling surface & litter-dwelling stratobionts from class Zoophaga, and the harpaloid geohortobionts from class Mixophytophaga.

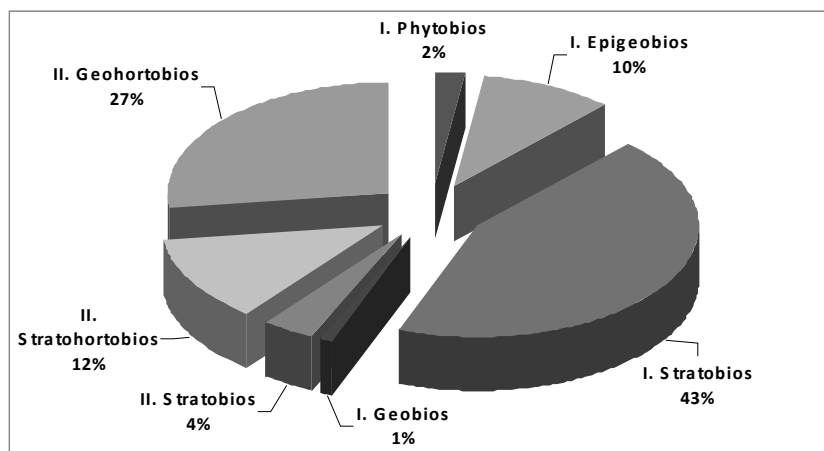


Fig. 4. Proportions of the subclasses of life forms in the carabid complex (I – class Zoophaga, II – class Mixophytophaga).

The analysis of distribution of life form subclasses in the various sampling areas showed that zoophagous stratobionts were the most abundant group in all sampling areas

with exception of the steppe-like habitat, where the mixophytophagous geohortobionts prevailed. This was in full concordance with the typical predominance of species with mixed feeding in the open territories. It was found (Teofilova *et al.* 2012b) that zoophagous stratobionts and mixophytophagous geohortobionts were most abundant in the region of the Bulgarian Black sea coast too. Similar patterns were found in study of the carabid fauna of South Dobrudzha (Kodzhabashev & Penev 2006).

Habitat preferences

Ground beetles were separated to 3 major groups, defined by the senior author: stenotopic (living in very restricted environmental conditions), oligo- and polytopic (inhabitants of several habitats), and eurytopic species (ecologically plastic inhabitants of all kinds of habitats).

The analysis of the preferred habitat types showed that the majority of the species (79 species, 59%) inhabit more than one type of habitat. A significant part of the carabid beetles however (42 species, 32%) are stenobionts and are bound to particular conditions, which makes them especially sensitive to any changes in environmental conditions. The group of ecologically plastic and resistant eurytopic species includes 12 species (only 9% of all) (**Fig. 5**). A similarly small amount of eurytopic species was characteristic for the Bulgarian Black sea coast (Teofilova *et al.* 2012b) and South Dobrudzha (Kodzhabashev & Penev 2006) too.

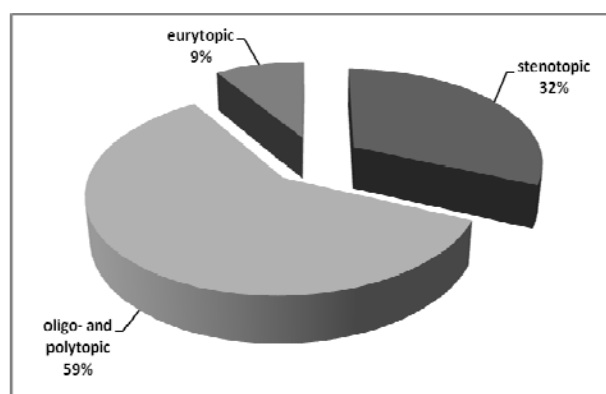


Fig. 5. Proportions of the species according to their habitat preferendum.

According to their occurrence in the studied habitats, carabids were further separated to: 1) dwellers of dry open habitats; 2) inhabitants of humid open habitats; 3) inhabitants of dry forest habitats; 4) inhabitants of humid forest habitats; 5) inhabitants of open, insolated banks; 6) inhabitants of canopy, shady banks; 7) halobionts; 8) bothrobionts; 9) eurybionts.

More than a half of the species are bound to open habitats (**Fig. 6**), as the majority of the species (48%) prefer arid areas and are representatives mainly of the Mediterranean and, to a lesser extent – of the European-Asian faunal complex. Forest species (19%) are mostly representatives of the European faunal complex. Eurybionts (9%) are mainly Holarctic or Palearctic elements (**Table 2**).

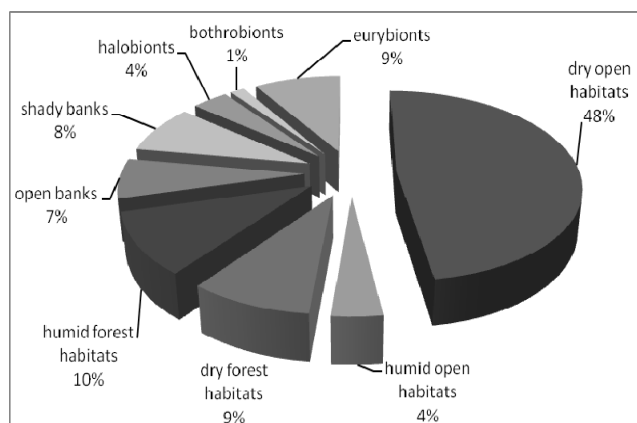


Fig. 6. Proportions of the species according to the type of preferred habitat.

Table 2. Distribution of the zoogeographical elements in the different habitat types (number of species).

Habitat	Northern Holarctic	European	European-Asiatic	Mediterranean (s. lato)	Total
Dry open	13	6	19	26	64
Humid open	1	1	2	1	5
Dry forest	3	7	1	1	12
Humid forest	2	7	3	2	14
Open banks	2	2	3	2	9
Shady banks	5	1	4	1	11
Halobionts	0	3	1	1	5
Bothrobionts	1	0	0	1	2
Eurybionts	6	1	3	2	12

Ecological groups in respect of the humidity

Division of the carabid beetles into environmental groups regarding their relation to humidity values show predominance of the xerophilic forms both by number of species (**Fig. 7**) and number of specimens (**Fig. 8**), and these include mainly representatives of tribes Harpalini, Amarini and some Sphodrini (58 species; 44%).

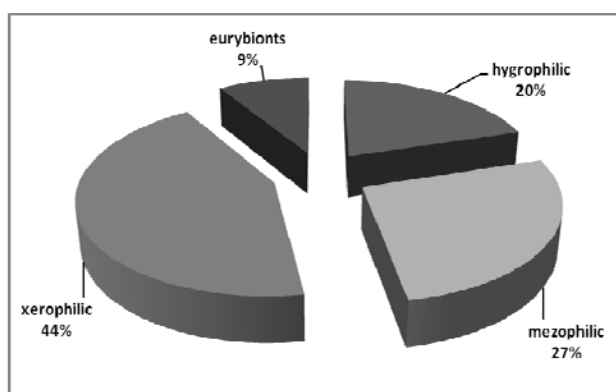


Fig. 7. Ecological groups of carabids in terms of humidity (number of species).

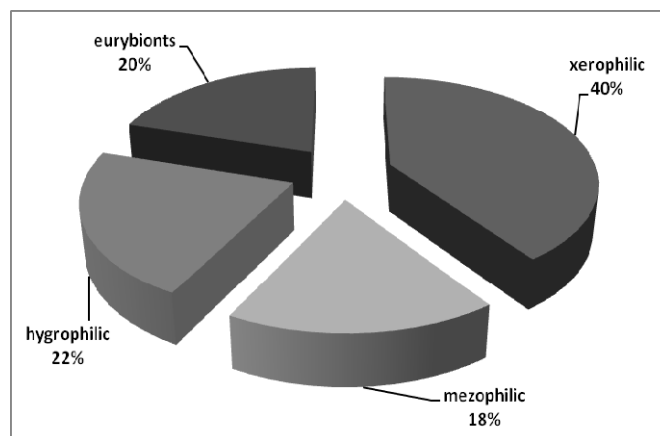


Fig. 8. Ecological groups of carabids in terms of humidity (number of specimens).

Mesophilic species are 36 (27%) and hygrophilic are 27 species (20%). They are represented primarily by the nemoral part of the tribes Carabini, Pterostichini, Platynini, Sphodrini and Brachinini and the typical shore inhabitants of Bembidiini.

The results could be explained by global climate changes, related to xerophytization of habitats and formation of conditions for the settlement of southern, in particular – Mediterranean, and xerophilic species (*Scybalicus oblongiusculus* (Dejean, 1829), *Harpalus pygmaeus* Dejean, 1829, *Ophonus ardosiacus* (Lutshnik, 1922), *Acinopus megacephalus* (Rossi, 1794), *Cymindis ornata*, *Microlestes negrita* (Wollaston, 1854), *Brachinus ejaculans* Fischer-Waldheim, 1829). The movement of southern species to the north, as well as in altitude, has been shown all across Europe (Ohlemüller *et al.* 2006).

Discussion

Taxonomic structure of the carabid coenose from the region of the study showed dominance of the tribe Harpalini, due to the large number of species typical for open territories (usually created or modified by human activity – arable lands and xerophilous steppe-like grasslands). This dominance was found in other areas of the country too (Teofilova *et al.* 2012b; Kodzhabashev & Penev 2006; Kostova 2004; Shishiniova *et al.* 2001; Kodzhabashev & Mollov 2000; Popov & Krusteva 1999).

Over the past decades intensified processes of secondary xerophytization have been seen, a possible consequence of global climate changes and destruction of natural ligneous vegetation with changes in its species and age structure (Ohlemüller *et al.* 2006). This successive degradation of the primarily habitats strongly reflects on the contemporary state of the fauna, which manifests through species impoverishment, severe dystrophy of the zoocoenoses and substitution of native species and communities with ecologically plastic, invasive elements. This state is evidenced by a gradual and successful penetration of Mediterranean and European-Asian species characteristic of open habitats and by a reduction of the number of the forest inhabiting European faunal elements.

Global drought, as a result of climatic and anthropogenic changes, has led to serious detrimental changes in the faunistic complexes with possible unforeseen alterations and trends in the future. A key characteristic is the expansion of the northern limits of distribution of some historically southern species (Aleksandrowicz 2011; Ohlemüller *et al.* 2006). Typical thermophilic species, found so far only in the Southern part of the coast (Teofilova *et al.* 2012b; Guéorguiev & Guéorguiev 1995), have been identified during the study: *Tachys fulvicollis* (Dejean, 1831), *Bembidion inoptatum*, *Bembidion castaneipenne*

Jacquelin-Duval, 1851, *Scybalicus oblongiusculus*, *Gynandromorphus etruscus* (Quensel, 1806), *Harpalus pygmaeus*, *Dixus obscurus* (Dejean, 1825), *Ophonus ardosiacus*, *Microlestes fulvibasis* Reitter, 1900, *Microlestes negrita*, *Cymindis ornata*. Also important trend appear to be the local degradation processes of anthropogenic origin, such as the substitution of natural biotopes with anthropogenic habitats, in particular wheat fields, which favours the penetration of Near Eastern faunal elements, which predominantly live in open habitats. The abandonment of previous agrocoenoses, on the other hand, leads to ruderalization of vegetation and its associated fauna.

A high percentage of Mediterranean elements showed their successful penetration along the Black Sea coast, where they settle primarily in extra- and intrazonal habitats (seashore, sand dunes, sunny aspects, etc.). Predominance of the Mediterranean species has already been demonstrated for the region of the Bulgarian Black sea coast (Teofilova *et al.* 2012b).

The low percentage of European species might be due to continued deforestation affecting large parts of the whole of Europe. Substitution of forests with open biotopes and arable lands was the reason for the displacement of the European nemoral complex by more adaptable European-Asian species attached to such types of habitats (Aleksandrowicz 2011; Kodzhabashev & Penev 2006; Desender & Turin 1989). Degradation of forest communities led to formation of new carabid complex, represented mainly by xerophilous species inhabiting open biotopes, while the old nemoral carabid complex (Carabini, Pterostichini, Platynini) has shifted to plots with preserved forest biotopes. Those remnants preserve forest elements in denuded and anthropogenized territories and their conservation is essential for maintaining a diverse fauna of mostly European and European-Siberian forest species (Kodzhabashev & Penev 2006).

The results from the zoogeographical analysis demonstrated high presence of southern forms, which together with the mixing of various biogeographical elements (Popov 2007; Tzonev *et al.* 2005), provided further evidence for the questionable qualification of Cape Emine as a border point for the separation of the biogeographic regions of Northern and Southern Black Sea coast.

The high percentage of stenobionts once again showed the urgent need to expand the protection of certain natural and semi-natural habitats in the region, such as significant biotopes and “islands” for the conservation of biodiversity: coastal and riparian habitats, marshes, swamps and lakes, floodplains, mesophilous and xerophilous forests, steppe-like and semi-steppe plots, as well as salinized habitats along the coast.

A relatively high percentage of sporadic and rare intra- and extrazonal and hygrophilous Carabidae species was found due to the specific climatic conditions and geographic location of Cape Emine, as well as to the set natural features in the area.

Conclusions

This paper represents the first study of the carabid fauna of the area of Cape Emine. A total of 134 species were found, which included two endemics and 59 new species.

Taxonomic structure of the carabid complex in the region of Cape Emine testified to the predominance of species associated with open biotopes – particularly representatives of the tribe Harpalini.

Zoogeographical characterization of the ground beetle fauna showed complicated composition of geographical elements and evidenced successful penetration of Mediterranean and European-Asiatic species with a smaller amount of European categories, which is probably the result of deforestation and global xerophytization of habitats.

The wide range of life forms demonstrated full utilization of existing ecological niches, and the distribution of species between the classes and groups testified about relative evolutionary completion of the region of Cape Emine (Lövei 2008, etc.).

The smaller number of species in anthropogenically affected habitats proved once again the negative impact of urbanization and anthropogenic phenomena on species richness (Weller & Ganzhorn 2004, etc.).

Measures are needed for rehabilitation and protection of the biodiversity as a whole. Special efforts should be undertaken for the restoration of the destroyed part of the bed of the Vaya River near Irakli site and for the revival of all affected riparian forests. That would ensure the microhabitat variegation, which defines the high variety of communities (Baiocchi *et al.* 2012).

Of extreme importance for the conservation of essential habitats is the reversion and maintenance of extensive farming practices in abandoned cultivated lands (grazing, extensive viticulture and gardening).

Despite of all negative anthropogenic activities (Teofilova *et al.* 2012a), we face with a rich, original and diverse ground beetle fauna, which reinforces the need to extend the network of protected areas so that they cover all of the main natural habitats of the region of Cape Emine.

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Appendix. List of Carabidae beetles, established in the area of Cape Emine (central Bulgarian Black sea coast)

No	Species	Material examined	Range type	Life form	Habitat	Faunistic records from the literature	Sampling area in which the species was established
1.	<i>Cicindela (Cylindera) germanica</i> Linnaeus, 1758	1♂	E-PAS	1.2.4.	1	H-W	VBd
2.	<i>Cicindela (Cicindela) campestris</i> Linnaeus, 1758	200, 3♂♂	PAL	1.2.4.	1	*	II, VBd
3.	<i>Leistus (Pogonophorus) rufomarginatus</i> (Duftschmid, 1812)	1500, 33♂♂, 1n	EUR	1.3 1.2.	4	*	Id, Ib, IrV, In1, VBb, VBd, Rd
4.	<i>Nebria (Nebria) brevicollis</i> [Fabricius, 1792]	88900, 978♂♂, 23n	E-PAS	1.3 1.2.	4	H-W	II, Id, Ib, IrV, In1, In2, I2e, VBb, VBd
5.	<i>Notiophilus interstitialis</i> Reitter, 1889	3♂♂	B-PAS	1.3 1.1.	1	H-W	In2, Fs, Rd
6.	<i>Notiophilus damieni</i> Reitter, 1897	1♀	B-PAS	1.3 1.1.	6	*	Rd
7.	<i>Notiophilus rufipes</i> Curtis, 1829	3600, 43♂♂	CE-PAS	1.3 1.1.	3	Apf.	Id, Ib, IrV, In1, VBb, Rd
8.	<i>Calosoma (Calosoma) sycophanta</i> [Linnaeus, 1758]	1700, 109♂♂	PAL	1.2.2 1.	4	*	Ib, VBd, Rs, Rd
9.	<i>Calosoma (Acalosoma) inquisitor</i> [Linnaeus, 1758]	1400, 49♂♂, 2n	PAL	1.2.2 1.	3	*	Id, Ib, In2, VBd, Rs, Rd
10.	<i>Carabus (Eucarabus) ulrichi</i> Germar, 1824	6500, 82♂♂	C-EE	1.2.2.	4	*	II, Id, Ib, IrV, In1, In2, I2e, VBd
11.	<i>Carabus (Autocarabus) cancellatus</i> Illiger, 1798	1400, 16♂♂	E-SI	1.2.2.	6	*	IrV, In1
12.	<i>Carabus (Trachycarabus) scabrinotatus</i> Olivier, 1795	2800, 37♂♂, 2n	CE-PAS	1.2.2.	1	*	Id, Ib, VBd, Rd
13.	<i>Carabus (Archicarabus) montivagus</i> Palliardi, 1825	200, 1♂	BAL-K	1.2.2.	3	*	Id
14.	<i>Carabus (Tomocarabus) convexus</i> Fabricius, 1775	13900, 130♂♂, 19n	E-PAS	1.2.2.	3	*	all without Fn and Rne
15.	<i>Carabus (Chaetocarabus) intricatus</i> Linnaeus, 1761	1♂	EUR	1.2.2.	4	*	VBd
16.	<i>Carabus (Procrustes) coriaceus</i> Linnaeus, 1758	63900, 574♂♂, 27n	E-PAS	1.2.2.	9	Apf., H-W	all
17.	<i>Cychrus semigranosus</i> Palliardi, 1825	10, 9♂♂, 1n	BAL-K	1.2.2.	4	*	VBb, VBd
18.	<i>Dyschirius (Dyschiriodes) rufipes</i> Dejean, 1825	1♀	CE-PAS	1.4.2 1.	5	***	IrV
19.	<i>Trechus quadristriatus</i> (Schränk, 1781)	11700, 197♂♂, 5n	E-CA-M	1.3 1.2.	9	H-W	all
20.	<i>Trechus crucifer</i> La Brulerie, 1875	1♀	B-PAS	1.3 1.2.	4	*	Id
21.	<i>Tachys (Paratachys) fulvicollis</i> [Dejean, 1831]	1♂	E-PA-M	1.3 1.4.	6	H-W	IrV
22.	<i>Porotachys bisulcatus</i> (Nicolaï, 1822)	10, 1♂	E-PA-M	1.3 1.1.	8	H-W	IrV
23.	<i>Asaphidion flavipes</i> [Linnaeus, 1761]	2300, 34♂♂	W-PAL	1.2.3.	6	*	IrV
24.	<i>Bembidion (Metalina) lampros</i> (Herbst, 1784)	1700, 25♂♂	OLA	1.3 1.1.	9	H-W	Id, IrV, In1
25.	<i>Bembidion (Metalina) properans</i> (Stephens, 1828)	300, 2♂♂	E-WSI	1.3 1.1.	5	H-W	IrV, In1, Rne
26.	<i>Bembidion (Philochthus) inoptatum</i> Schaum, 1857	1♂	E-PAS	1.3 1.1.	7	H-W	IrV
27.	<i>Bembidion (Philochthus) humulatum</i> [Fourcroy, 1785]	200, 4♂♂	E-MED	1.3 1.1.	5	*	Ib, VBb, VBd
28.	<i>Bembidion (Talanus) subfasciatum</i> Chaudoir, 1850	1♂	B-PAS	1.3 1.1.	7	*	VBd
29.	<i>Bembidion (Leja) articulatum</i> (Panzer, 1796)	1♂	PAL	1.3 1.1.	5	H-W	VBd
30.	<i>Bembidion (Euperiphys) combustum</i> Ménétrés, 1832	1♀	B-CAS	1.3 1.1.	5	*	IrV
31.	<i>Bembidion (Peryphanes) dalmatinum</i> Dejean, 1831	400, 2♂♂	CE-PAS	1.3 1.1.	6	H-W	Id, Ib, In1, Fn
32.	<i>Bembidion (Peryphanes) castaneipennis</i> Jacquelin-Duval, 1851	2♂♂	B-PAS	1.3 1.1.	5	H-W	I2e, Fn
33.	<i>Stomis pumicatus</i> [Panzer, 1796]	10, 2♂♂	E-PAS	1.3 1.2.	4	*	IrV
34.	<i>Myas chalybaeus</i> (Palliardi, 1825)	4100, 24♂♂, 1n	BAL-K	1.3 2 1.	3	*	Id, Ib, IrV, In2, VBb, VBd, Rs, Rd
35.	<i>Poecilus (Poecilus) cupreus</i> [Linnaeus, 1758]	200, 1♂	E-AS	1.3 2 1.	1	H-W	IrV, In1, Rne
36.	<i>Pterostichus (Melaninus) nigrita</i> (Paykull, 1790)	1♀	PAL	1.3 2 1.	4	*	In1

37.	<i>Pterostichus (Feronidius) melas</i> (Creutzer, 1799)	410♂♂, 408♂♂, 62n	E-PAS	1.3(2).1.	2	*	all without Rs
38.	<i>Pterostichus (Pterostichus) merkii</i> Fivaldsky, 1879	1♀, 3♂♂	BGE	1.3(2).1.	4	*	IrV
39.	<i>Abax carmiatus</i> (Duftschmid, 1812)	1♀	C-EE	1.3(2).1.	4	*	IrV
40.	<i>Calathus (Calathus) attinguendus</i> Chaudoir, 1846	36♀♀, 24♂♂, 2n	B-PAS	1.3(1).2.	1	*	In1, VBb, Rs, Rd
41.	<i>Calathus (Calathus) fuscipes</i> (Goeze, 1777)	292♀♀, 283♂♂, 48n	PAL	1.3(1).2.	1	H-W	all without Rn
42.	<i>Calathus (Calathus) longicollis</i> Motschulsky, 1864	11♀♀, 25♂♂	B-PAS	1.3(1).2.	1	*	II, IrV, Rs, Rd
43.	<i>Calathus (Neocalathus) ambignus</i> (Paykull, 1790)	24♀♀, 5♂♂	E-AS	1.3(1).2.	1	H-W	II, Ib, I2e, VBb, VBd, Rs, Rd
44.	<i>Calathus (Neocalathus) melanocephalus</i> [Linnaeus, 1758]	41♀♀, 23♂♂	OLA	1.3(1).2.	1	*	Ib, In2, I2e, VBd, Rs, Rd
45.	<i>Calathus (Neocalathus) mollis</i> Marsham, 1802	1♀	NM-CAS	1.3(1).2.	1	H-W	Rs
46.	<i>Calathus (Neocalathus) cinctus</i> [Motschulsky, 1850]	83♀♀, 55♂♂, 2n	E-PAS	1.3(1).2.	1	*	II, Id, Ib, In2, VBb, VBd, Rs, Rd
47.	<i>Laemostenus (Laemostenus) venustus</i> (Dejean, 1828)	1♀	E-MED	1.3(1).1.	3	*	Id
48.	<i>Laemostenus (Laemostenus) janthinus</i> [Duftschmid, 1812]	2♀♀	C-EE	1.3(1).1.	3	***	Ib
49.	<i>Laemostenus (Fristonychus) terricola</i> (Herbst, 1783)	50♀♀, 25♂♂, 11n	W-PAL	1.3(1).6.	8	*	II, Id, Ib, VBb, VBd, Rs, Rd
50.	<i>Agonum (Europhius) sp.</i>	96♀♀, 49♂♂, 2n	PAL	1.3(1).1.	2	*	II, In1, VBd, Rs, Rd, Rn, Rne
51.	<i>Anchomenus dorsalis</i> [Pontoppidan, 1763]	269♀♀, 129♂♂, 1n	PAL	1.3(1).1.	1	*	IrV, In1, VBd
52.	<i>Amara (Zezsa) reflexicollis</i> Motschulsky, 1844	1♂	E-PA-M	2.2.1.	1	H-W	I2e
53.	<i>Amara (Amara) aenea</i> (De Geer, 1774)	31♀♀, 16♂♂, 1n	OLA	2.3.1.	1	Apf., H-W	all without Id n IrV
54.	<i>Amara (Amara) anthobia</i> Villa, 1833	15♀♀, 13♂♂	E-PAS	2.1.1.	1	*	II, Id, Ib, IrV, VBb, Rs, Rd
55.	<i>Amara (Amara) communis</i> Panzer, 1797	4♀♀, 2♂♂	E-SI	2.3.1.	1	**	In1, VBd, Rs
56.	<i>Amara (Amara) familiaris</i> (Duftschmid, 1812)	8♀♀, 11♂♂	OLA	2.1.1.	9	*	II, Ib, In1, VBb, VBd, Rs, Rd
57.	<i>Amara (Amara) lucida</i> (Duftschmid, 1812)	1♀, 1♂	E-PA-M	2.3.1.	9	H-W	II, Rs
58.	<i>Amara (Amara) simulata</i> (Gyllenhal, 1810)	1♂	E-CA-M	2.3.1.	1	H-W	Rn
59.	<i>Amara (Celia) ingenua</i> (Duftschmid, 1812)	1♀, 1♂	E-AS	2.3.1.	1	*	In1, In2
60.	<i>Amara (Bradytus) apricaria</i> (Paykull, 1790)	1♂	OLA	2.3.1(1).	1	Apf., H-W	Rd
61.	<i>Amara (Bradytus) majuscula</i> (Chaudoir, 1850)	1♀	PAL	2.3.1(1).	1	G-G, H-W	Rs
62.	<i>Zabrus (Pelor) graecus</i> Dejean, 1828	1♂	B-PAS	2.3.2.	4	**	VBd
63.	<i>Scybalius oblongiscutis</i> (Dejean, 1829)	1♀, 1♂	MED	2.3.1.	1	G-G, H-W	In1, Rs
64.	<i>Gynandromorphus etruscus</i> (Quense, 1806)	5♂♂	NMED	2.2.1.	1	*	Rs, Rn, Rne
65.	<i>Stenolophus mixtus</i> (Herbst, 1784)	1♂	PAL	2.1.1.	6	H-W	IrV
66.	<i>Acupalpus (Ancylotria) interstitialis</i> Reitter, 1884	1♂	B-PAS	2.1.1.	5	H-W	Rd
67.	<i>Acupalpus (Acupalpus) meridamus</i> [Linnaeus, 1767]	1♀, 1♂	E-PAS	2.1.1.	5	H-W	I2e, Rn
68.	<i>Parophonus (Tachyophonus) laeviceps</i> (Ménétriés, 1832)	1♀, 3♂♂	B-PAS	2.2.1.	1	G-G, H-W	IrV, In1, Rn
69.	<i>Parophonus (Tachyophonus) mendax</i> [Rossi, 1790]	25♀♀, 23♂♂	B-PAS	2.2.1.	2	*	II, IIp, In1, In2, I2e, Rs, Rd, Rn, Rne
70.	<i>Pseudophonus rufipes</i> (Degeer, 1774)	7♀♀, 6♂♂, 4n	PAL	2.2.1.	9	H-W	Ib, In1, I2e, VBd, Rd, Rne
71.	<i>Pseudophonus calceatus</i> (Duftschmid, 1812)	1♀	E-AS	2.3.1.	1	H-W	Rs
72.	<i>Harpalus (Cryptophonus) melancholicus</i> Dejean, 1829	1♀	E-PAS	2.3.1.	7	H-W	Rs
73.	<i>Harpalus (Harpalus) rufipalpis</i> Sturm, 1818	1♀	W-PAL	2.3.1.	1	*	II
74.	<i>Harpalus (Harpalus) honestus</i> (Duftschmid, 1812)	1♀	E-SI	2.3.1.	1	*	Rs
75.	<i>Harpalus (Harpalus) rubripes</i> (Duftschmid, 1812)	8♀♀, 13♂♂	OLA	2.3.1.	9	H-W	II, Id, In1, I2e, VBd, Rs, Rd, Rn
76.	<i>Harpalus (Harpalus) attenuatus</i> Stephens, 1828	19♀♀, 20♂♂	MED	2.3.1.	4	H-W	II, Id, Ib, Rs, Rd
77.	<i>Harpalus (Harpalus) atratus</i> Latreille, 1804	2♂♂	E-CAS	2.3.1.	9	*	Id
78.	<i>Harpalus (Harpalus) quadripunctatus</i> Dejean, 1829	1♀	OLA	2.3.1.	3	**	Rs
79.	<i>Harpalus (Harpalus) serripes</i> (Quense, 1806)	1♀, 2♂♂	PAL	2.3.1.	1	H-W	IrV, In2, I2e
80.	<i>Harpalus (Harpalus) politus</i> Dejean, 1829	1♂	E-SI	2.3.1.	3	Apf.	II
81.	<i>Harpalus (Harpalus) flavicornis</i> Dejean, 1829	31♀♀, 77♂♂	CE-PAS	2.3.1.	3	H-W	II, Id, IrV, In1, VBd, Rn, Rne

		1♂, 1♂	2.3.1.	1	H-W	VBd
82.	<i>Harpalus (Actephilus) pumilus</i> (Sturm, 1818)	1♂, 1♂	2.3.1.	1	H-W	VBd
83.	<i>Harpalus (Harpalus) subcybndricus</i> Dejean, 1829	1♂	2.3.1.	9	*	II
84.	<i>Harpalus (Harpalus) tardus</i> (Panzer, 1797)	25♂♂, 23♂♂, 1n	2.3.1.	9	*	II, Id, IrV, In1, In2, I2e, VBb, VBd, Rd
85.	<i>Harpalus (Harpalus) albarnicus</i> Reitter, 1900	2♂♂, 2♂♂	2.3.1.	1	H-W	IrV, Rs
86.	<i>Harpalus (Harpalus) cupreus</i> Dejean, 1829	13♂♂, 13♂♂	2.3.1.	1	*	Id, IrV, In1, I2e, Rn, Rne
87.	<i>Harpalus (Harpalus) dimidiatus</i> (Rossi, 1790)	269♂♂, 277♂♂, 9n	2.3.1.	3	H-W	all
88.	<i>Harpalus (Harpalus) metallinus</i> Ménétrés, 1838	19, 5♂♂	2.3.1.	4	H-W	Rs
89.	<i>Harpalus (Harpalus) pygmaeus</i> Dejean, 1829	2♂♂, 6♂♂	2.3.1.	1	*	II
90.	<i>Harpalus (Harpalus) hospes</i> Sturm, 1818	14♂♂, 19♂♂	2.3.1.	7	Apf.	I2e, VBb, Rs, Rn
91.	<i>Harpalus (Harpalus) distinguendus</i> (Duftschmid, 1812)	8♂♂, 14♂♂	2.3.1.	9	Apf., H-W	II, Ib, IrV, I2e, Rs, Rd, Rn, Rne
92.	<i>Harpalus (Harpalus) oblitus</i> Dejean, 1829	1♂	2.3.1.	1	*	VBd
93.	<i>Acinopus (Oedematicus) megacephalus</i> (Rossi, 1794)	86♂♂, 188♂♂, 3n	2.3.2.	1	H-W	II, Id, Ib, IrV, In1, In2, VBd, Rs, Rd
94.	<i>Ophonus (Metophonus) nitidulus</i> Stephens, 1828	5♂♂, 1♂	2.2.1.	1	*	IrV, In1, Rs
95.	<i>Ophonus (Metophonus) ruficollis</i> (Sturm, 1818)	1♂	2.2.1.	1	H-W	Rs
96.	<i>Ophonus (Metophonus) puncticeps</i> (Stephens, 1828)	6♂♂, 4♂♂	2.2.1.	1	H-W	II, Rs, Rd
97.	<i>Ophonus (Metophonus) parallelus</i> (Dejean, 1829)	1♂	2.2.1.	1	G-G, H-W	Rs
98.	<i>Ophonus (Metophonus) brevicollis</i> (Serville, 1821)	1♂	2.2.1.	1	H-W	Rne
99.	<i>Ophonus (Hesperophonus) similis</i> (Dejean, 1829)	2♂♂, 3♂♂	2.2.1.	1	G-G, H-W	In1, VBb, Rs
100.	<i>Ophonus (Hesperophonus) azureus</i> (Fabricius, 1775)	48♂♂, 68♂♂, 2n	2.2.1.	1	H-W	II, Ib, In1, I2e, VBd, Rs, Rd, Rn, Rne
101.	<i>Ophonus (Hesperophonus) subquadratus</i> (Dejean, 1829)	1♂	2.2.1.	1	H-W	VBd
102.	<i>Ophonus (Hesperophonus) cribricollis</i> Dejean, 1829	4♂♂	2.2.1.	1	H-W	VBd
103.	<i>Ophonus (Ophonus) ardosiacus</i> (Lutshnik, 1922)	5♂♂, 4♂♂	2.2.1.	1	G-G	II, Rs
104.	<i>Ophonus (Ophonus) sabulicola</i> (Panzer, 1796)	15♂♂, 25♂♂	2.2.1.	1	H-W	II, I2e, Rs, Rd, Rne
105.	<i>Ophonus (Macrophonus) oblongus</i> (Schaum, 1858)	19, 2♂♂	2.2.1.	1	*	Rs, Rn
106.	<i>Carterus (Carterus) rufipes</i> Chaudoir, 1843	2♂♂, 3♂♂	2.3.3.	1	*	Rs, Rne
107.	<i>Carterus (Pristocarterus) angustipennis</i> Chaudoir, 1852	2♂♂	2.3.3.	1	H-W	Rs
108.	<i>Dinomus calydonicus</i> (Rossi, 1790)	12♂♂, 4♂♂	2.3.3.	1	H-W	Ib, Rs, Rd, Rn, Rne
109.	<i>Dinox obscurus</i> (Dejean, 1825)	2♂♂, 1n	2.3.3.	1	H-W	II, Ib, Rd
110.	<i>Dinodes decipiens</i> (Dufour, 1820)	19, 2♂♂	1.3(1).1.	1	H-W	II, Rs
111.	<i>Chlaenius (Chlaenius) festinus</i> (Panzer, 1796)	4♂♂, 6♂♂, 2n	1.3(1).1.	6	H-W	IrV, In1
112.	<i>Chlaenius (Chlaenius) nitidulus</i> (Schränk, 1781)	998♂♂, 903♂♂, 26n	1.3(1).1.	6	*	all without IrV n VBd
113.	<i>Chlaenius (Chlaenius) nigricornis</i> (Fabricius, 1787)	1♂	1.3(1).1.	6	H-W	IrV
114.	<i>Chlaenius (Chlaenius) vestitus</i> (Paykull, 1790)	1♂	1.3(1).1.	6	H-W	IrV
115.	<i>Badister (Badister) bullatus</i> (Schränk, 1798)	1♂	1.3(1).1.	6	H-W	IrV
116.	<i>Lebia (Lampyrus) cyanocephala</i> (Linnaeus, 1758)	1♂, 1♂	1.1.3.	1	*	Ib, Rd
117.	<i>Paradromius (Manodromius) linearis</i> (Olivier, 1759)	1♂	1.3(1).5.	5	G-G, H-W	II
118.	<i>Philorhizus (Philorhizus) notatus</i> (Stephens, 1827)	3♂♂, 2♂♂	1.3(1).3.	1	*	In2, Rs, Rd, Rne
119.	<i>Syntomus obscuriguttatus</i> (Duftschmid, 1812)	6♂♂, 4♂♂	1.1.2.	1	H-W	II, IrV, In1, Rd, Rne
120.	<i>Syntomus pallipes</i> (Dejean, 1825)	3♂♂, 2♂♂	1.1.2.	1	*	IrV, In1, VBd
121.	<i>Microlestes fissuralis</i> Reitter, 1901	5♂♂, 7♂♂	1.3(1).3.	1	*	II, In2, I2e, Rs
122.	<i>Microlestes fulvibasis</i> Reitter, 1900	19, 2♂♂	1.3(1).3.	7	G-G, H-W	In2, Rs
123.	<i>Microlestes maurus</i> (Sturm, 1827)	15♂♂, 36♂♂	1.3(1).3.	1	H-W	II, Ib, IrV, In1, I2e, Rs, Rd, Rn
124.	<i>Microlestes minutulus</i> (Goeze, 1777)	20♂♂, 44♂♂	1.3(1).3.	1	H-W	II, Ib, In2, I2e, Rs, Rd
125.	<i>Microlestes nigrita</i> (Wollaston, 1854)	48♂♂, 48♂♂	1.3(1).3.	1	H-W	all without Id
126.	<i>Cymindis (Cymindis) ornata</i> Fisher-Waldheim, 1824	5♂♂, 6♂♂	1.3(1).3.	1	*	Ib, Rs, Rd

127.	<i>Brachinus alexandri</i> F. Battoni, 1984	799, 833	P-SMED	1.3 1 .3.	1	G-G, H-W	Rs, Rne
128.	<i>Brachinus berytensis</i> Reiche, 1855	1499, 833	B-PAS	1.3 1 .3.	1	*	II, In1, Rs, Rd, Rn, Rne
129.	<i>Brachinus brevicollis</i> Motschulsky, 1844	2199, 1533	B-CAS	1.3 1 .3.	3	G-G	Rs, Rd
130.	<i>Brachinus crepitans</i> [Linnaeus, 1758]	76199, 43933, 13n	PAL	1.3 1 .3.	1	H-W	IrV, In1, In2, I2e, Rs, Rd, Rn, Rne
131.	<i>Brachinus ejaculans</i> Fischer-Waldheim, 1829	299, 233	B-CAS	1.3 1 .3.	2	H-W	Rs, Rd
132.	<i>Brachinus exfoliatus</i> Duftschmid, 1812	4999, 3033	E-CA-M	1.3 1 .3.	1	H-W	IrV, In1, In2, I2e, Rs, Rd, Rne
133.	<i>Brachinus plagiatus</i> Reiche, 1868	499, 133	P-SMED	1.3 1 .3.	2	*	Rs
134.	<i>Brachinus psophia</i> Serville, 1821	30199, 15733	E-CAS	1.3 1 .3.	1	H-W	IrV, In1, In2, Rs, Rd, Rne

Explanations to the Appendix

Column № 1. Consecutive number.

Column № 2. List of the species recorded from the area of Cape Emine.

Column № 3. Numbers of examined specimens.

♀ - female; ♂ - male; n - not determined.

Column № 4. Zoogeographical categories and faunal types:

I. Northern Holarctic and Euro-Siberian faunal type:

OLA - Holarctic; PAL - Palearctic; W-PAL - Western Palearctic; E-SI - Eurosiberian; E-WSI - Euro-Westsiberian.

II. European faunal type:

EUR - European; E-PAS - European-Neareastern; CE-PAS - Central European and Neareastern; CEE-PA - Central and Eastern European and Neareastern; C-EE - Central and Eastern European; CEUR - Central European; BAL-K - Balkan-Carpathian.

III. Euroasiatic faunal type:

E-AS - Euroasiatic steppe complex; E-CAS - European and Central Asian; B-CAS - Balkan and Central Asian; B-PAS - Balkan-Neareastern (+ Balkan-Anatolian).

IV. Mediterranean (s. lato) faunal type (species of the Ancient Mediterranean):

E-CA-M - European-Centralasian-Mediterranean; E-PA-M - European-Neareastern-Mediterranean; CA-MED - Mediterranean-Centralasian; MED-PAS - Mediterranean-Neareastern; MED - Mediterranean; E-MED - Eastmediterranean; P-SMED - Pontic-Submediterranean; PON - Pontic; SE - South European; NMED - Northmediterranean; NM-CAS - Northmediterranean-Centralasian; BAL - Balkan; BgE - Bulgarian endemic.

Column № 5. Explanation to the indexes of the life forms:

The first figure in the index shows the class of life form, the second – the subclass, the third – the life form group. In brackets after the subclass the series is shown, when it exists.

Life form class 1. Zoophagous.

Life form subclass: 1.1 - Phytobios; 1.2 - Epigeobios; 1.3 - Stratobios; 1.4 - Geobios; 1.5 - Psammocolimbets.

Life form groups: 1.1.1 - dendrobionts; 1.1.2 - stem-dwelling hortobionts; 1.1.3 - leaf-dwelling dendrohortobionts; 1.2.1 - small walking epigeobionts; 1.2.2 - large walking epigeobionts; 1.2.2(1) - large walking dendroepigeobionts; 1.2.3 - running epigeobionts; 1.2.4 - flying epigeobionts; 1.3(1) - series crevice-dwelling stratobionts; 1.3(1).1 - surface & litter-dwelling; 1.3(1).2 - litter-dwelling; 1.3(1).3 - litter & crevice-dwelling; 1.3(1).4 - endogeobionts; 1.3(1).5 - litter & bark-dwelling; 1.3(1).6 - bothrobionts; 1.3(1).7 - troglobionts; 1.3(2) - series digging stratobionts; 1.3(2).1 - litter & soil-dwelling; 1.3(2).2 - litter & crevice-dwelling; 1.3(2).3 - bothrobionts; 1.3(2).4 - troglobionts; 1.4.1 - running & digging geobionts; 1.4.2(1) - small digging geobionts; 1.4.2(1) - large digging geobionts 1.5.1 - shore psammobionts.

Life form class 2. Mixophytophagous.

Life form subclass: 2.1 - Stratobios; 2.2 - Stratohortobios; 2.3 - Geohortobios.

Life form groups: 2.1.1 - crevice-dwelling stratobionts; 2.2.1 - stratohortobionts; 2.3.1 - harpaloid geohortobionts; 2.3.1(1) - crevice-dwelling harpaloid geohortobionts; 2.3.2 - zabroid geohortobionts; 2.3.3 - dytomeoid geohortobionts.

Column № 6. Habitat preferences of the species in the region of the Cape Emine:

1 – inhabitants of dry open habitats; 2 – inhabitants of humid open habitats; 3 – inhabitants of dry forest habitats; 4 – inhabitants of humid forest habitats; 5 – inhabitants of the open, sunny coasts; 6 – the inhabitants of canopy, shady shores; 7 – halobionts; 8 – bothrobionts; 9-eurybionts.

Column № 7: Abbreviations of the publications containing records on ground beetles from the region:

Apf. – Apfelbeck (1904); G-G – Guéorguiev, Guéorguiev (1995); H-W – Hieke, Wrase (1988); *** - new for Bulgaria, ** - new for the Bulgarian Black Sea coast, * - new for Cape Emine.

Column № 8: Abbreviations of the sampling areas:

Il-Irakli, abandoned vineyard; Id – Irakli, oak forest; Ib – Irakli, shore – ecotone; IrV-Irakli, River Vaya; In1 – Irakli, wheat field 1; In2 – Irakli, wheat field 2; I2e – Irakli, field 2 – ecotone; VBd – Military base, gully; VBB-Military base, pine forest; Rs – Signal repeater, steppe-like habitat; Rd – Signal repeater, oak forest; Rn - Signal repeater, field; Rne – Signal repeater, field – ecotone.

On the activity of two medium-sized canids: the Golden Jackal (*Canis aureus*) and the Red Fox (*Vulpes vulpes*) in the Natural Park “Sinite Kamani” (Bulgaria) revealed by camera traps

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Abstract. Daylight activity in both species was often registered during the cold seasons. Foxes were registered to be active between 7-9h and 12-14h, while the jackals – during the whole day. Activity of both predators was rising during the night. The number of photographs increased from around 17h and activity was high until morning around 9-10h.

Key words: *Canidae*, photographs, activity.

Introduction

The activity of the carnivore mammals is mainly nocturnal when they manage to catch their prey more successfully than during the daylight. The other reason for such behavior is the anthropogenic pressure forcing these mammals to avoid contact with humans. The activity of the predatory mammals depends also on species and individual characteristics, climate, relief, prey abundance and availability, and other (Servin *et al.*, 1991; Jaeger *et al.*, 2007; Ilemine & Gürkan, 2010; Racheba *et al.*, 2012; Kachamakova & Zlatanova, 2014).

In this paper we represent a short note on the daily activity of two canids, studied with camera traps in the Sinite Kamani Nature Park, Bulgaria.

Material and Methods

The analysis was made on the base of 2785 pictures of animals taken by 10 digital camera traps (Ltl-5210A) during one year, from October 2013 till October 2014. The cameras were situated throughout the park in 10 different areas (Fig. 1) and checked monthly. Pictures were taken every 30 seconds.

From all carnivores the most abundant registrations were those of the Red Fox (n=1014), and the Golden Jackal (n=283). The rest of the photos represented other mammalian species occurring in the park as *Martes foina*, *Capreolus capreolus*, *Cervus*

elaphus, *Sus scrofa*, *Felis silvestris*, *Canis lupus*, *Erinaceus roumanicus*, *Sciurus vulgaris*, and many unidentified mice (*Muridae* and *Cricetidae*).

To attract the animals in front of the cameras, dog or cat food granules (occasionally fish and other stuff) were spread.

Study Area

The Sinite Kamani Natural Park is 11380.8 hectares situated on the south slope of the east chain of Stara Planina Mountain (Fig. 1). The main habitats are broad leaf forests of *Quercus* spp. at low altitudes and *Fagus sylvatica* at above 1000 m a.s.l. On its southern part the terrain consists of rocky areas occupied by grass or shrub vegetation.

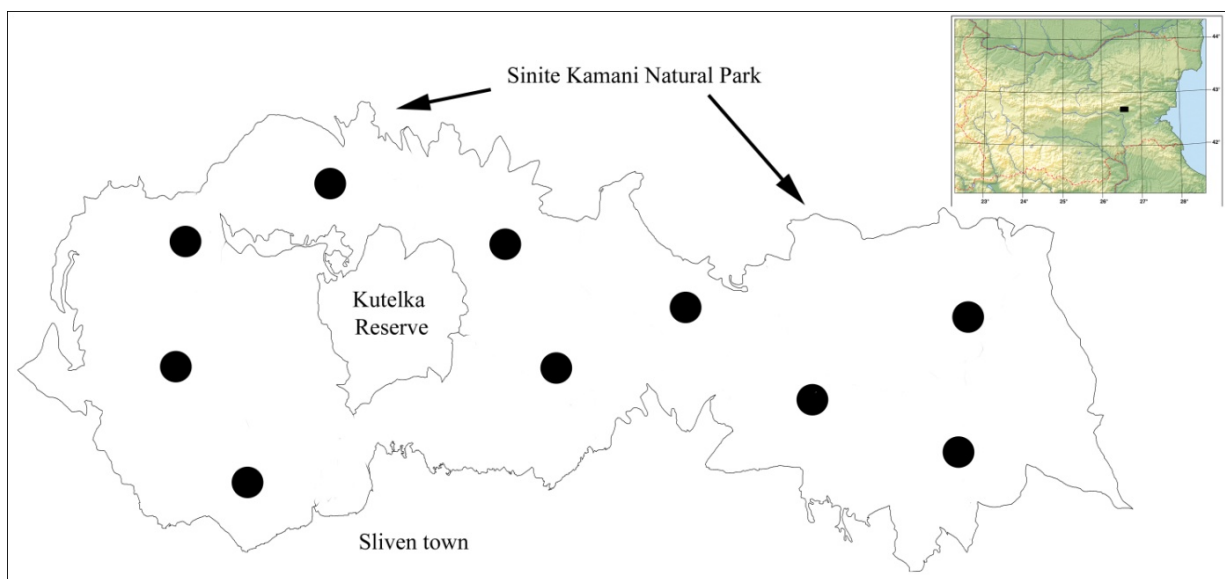


Fig. 1. Location of Sinite Kamani Natural Park and schematic position of the camera traps through the park (black circles).

Results

Daylight activity in both species was often registered during the cold seasons (autumn-winter) (Fig 2, 3, 4). Foxes were registered to be active between 7-9h and 12-14h, while the jackals – during the whole day. Photos of foxes were rare during daytime in the warm seasons, and those of jackals were lacking.

Activity of both predators was rising during the night (more photos were taken by the camera traps). The number of photographs taken was rising from around 17h and were high in number until morning, till around 9-10h. After this period the number of photos was low or lacking. Occasional peak concerning the fox was observed due to one single animal feeding in front of the camera between 11-13h (Fig. 2).

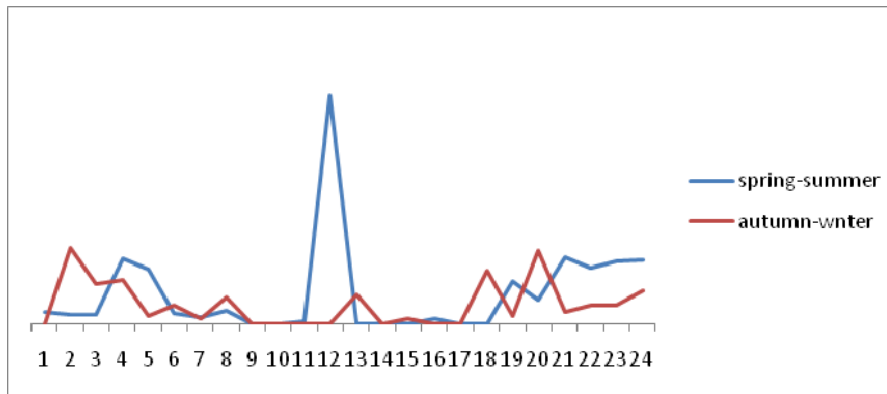


Fig. 2. Number of all pictures of Red Fox (*Vulpes vulpes*) taken during 24h (expressed for one hour time interval) during seasons.

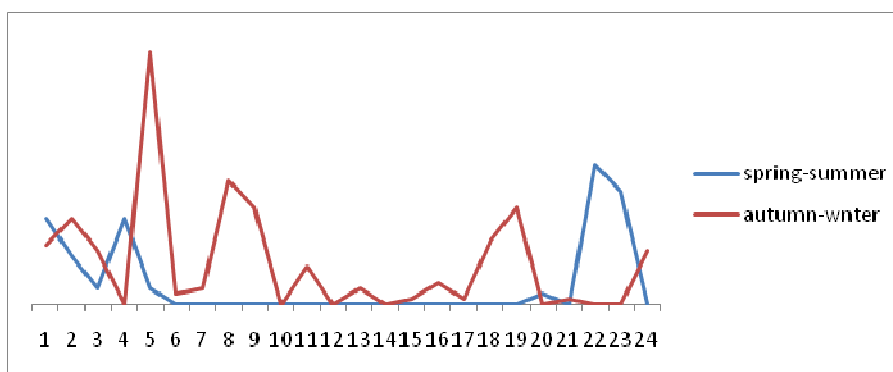


Fig. 3. Number of all pictures of Golden Jackal (*Canis aureus*) taken during 24h (expressed for one hour time interval) during seasons.



Fig. 4. Daytime activity (feeding on dog food) of the Red Fox and the Golden Jackal registered by camera traps at the Sinite Kamani Natural Park.

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Movement and activity pattern of a brown bear (*Ursus arctos* L.) tracked in Central Balkan Mountain, Bulgaria

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Abstract. First data about movement of brown bear in Bulgaria, based on 10 days tracking period of female without cubs on the territory of Central Balkan, Stara planina Mountain. Bear is active 46% of a 24-hour period, traveling 4 km per day in average. Speed is varying in different habitats and is up to 6.5 km/h in the grasslands.

Key words: *Ursus arctos*, brown bear, movement, speed, Central Balkan Mountain.

Introduction

Little is known about the movement and activity of the brown bears in Bulgaria. Knowledge so far is empirical, gathered from observations made by hunters, game breeding specialists or zoologists (Peshev *et al.* 2004, Spassov 2007). Most of the knowledge is based on assumptions because bears are hard to observe and follow. When alarmed by human presence they change the rate of movement or try to hide, which changes the behaviour and biases the results (Nellemann *et al.* 2007, Martin *et al.* 2010, Ordiz *et al.* 2013). In this case GPS telemetry is valuable method giving accurate data and avoiding bear's disturbance and change in behaviour.

The censuses of bears in Bulgaria that have been done by counting bears on high stands without taking in account the movement and activity characteristics of the species gives poor results and biases the study. Bear's activity and movement patterns are one of the main sources of information about the bears ecology and use of habitat. It is very important when management decisions have to be made.

The aim of the study is to determine the traveled distance, average speed and movement through different habitats by GPS-collared female bear in Bulgaria.

Material and Methods

The study includes data collected from 1 animal with GPS-GSM collar (Vectronics, model GPS PRO Light-3) in the period 2007-2008.

The bear is 3-4 year old, female without cubs, caught on the territory of Central Balkan Mt., Mazalat Game Breeding Station (Mazalat GBS).

The animal was caught with Aldrich foot snare close to high stand in the Mazalat GBS, with maze as bait. It was tranquilized with dart gun with 500 mg Zoletyl and fitted with GPS collar with adaptable rate of GPS-locations (fixes) take-down.

For the presented study 10 minutes intervals between fixes for 10 days period were taken (13.06.2008 - 22.06.2008). For the calculation of the animal movement speed between two fixes, the path traveled is divided by time interval of 10 min and recalculated to kilometer per hour.

The time interval between 06:00 and 21:00 was assumed as diurnal activity and for nocturnal activity - the interval between 21:00 and 06:00.

All locations with stationary behavior (sleeping or feeding on concentrated food source) were excluded from the analysis on the following basis: all locations 10 m or less from one another were considered inactive because of the GPS-error and a “cloud” of fixes in a 10 m radius (20 m in diameter) shows stationary behavior.

For distance traveled and speed in different habitat types only the path within the habitat was taken into account. Transitional segments (when the start point and end point are in different habitats) were excluded from the analysis, because the time of the transition cannot be identified properly.

Results and Discussion

Bear's activity, distance traveled and speed

Bears are active both during the day and the night. The pattern of rest and activity is complex and is determined by a lot of factors (type and availability of food sources, disturbance, weather conditions, intra- and interspecies relations, etc.).

During the study period the tracked bear was active average 6h 40 min during the day (44.2 % of the time; min 4h 20 min; max 9h 10 min) and 4h 30 min during the night (50.6 % of the time; min 2h 50 min; max 4h 50 min). Average distance traveled was 2.3 km (2322 meters; min 1431.8 m; max 3695.6 m) for the daytime and 1.7 km (1661 m; min 224.2 m; max 3842.5 m) for the nighttime. Average 24-hours distance traveled was 4 km (3984 m; min 2216 m; max 5675.8 m).

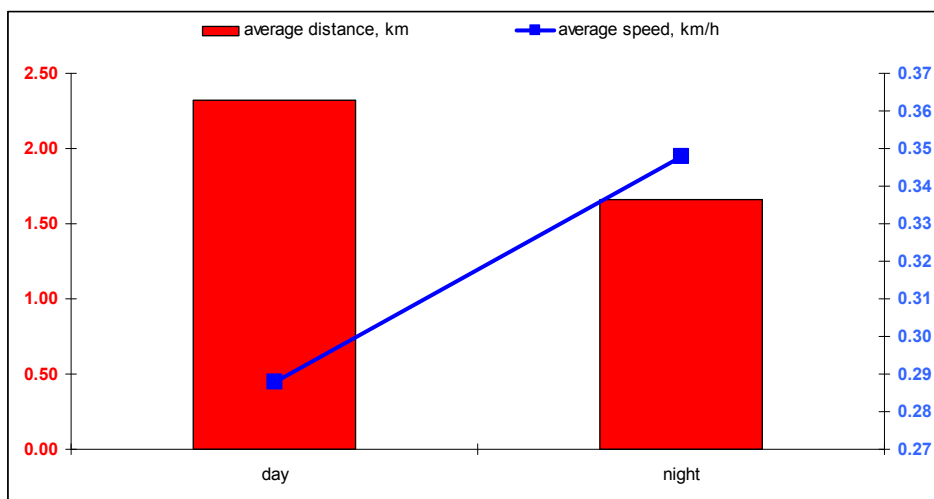


Fig. 1 Average speed and distance traveled.

Average day-time speed is lower than night-time speed, but the average distance traveled is higher. This relates to slow, but constant animal movement during the day. Resting is short in duration but on a numerous different locations. During the night the movement is fast, but the distance traveled is shorter which indicates relatively quick movement between points of interest and longer stationary feeding or resting behavior (Fig.1).

Average speed in different habitat types

Within the study period the tracked bear uses three habitat types: broad-leaved forests, natural grasslands, and transitional woodland/shrub according to Corine land cover nomenclature (Commission of European Communities 1994).

The bear moves with different speed in different habitats, fast in the grasslands (average speed 1.2 km/h), slower in the forest (average speed 0.7 km/h), and slowest in the shrubs (average speed 0.3 km/h) (Fig. 2).

The lowest speed is observed in forests and grasslands. This is determined by the fact that bears often feed in these habitats, slowly moving and picking berries, turning stones or logs in search of insects, etc.

The highest speed is observed in the grasslands (2.4 km/h) which is determined by the animal's strive to pass through uncovered areas as quickly as possible (Stelmock & Dean 1986).

Broad-leaved forests in the region are generally beech forests, which provide more cover than the grassland, but less than coniferous forest or shrubs. Animals can move through it easy but not as fast as in the open grassland. The average speed in this habitat is 1.45 km/h.

Bear's speed in transitional woodland/shrub is constant and slow. This is determined by the fact that the minimum and maximum speed are in close margins, and average speed is lower than average speed in the forest or the grassland. Bear is moving through the shrubs with constant low speed, picking berries or hiding.

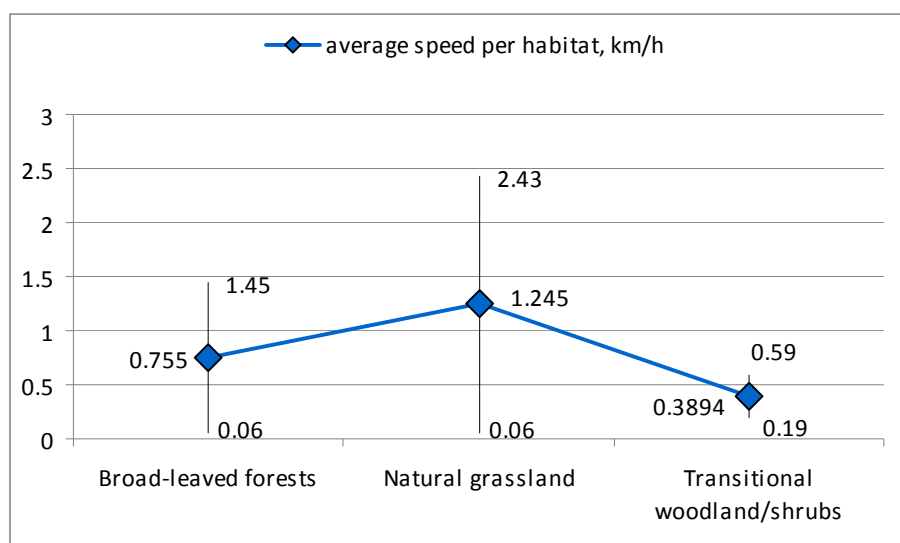


Fig. 2. Minimum, maximum and average speed of the bear in different habitats.

Top speed of the bear is 6.53 km/h measured when bear traveled between two forest patches through grassland during the night (22:40-22:50). These results are similar to those of Croatia (Huber & Roth 1993) and Greece (Merdzanis 2008).

Conclusions

Bears have flexible activity and can be diurnal, nocturnal or crepuscular in different situations. Bears are active around 50% of the time.

Bears use different habitats in search of food, shelter, denning, which affects the bear's speed and movement.

Fastest movement is in the grasslands especially when passing between forest patches. It is in order to pass quickly through dangerous habitat or to reach desirable location faster.

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Ground beetles (Coleoptera: Carabidae) from the region of Cape Emine (central Bulgarian Black sea coast). Part II. Ecological parameters and community structure

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Abstract. For the first time an ecological study of the carabid fauna of the area of Cape Emine (Bulgarian Black sea coast) was conducted. Over the period 2010 – 2011 adult carabid beetles were collected. The investigation was carried out at 7 sampling sites and pitfall traps were used. During the study altogether 6245 specimens were captured. The greatest number of both species and specimens was found in the steppe-like habitat, least – in the pine (*Pinus nigra* J. F. Arnold) plantation. Indices for α - and β -diversity were calculated. The dominant structure of the whole carabid complex showed the presence of two eudominant, three dominant, two subdominant, eleven recedent and 85 subrecedent species. The analysis of the sex structure showed the total prevalence of males over females. Cluster analysis indicated a low percentage of taxonomic similarity between the communities, which reflected the diversity of the landscape.

Keywords: Carabidae, Cape Emine, α - and β -diversity, dominance structure, sex structure, taxonomic similarity, occurrence

Introduction

Synecological studies are often used to characterize the influence of the biota on the environment and the impact of human activity on the functioning, productivity and changes in the ecosystems. In this respect, ecological parameters of the communities can be used in assessing the state of the environment and predicting the trends in the future development of the ecological systems.

Ground beetles (Coleoptera: Carabidae) are a frequent and convenient object for synecological studies. Carabids and their communities are widely used as bioindicators of terrestrial environment in the system of biological monitoring (Desender & Baert 1995; Luff 1996; Cranston & Trueman 1997; Pearsall 2007). Furthermore, they are easily trapped and their high taxonomic richness, large numbers and diverse life specializations are the reasons that they cover the entire environmental spectrum of fundamental natural gradients. Carabids have cosmopolitan distribution and decisive importance for the functioning of ecosystems (Brumwell *et al.* 1998).

So far there were no ecological studies, related to the ground beetles from the region of Cape Emine, which is with high conservation status (Teofilova *et al.* 2012). This study was the first which performed an ecological analysis of the carabid communities in some of the main types of habitats in the area. The survey aimed to contribute for the better

acquaintance and more complete biomonitoring use of this undoubtedly beneficial group of insects.

Material and Methods

In connection with the participation in a project for biological monitoring studies in the area, a series of observations and samplings were carried out in two years period during 2010 – 2011. Catches were carried out during the three vegetative periods – spring, summer and autumn – of both years. Ground beetles were collected by the approved method with terrestrial pitfall traps (Dahl 1896; Hertz 1927; Barber 1931). The traps were made of plastic bottles, buried at the level of the ground surface. As fixation fluid a 4% solution of formaldehyde was used. The investigations were performed at 7 sample sites. Specimens were identified according to: Kryzhanovskij (unpublished data), Arndt *et al.* (2011), Lindroth (1974), Hůrka (1996), Reitter (2006), Trautner & Geigenmüller (1987) and are deposited in the Carabidae collection of the Institute of Biodiversity and Ecosystem Research.

Study area

Cape Emine marks the end of the Stara planina Mts. range and conditionally separates Northern and Southern Bulgarian Black sea coast (Galabov 1956). However, it is questionable whether the Cape is also the biogeographical boundary of the coast, as claimed (Josifov 1988, etc.), because the mixing of many biogeographical elements has been found (Tzonev *et al.* 2005; Popov 2007; Teofilova 2013; Teofilova *et al.* 2015). The Cape is located 79 km south of the town of Varna and 54 km north of the town of Bourgas. Stara planina Mts. coast has a length of 52 km, of which beaches occupy over 32%. The coast is almost completely steep and rocky, and between Cape Emine and the town of Obzor is situated one of the few places in Bulgaria where the forest goes immediately to the sea shore.

The geostrategic location of the studied territory, its diverse topography, formed by the “meeting” of the mountain with the sea, and the contingent climate with influence of three climatic zones, contribute to the mixing of the representatives of various ecological and biogeographical complexes, which has led to the formation of peculiar biocoenoses.

The working sampling sites are included within the territory of the protected zones BG0001004 „Emine – Irakli” under Directive 92/43 for the protection of natural habitats and wild flora and fauna and BG0002043 „Emine” under Directive 79/409 for the protection of birds. They are representative ecosystems, characteristic for the region. A brief description of the sampling sites is given in Table 1. Further on in the text, the abbreviated expressions given in the table were used.

In all sampling areas 10 – 14 pitfall traps were set.

Data analysis

The classical four-level classification of Tischler (1949) for soil invertebrates, modified by Sharova (1981) with the initiation of a 5th category “eudominant”, was used for determination of the **dominance structure** of the communities: eudominants (with degree of dominance over 10%), dominants (5 to 10%), subdominants (2 to 5%), recedents (1 to 2%), subrecedents (< 1%). The **sex structure** of the individual communities and of the carabid complex as a whole was determined.

Indices for **α -diversity** reflect the biological diversity within the community or the habitat. The following indices were used: concentration of dominance (Simpson 1949), species richness of Margalef (1958), total species diversity (Shannon 1949, 1963), evenness (Pielou 1966). The index of Hill was calculated for comparison.

The indices for **β -diversity** reflect the diversity between the individual communities or habitats and may serve as a measure of the value and the rate of change in species on a gradient from one habitat to another. For determination of the taxonomic similarity between the faunistic complexes in the different ecosystems the coefficients of Jaccard (1908) and

Sørensen (1948) were used. Cluster analysis was performed with the program PRIMER6 (Clarke & Gorley 2005). The similarity in population density was determined by the coefficient of Jaccard-Naumov (Chernov 1975). Species **occurrence** was established according to the three-level classification of Bodenheimer (1955) and Balogh (1958).

Table 1. Abbreviation, name and description of the sampling sites.

Abbr.	Name	Description of the sampling site	Altitude	Coordinates
IrL	„Irakli, vineyard”	An abandoned vineyard with initial stages of a autochthonous forest recovering succession	26 – 34 m a. s. l.	N 42°45' E 27°53'
IrD	„Irakli, oak forest”	Mesophilous oak forest near the Irakli site	45 – 54 m a. s. l.	N 42°45' E 27°53'
IrB	„Irakli, shore ecotone”	Immediately upon the rocky edge above the sea shore, near a mixed pine-oak forest	38 – 47 m a. s. l.	N 42°45' E 27°53'
VBd	„Military base, Domuskolak gully”	On the open path from the oak forest to the beach and the mouth of a brook drying up in summer	8 – 13 m a. s. l.	N 42°47' E 27°53'
VBb	„Military base, pine forest”	Black pine plantation with accompanying bush-grass vegetation	13 – 25 m a. s. l.	N 42°47' E 27°53'
Rs	„Signal repeater, steppe”	Steppe-like habitat at the crest meadow above site Rd	114 – 117 m a. s. l.	N 42°46' E 27°53'
Rd	„Signal repeater, oak forest”	Old oak forest with dense undergrowth of spiny shrubs and moderately xerothermic conditions	112 – 121 m a. s. l.	N 42°46' E 27°53'

Results

During the study altogether 6245 specimens were captured (2163 ex. in 2010 and 4082 ex. in 2011). They belong to 103 species, 34 genera, 15 tribes, 3 subfamilies.

Carabus coriaceus Linnaeus, 1758 (with 950 indivs.) and *Chlaenius nitidulus* (Schrank, 1781) (with 1256 ex.) are the most abundant species. Thirty-one species (30%) are represented by only one individual. The largest number of such species was found in the sample sites Rs (8 species) and VBd (7 species).

For further information about species composition, taxonomic structure etc., see Teofilova (2013) and Teofilova *et al.* (2015).

The analysis of the dominance structure showed the presence of 2 eudominants (*Carabus coriaceus* and *Chlaenius nitidulus*) with total numbers of 35% of all captured specimens, 3 dominants (*Pterostichus melas* (Creutzer, 1799), *Calathus fuscipes* (Goeze, 1777) and *Brachinus crepitans* (Linnaeus, 1758), 2 subdominants (*Harpalus dimidiatus* (Rossi, 1790) and *Acinopus megacephalus* (Rossi, 1794), 11 recedents (*Calosoma sycophanta* (Linnaeus, 1758), *Carabus ullrichi* Germar, 1824, *Carabus convexus* Fabricius, 1775, *Trechus quadristriatus* (Schrank, 1781), *Myas chalybaeus* (Palliard, 1825), *Calathus cinctus* (Motschulsky, 1850), *Laemostenus terricola* (Herbst, 1783), *Agonum (Europhilus) sp.* Chaudoir, 1859, *Harpalus flavicornis* Dejean, 1829, *Ophonus azureus* (Fabricius, 1775) and *Microlestes negrita* (Wollaston, 1854), and 85 subrecedents (Fig. 1). The number of species of various categories for the individual sampling sites is shown in Table 2.

Table 2. Categories of dominance of the Carabidae species in the sampling sites (number of species).

Category	IrL	IrD	IrB	VBd	VBb	Rs	Rd	Total
eudominants	3	2	2	2	2	1	4	2
dominants	3	1	0	2	3	3	1	3
subdominants	0	3	2	3	2	3	2	2
recedents	7	7	11	11	4	9	10	11
subrecedents	25	14	15	22	11	50	24	85

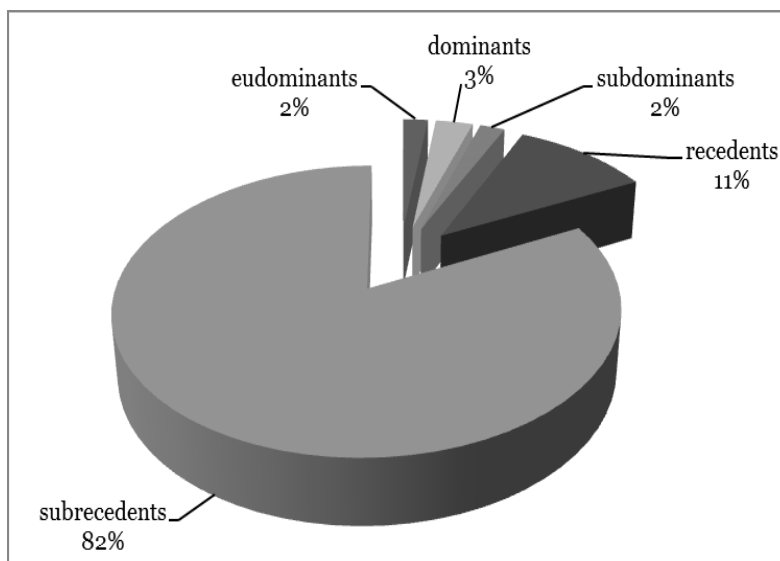


Fig. 1. Dominance structure of the entire carabid complex (number of species).

Eudominants in the individual sampling sites are between 1 and 4, and their total numbers amount to over 50% in all sampling sites with the exception of Rs, where eudominant is one species (*Chlaenius nitidulus*) with numbers of around 40%. For the whole carabid complex eudominants cover 35% of the total numbers. *Carabus coriaceus* can be determined as a permanent eudominant (i. e. established in all of the points), as it belongs to the latter category in all of the sampling sites except one (Rs), where it belongs to the category of dominants, and also is generally a characteristic representative of species with a high degree of dominance (Polak 2004; Varvara & Zugravu 2004). *Calathus fuscipes* has been also established as a dominant in an ecological study of carabid communities from Northern Poland (Aleksandrowicz *et al.* 2009).

In all sampling sites the number of the subrecedents is the highest and it represents over 50% of the number of species. In sampling site Rs they reach 76% of all species.

In relation to the distribution of the number of specimens over the categories of dominance, the biggest share of eudominants and dominants (forming together a 60% of the total population) should be highlighted (Fig. 2).

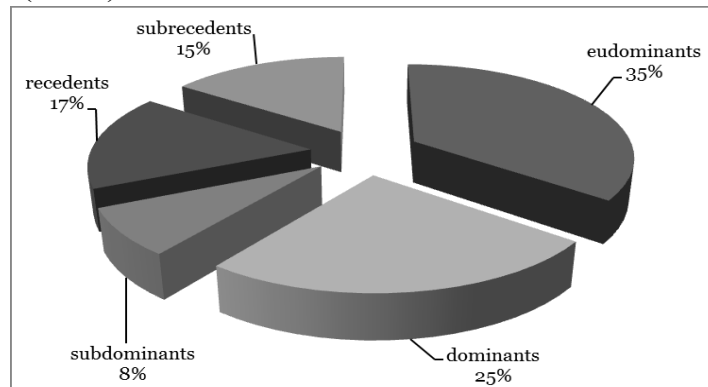


Fig. 2. Distribution of specimens of ground beetles on the levels of the dominant structure for the entire carabid complex (number of specimens).

The **sex structure** of the biocoenose reflects the ratio between individuals of different genders. The analysis of the results showed the total predominance of male over female specimens. The dominance of male beetles is expressed in all catches, with the exception of the summer collection in 2011, when the greatest total catch was also observed (Fig. 3). The least number of caught carabids of both sexes is during the spring collection of 2011, followed by the same in 2010. This pattern appears to be normal and is associated with the natural development of beetles and the absence of activity in the winter season. Distribution of carabids in the individual sampling sites is shown in Fig. 4.

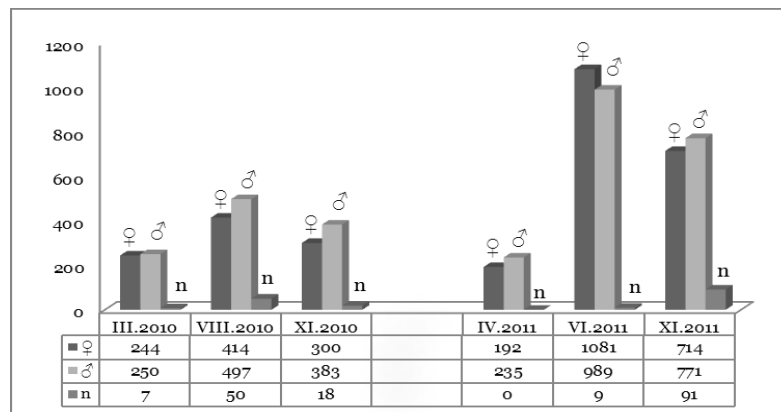


Fig. 3. Distribution of the specimens by gender during the different seasons (♀ – female, ♂ – male, n – not determined) (number of specimens).

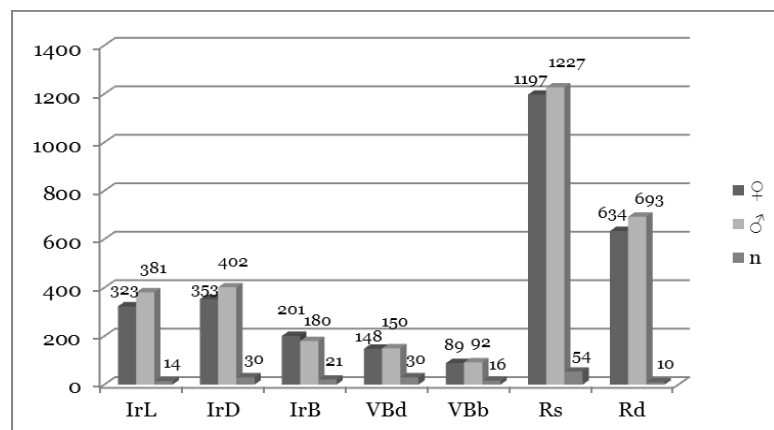


Fig. 4. Distribution of the specimens by gender in the individual sampling sites (♀ – female, ♂ – male, n – not determined) (number of specimens).

The analysis of biodiversity (Table 3) showed, that the largest number of species (S) and specimens (N) is found in the site Rs (S = 66; N = 2478), and the least – in VBb (S = 22; N = 197). Similar pattern is found for pine forest assemblages in the Carpathian Mountains (Balog *et al.* 2012).

The parameters of the **α -diversity** indicated that the seashore in Irakli site (IrB) where dominant species are missing and the category of eudominants is represented by two species, is characterized with the highest concentration of dominance of Simpson (hence, impaired dominant structure). This value is lowest in the old oak forest near signal repeaters north of Irakli site (Rd). The diversity of Margalef, the function of the Shannon and the Pielou's evenness are with the highest values in sampling sites Rd, VBd and Rs (Fig. 5).

Calculated indices of Jaccard and Sørensen, reflecting the **β -diversity**, showed an average or low taxonomic similarity between the individual sampling sites, which indicates a significant heterogeneity of the studied communities.

Table 3. Numerical values of the ecological indices of α -diversity.

Index	IrL	IrD	IrB	VBd	VBb	Rs	Rd
number of species (S)	38	27	30	40	22	66	41
number of specimens, abundance (N)	718	785	402	329	197	2478	1336
Margalef	5,626	3,901	4,836	6,729	3,975	8,317	5,558
Pielou's Evenness	0,68	0,615	0,618	0,696	0,669	0,593	0,712
Shannon	2,474	2,025	2,104	2,566	2,067	2,486	2,644
Simpson's dominance	0,125	0,236	0,269	0,155	0,199	0,191	0,111
Simpson's diversity	0,875	0,764	0,731	0,845	0,801	0,809	0,889
Hill	11,87	7,577	8,196	13,01	7,904	12,01	14,07

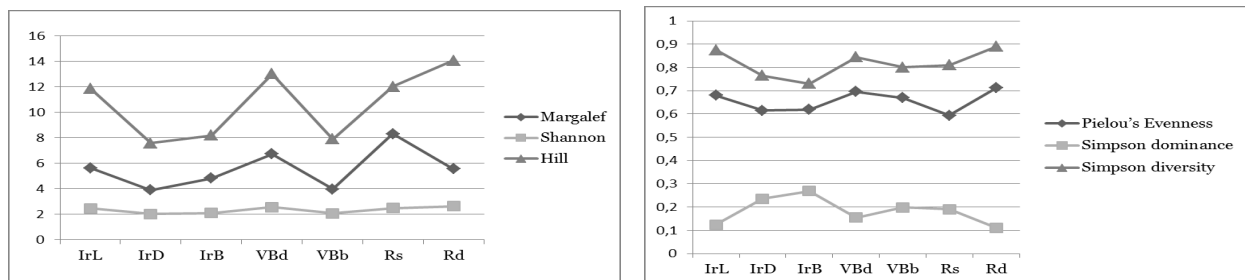


Fig. 5. Indices of α -diversity: Species diversity of Margalef, Shannon and Hill (left) and Evenness, Simpson's concentration of dominance and diversity (right).

Cluster analysis (Fig. 6) showed a lower rate of taxonomic similarity of associations, compared for example with the values obtained in agrocoenoses near the town of Sofia (Kostova 2004; Shishinova *et al.* 2001). Higher is the similarity between the coastal habitats, as well as between the situated in close proximity to each other sampling areas Rs and Rd. Analysis demonstrated the separation of the two main groups, which might be characterized as "forest habitats" and "ecotones".

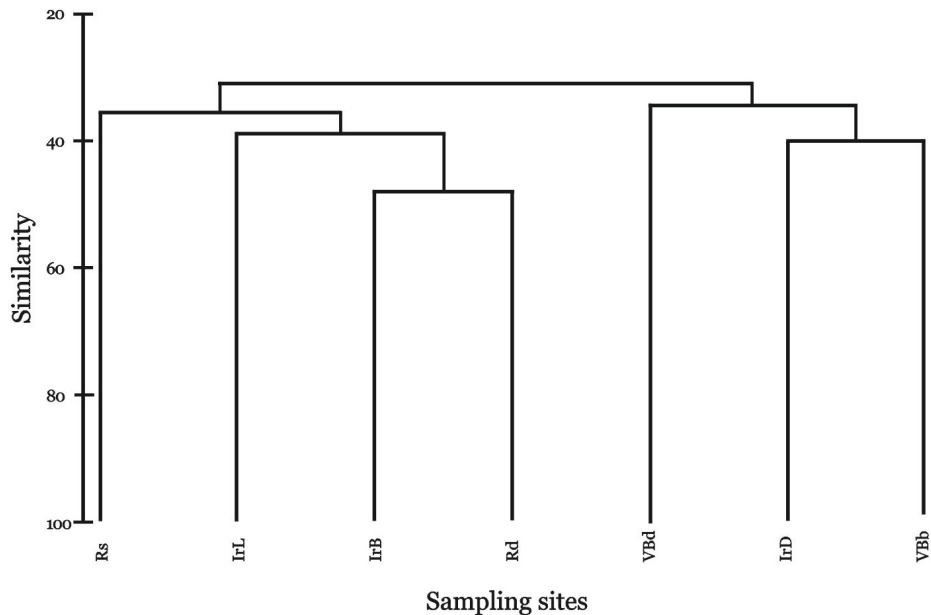


Fig. 6. Cluster analysis of the carabid assemblages of the region of Cape Emine, using the index of similarity of Jaccard.

The results of the analysis of **quantitative data** (Jaccard-Naumov index) also showed the greatest taxonomic similarity between the coastal communities. With high similarity in quantity are both sampling sites located on the territory of the military base (VBd and VBb), followed by the Rs and Rd areas. The lowest quantitative similarity is found between the pine forest (VBb) and the steppe-like habitat (Rs).

The **occurrence** reflects the uniformity or the evenness of the distribution of species in space. The division of species of the entire carabid complex according to their occurrence showed the highest share of rare, random species that occur in less than 20% of the sampling sites (Table 4). Species that were found in all sampling sites and had 100% of occurrence are five: *Carabus coriaceus*, *Trechus quadristriatus*, *Calathus fuscipes*, *Laemostenus terricola* and *Harpalus dimidiatus*.

Table 4. Categories of occurrence of the ground beetles.

Bodenheimer, Balogh	Number of species	Species
permanent (> 50%)	26	<i>Nebria brevicollis</i> , <i>Calosoma sycophanta</i> , <i>Calosoma inquisitor</i> , <i>Carabus ullrichi</i> , <i>Carabus convexus</i> , <i>Carabus coriaceus</i> , <i>Trechus quadristriatus</i> , <i>Myas chalybaeus</i> , <i>Pterostichus melas</i> , <i>Calathus fuscipes</i> , <i>Calathus ambiguus</i> , <i>Calathus melanocephalus</i> , <i>Calathus cinctus</i> , <i>Laemostenus terricola</i> , <i>Amara aenea</i> , <i>Amara anthobia</i> , <i>Amara familiaris</i> , <i>Harpalus rubripes</i> , <i>Harpalus attenuatus</i> , <i>Harpalus tardus</i> , <i>Harpalus dimidiatus</i> , <i>Acinopus megacephalus</i> , <i>Ophonus azureus</i> , <i>Chlaenius nitidulus</i> , <i>Microlestes maurus</i> , <i>Microlestes negrita</i>
auxiliary (25 to 50%)	29	<i>Leistus rufomarginatus</i> , <i>Notiophilus rufipes</i> , <i>Carabus scabriusculus</i> , <i>Cychrus semigranosus</i> , <i>Bembidion lunulatum</i> , <i>Calathus distinguendus</i> , <i>Calathus longicollis</i> , <i>Agonum sp.</i> , <i>Amara communis</i> , <i>Parophonus mendax</i> , <i>Pseudoophonus rufipes</i> , <i>Harpalus flavicornis</i> , <i>Harpalus hospes</i> , <i>Harpalus distinguendus</i> , <i>Ophonus puncticeps</i> , <i>Ophonus similis</i> , <i>Ophonus ardosiacus</i> , <i>Ophonus sabulicola</i> , <i>Ditomus calydonius</i> , <i>Dixus obscurus</i> , <i>Dinodes decipiens</i> , <i>Microlestes fissuralis</i> , <i>Microlestes minutulus</i> , <i>Cymindis ornata</i> , <i>Brachinus berytensis</i> , <i>Brachinus brevicollis</i> , <i>Brachinus crepitans</i> , <i>Brachinus explodens</i> , <i>Brachinus psophia</i>
random (< 25%)	48	all the rest

Discussion

The results of the survey showed strong variation in number of species and specimens in the individual communities, and in the VBb both had lowest values. Poor species composition is typical for pine cultures (Hengeveld 1980; Niemelä 1993; Rainio 2009; Balog *et al.* 2012), while the richest species composition of the steppe-like habitat is likely due to the presence of some extrazonal (i.e. forest) and intrazonal (halophilic) and Mediterranean species (Putchkov 2011). Furthermore, it has been demonstrated, that species diversity decreases towards the affected habitats along with the gradient of urbanization (Niemelä *et al.* 2002, etc.). In addition, anthropogenic interference affects indirectly the dynamics of the ground beetles distribution, causing the homogenization of habitats and thus affecting the availability of the beetles' prey (McKinney 2006).

The large percentage of species, which were represented by a single specimen did not appear to be unusual, as it was also established by other authors (Coddington *et al.* 2009; Ferro *et al.* 2012).

The analysis of the dominance structure showed the presence of eudominant species, which is typical for anthropogenically-influenced and unsustainable ecosystems and was also established by Kodzhabashev & Mollov (2000), Kostova (2004) and Baranová *et al.* (2013). Recurring ecological model in biocoenotic researches is the presence of a few abundant species and the predominance of the variety of rare species (Preston 1962), which is confirmed by the results of this study, showing the greatest number of species from the category of the subprecedents.

In terms of the sex structure, it could be said that the predominance of males over females is completely understandable, because males are more active, and therefore are more likely to fall into the traps.

Low species diversity and high values for Simpson's concentration of dominance in IrD and IrB probably originate from the stronger anthropogenic influence in that areas, especially during the summer season when tourism pressure increases.

Analysis of β -diversity demonstrated the separation of the two main groups – “forest habitats” and “ecotones”. The inclusion of Rd in the second group can be explained by its proximity with the steppe-like habitat (Rs) and the corresponding influx of open habitat dwelling forms in it. Clearly notable is the separation of the Rs site, probably originating from the highest species diversity in this biotope. The immediate statistical vicinity of the abandoned vineyard (IrL) could be resulting from a succession, taking place with similar speed in both sampling sites, despite the spatial distance.

The results of this study indicate, that the grouping of the clusters somewhat follows the humidity gradient. However, it is more likely that the determining factor in the studied case is the synanthropic effect. Similar results were obtained by Kostova (2004) and Shishinova *et al.* (2001), where the first environmental gradient was determined by the type of cultivated vegetation (moisture, respectively), and the second reflected the degree of anthropogenic load. The low percentage of taxonomic similarity of the groups reflects the diversity of the landscape.

Conclusions

For the first time main coenotic structure parameters of the carabid communities in different types of ecosystems from the region of Cape Emine were determined and analyzed.

The dominant structure showed the presence of two eudominants, which is typical for anthropogenically-influenced and unstable ecosystems.

The analysis of biodiversity indices proved: 1) the importance of coastal habitats, associated with the primary hygromesophylic preferences of the ground beetles; 2) the role of steppe-like habitats in the maintenance of the populations of rare and stenotopic species; 3) the negative impact of anthropogenization, expressed as a direct concern of the species and as an altering or destroying of their habitats.

Cluster analysis indicated a low percentage of taxonomic similarity of the coenoses, reflecting the diversity of the biotopes, and also confirmed the importance of soil and air humidity and water balance (Thiele 1977; Fuellhaas 2000; Antvogel, Bonn 2001; Eyre *et al.* 2005), as well as the influence of various anthropogenic activities (Kostova 2004; Teofilova *et al.* 2012) as an additional environmental gradient in the distribution of ground beetles.

Although the territory has a high conservation status, the human element continues to play a negative role in the functioning of the ecosystems (Teofilova *et al.* 2012). Given its determinative importance in the distribution of ground beetles, the imposition of measures for the protection of biodiversity as a whole is needed (Teofilova 2013).

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Soluble proteins in *Messor structor* (Latreille, 1798) (Hymenoptera: Formicidae) populations from Bulgaria – genetic variability and possible usage as population-genetic markers

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Abstract. The genetic variability of ant species determined as *Messor structor* (Latreille, 1798) or close to it (“*M. cf. structor*”) from Bulgaria has been studied using polyacrylamide gel electrophoresis analysis of five soluble protein systems (Sp-1, Sp-2, Sp-3, Sp-4 and Sp-5) corresponding to 5 loci. Four of the studied loci were found to be polymorphic. Two alleles were detected at Sp-1 and Sp-2 loci and three – at Sp-3 and Sp-5. The observed and expected heterozygosities (H_o and H_e) ranged from 0.0 (Yambol) to 0.140 (Topolovo) and from 0.170 (Nova Zagora) to 0.401 (Tvarditsa), respectively. The calculated mean value of inbreeding coefficient (F_{IS}) was 0.8263 and demonstrated high level of inbreeding within populations, which correlated with a low level of observed heterozygosity compared to the expected one. The estimated mean fixation index (F_{ST}) value was 0.2746. Allele frequencies of soluble protein loci were used to estimate Nei’s (1972) genetic distance and to obtain the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Neighbor-Joining (NJ) dendrograms, where Topolovo and Nova Zagora populations were grouped separately than other populations.

Key words: *Messor structor*, soluble proteins, genetic variability, phylogeny.

Introduction

The harvester ant *Messor structor* (Latreille, 1798) is well adapted to different climatic conditions. Being one of the most widespread steppe species, it occurs throughout the European continent. In Bulgaria this species could be found in medium sized populations along all over the country. Harvester ant occur in plain and mountainous terrains – slopes, outskirts, different woodlands, abandoned fields, pastures, unkept lawns, road right of ways. As haploid social insects, they could be an interesting model for different investigations dedicated to behavior ecology, population genetics and phylogeny. (Brian 1983, Cantagali *et al.* 2010, Hölldobler & Wilson 1990).

Although different DNA and isozyme analysis have been done for the ant genetic characterization, (Cantagali *et al.* 2010, Diel *et al.* 2002, Hagen *et al.* 1988, Krieger *et al.* 1999, Pamilo *et al.* 1975, Ross *et al.* 2003, Schlick-Steiner *et al.* 2005, Tomaszewski *et al.* 1973), *M. structor* populations have not been enough investigated. Schlick-Steiner *et al.* (2006) have studied genetic diversity of ants determined as *Messor structor*, collected from different countries in Central Europe and have found out two major mtDNA lineages with partial overlapping distribution. According to this study, specimens from both lineages occur in Bulgaria. These results lead to the idea of possible existence of cryptic species within *M. structor* group.

As a simple method, electrophoresis has been applied for studying the protein and isozyme polymorphisms which exist at levels high enough (about 30% of all loci) for the population genetics aims (Kimura 1968, Tomaszewski *et al.* 1973). There was not much information found concerning population-genetic studies of soluble proteins in different eusocial Hymenoptera species – electrophoretic surveys of available polymorphic protein markers have been conducted for three fire ant species and for two forms of *Solenopsis invicta* (Buren) (Ross *et al.* 1987, Shoemaker *et al.* 1992). In this aspect and having this lack of information in mind, the aim of the study was to evaluate soluble proteins as suitable genetic markers for characterization of genetic variability among populations of harvester ant *M. cf. structor* in Bulgaria.

Material and Methods

***Messor structor* samples**

Totally 310 *M. structor* workers from ten nests, each from different unicolonial geographically distinct population from Southern part of Bulgaria were tested in this study (Fig. 1). The workers were collected directly from the nest. Three to five of the collected ants from a nest were used for the species identification on the base of classical morphometry. According to key of Agosti & Collingwood (1987) and Atanasov & Dluskyi (1992), workers were determined as belonging to *Messor structor* or close to it ("*Messor cf. structor*"). Other individuals were stored at -20°C until electrophoresis. From 30 to 36 workers per population were analyzed for electrophoretic spectrum of soluble proteins (Table 1).

Electrophoretic analysis

The ants were squashed with quartz sand in 0.8 M tris-phosphate buffer at pH 6.7 and left for extraction for 18 hrs at 4 °C. The total body extracts were centrifuged for 15 min at 5 000 rpm at 4 °C and the individual samples were turned to use for electrophoretic analyses. The electrophoretic separation was done in 7.5% polyacrylamide vertical gel (pH 8.9) at 4.5 mA/cm for 3 hrs, together with 3.3% concentrating gel (pH 6.7) and 0.05 M tris-0.2 M glycine electrode buffer at pH 8.3 (Davis 1964, Maurer 1971, Ivanova *et al.* 2000). Soluble proteins were displayed by staining with 5% Commassie Brilliant Blue R250 in 14% trichloroacetic acid.



Fig. 1. Sampling locations.

Table 1. Populations, number of sampled workers tested and allele frequencies (N – number of workers sampled).

Population	N	Locus										
		Sp-1		Sp-2		Sp-3			Sp-4	Sp-5		
		Sp-1 ¹⁰⁰	Sp-1 ⁹⁷	Sp-2 ¹⁰⁰	Sp-2 ⁹⁶	Sp-3 ¹⁰⁰	Sp-3 ⁹⁵	Sp-3 ⁸⁷	Sp-4 ¹⁰⁰	Sp-5 ¹⁰⁰	Sp-5 ⁸⁹	Sp-5 ⁷⁷
Nova Zagora	30	0.933	0.067	0.050	0.950	0.867	0.133	0	1	0.759	0.190	0.052
Elenovo	36	0.743	0.257	0.181	0.819	0.361	0.639	0	1	0.389	0.542	0.069
Yambol	31	1	0	1	0	0.097	0.774	0.129	1	0.387	0.613	0
Plovdiv	32	1	0	0.786	0.214	0.313	0.406	0.281	1	0.625	0.250	0.125
Pesnopoy	31	0.968	0.032	0.048	0.952	0.194	0.806	0	1	0.625	0.250	0.125
Tsalapitsa	30	1	0	0.517	0.483	0.037	0.630	0.333	1	0.083	0.833	0.083
Topolovo	30	0.233	0.767	0	1	0.283	0.517	0.200	1	0.500	0.067	0.433
Velinograd	30	0.933	0.067	0.283	0.717	0.167	0.733	0.100	1	0.167	0.517	0.317
Tvarditsa	30	0.467	0.533	0.383	0.617	0.067	0.667	0.267	1	0.357	0.589	0.054
Merichleri	30	0.717	0.283	0.383	0.617	0.217	0.783	0	1	0.583	0.167	0.250

Statistical analyses

Allele frequencies, mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium, Nei's genetic distance (D), (Nei 1972) and F_{ST} values, (Wright 1965) were calculated using BIOSYS-1 (Swofford 1981) software package. Phylogenetic UPGMA, (Sneath *et al.* 1973) and neighbor-joining, (Saitou & Nei 1987) trees were constructed by usage of Nei's genetic distance, (Nei 1972) and the PHYLIP, (Felsenstein 1993) software package.

Results and Discussion

Samples of protein electrophoretic spectrums could be seen in Fig. 2.

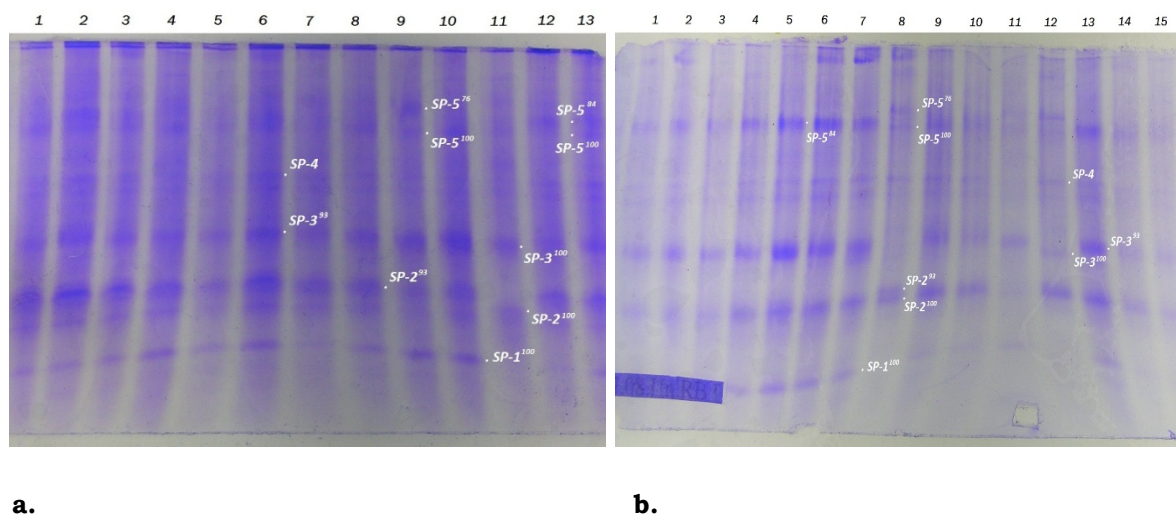


Fig. 2. Spectrum of soluble proteins of *M. structor* workers (7.5% PAGE): **a.** Elenovo population; **b.** Tsalapitsa population.

The allele frequencies calculated are presented in Table 1. Four of the studied protein loci (marked as Sp-1, Sp-2, Sp-3 and Sp-5) were polymorphic in almost of all of the populations, at the 95% level. Sp-4 locus was fixed in all studied populations. In total, two alleles were detected at Sp-1 (Sp-1¹⁰⁰ and Sp-1⁹⁷) and Sp-2 (Sp-2¹⁰⁰ and Sp-2⁹⁶) loci and three alleles – at Sp-3 (Sp-3¹⁰⁰, Sp-3⁹⁵ and Sp-3⁸⁷) and Sp-5 (Sp-5¹⁰⁰, Sp-5⁸⁹ and Sp-5⁷⁷) loci.

The most frequent alleles in the most of the populations were Sp-1¹⁰⁰ (except in Topolovo and Tvarditsa), Sp-2⁹⁶ (except in Yambol, Plovdiv and Tsalapitsa) and Sp-3⁹⁵ (except in Nova Zagora, where Sp-3¹⁰⁰ was more frequent allele). Sp-5¹⁰⁰ was detected as more or the most frequent allele in Nova Zagora, Plovdiv, Pesnopoy, Topolovo and Merichleri. Sp-5⁸⁹ was found as more or the most frequent allele in Elenovo, Yambol, Tsalapitsa, Velingrad and Tvarditsa. Differently than Sp-4 locus which was monomorphic in all of the studied populations with fixed Sp-4¹⁰⁰ allele, Sp-1¹⁰⁰ allele was fixed in populations of Yambol, Plovdiv and Tsalapitsa, Sp-2¹⁰⁰ – in Yambol population and Sp-2⁹⁶ – in Topolovo population.

Mean sample size per locus, mean number of alleles per locus, proportions of polymorphism, observed and expected heterozygosity in the populations tested are presented in Table 2.

Table 2. Mean sample size per locus, mean number of alleles, percentage of polymorphic loci, observed and expected heterozygosity in the populations tested (Standard Errors are included).

Population	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity	
				(Ho)	(He)
Nova Zagora	29.8±0.2	2.0±0.3	80.0	0.014±0.008	0.170±0.067
Elenovo	35.8±0.2	2.0±0.3	80.0	0.094±0.065	0.343±0.096
Yambol	31.0 ±0.0	1.6±0.4	40.0	0.000±0.000	0.173±0.107
Plovdiv	31.2±0.8	2.0±0.4	60.0	0.013±0.013	0.310±0.137
Pesnopoy	31.2±0.2	2.0±0.3	40.0	0.083±0.061	0.203±0.100
Tsalapitsa	29.4±0.6	2.0±0.4	60.0	0.028±0.014	0.261±0.113
Topolovo	30.0±0.0	2.0±0.4	60.0	0.140±0.116	0.311±0.134
Velingrad	30.0±0.0	2.2±0.4	80.0	0.013±0.008	0.317±0.111
Tvarditsa	29.6±0.4	2.2±0.4	80.0	0.054±0.017	0.401±0.101
Merichleri	30.0 ±0.0	2.0±0.3	80.0	0.040±0.016	0.364±0.099

According to the results of this study, the mean number of alleles per locus varied from 1.6 (Yambol) to 2.2 (Velingrad and Tvarditsa). The estimated percentage of polymorphic loci was 40% in Yambol and Pesnopoy, 60% in Plovdiv, Tsalapitsa and Topolovo, 80% in all other populations, using the 0.95 criterion (Table 3). The observed and expected heterozygosities (H_o and H_e) ranged from 0.0 (Yambol) to 0.140 (Topolovo) and from 0.170 (Nova Zagora) to 0.401 (Tvarditsa), respectively (Table 3). There are significant deviations of genotype frequencies from Hardy-Weinberg expectations at all of the loci in most populations ($0.005 \geq P$). Chi-Square (df: 1-3) tests showed that the deviations were generally in favour of homozygotes.

F statistics for the polymorphic loci are presented in Table 3. The estimated mean F_{ST} and F_{IS} values were 0.2746 and 0.8263, respectively. Allele frequencies of soluble protein loci were used to estimate Nei's genetic distance and to obtain the UPGMA and NJ dendrograms, where Topolovo and Nova Zagora populations were grouped separately than other populations (Fig. 3).

Proteins were used fragmentary as electrophoretic markers for genetic characterization of some ants. Shoemaker *et al.* (1992) surveyed enzyme and protein activity in red fire ant *Solenopsis invicta* (Buren) in order to estimate the heterozygosity in populations of these social insects. A total of 110 putative loci were revealed for the fire ants, 15 of which were polymorphic at the 95% level. No polymorphic protein loci were reported in this study (Shoemaker *et al.* 1992). Ross *et al.* (2007) conducted comparative biochemical genetic investigation on three fire ant species in North America with special preference to the two social forms of *S. invicta*. Nineteen enzyme and general protein systems were surveyed yielding the products of 26 presumptive loci. Three of the studied loci were protein – Pro-1, Pro-2 and Pro-3. Rare alleles (frequency less than 0.006) were found at Pro-1 and Pro-3 loci. Pro-3 was defined as polymorphic in *Solenopsis richteri* (Forel) but without useful variability ($p_1 - 0.983$, $q_2 - 0.017$) (Ross *et al.* 2007). Differently than in mentioned surveys, in our study, we found most soluble protein loci as polymorphic. Only Sp-4¹⁰⁰ was fixed in all the studied Bulgarian populations. Sp-1¹⁰⁰, Sp-2¹⁰⁰ and Sp-2⁹⁶ alleles were fixed in only some of populations (Table 1) which indicates them as possible genetic markers. It must be also noted that differently that in surveys mentioned above, in all Bulgarian populations, high percentage of polymorphic loci was found (40% - 80%). Data

established by us concerning the polymorphism of protein loci are in agreement with these concerning genetic polymorphism found in the mitochondrial *COI* gene in *Messor* cf. *structor* workers collected from different countries in Central Europe including Bulgaria (Schlick-Steiner *et al.* 2006). Schlick-Steiner *et al.* (2006) founded out two major mtDNA lineages. Polymorphism in *M. structor* populations from the region near Retz (Austria) was described by Arthofer *et al.* (2005), too. On the base of the microsatellite polymorphism found (seven microsatellite loci with 21 alleles), the authors determined *M. structor* as a model organism for studying the habitat fragmentation influence on social and population structure of social insects (Arthofer *et al.* 2005).

Table 3. F-statistics for polymorphic loci studied.

F - statistics for individual alleles		F(IS)	F(ST)	F(IT)
Locus:Sp-1	Sp-1 ¹⁰⁰	0.9175	0.3847	0.9492
	Sp-1 ⁹⁷	0.9175	0.3847	0.9492
	Mean	0.9175	0.3847	0.9492
Locus:Sp-2	Sp-2 ¹⁰⁰	0.8401	0.4218	0.9076
	Sp-2 ⁹⁶	0.8401	0.4218	0.9076
	Mean	0.8401	0.4218	0.9076
Locus:Sp-3	Sp-3 ¹⁰⁰	0.8197	0.2558	0.8658
	Sp-3 ⁹⁵	0.8528	0.1632	0.8769
	Sp-3 ⁸⁷	0.9659	0.1388	0.9706
	Mean	0.8665	0.1914	0.8921
Locus:Sp-5	Sp-5 ¹⁰⁰	0.7183	0.1611	0.7637
	Sp-5 ⁸⁹	0.8777	0.2276	0.9055
	Sp-5 ⁷⁷	0.5875	0.1325	0.6422
	Mean	0.7485	0.1812	0.7941
Summary of F - statistics at all loci		F(IS)	F(ST)	F(IT)
Locus: Sp-1		0.9175	0.3847	0.9492
Locus: Sp-2		0.8401	0.4218	0.9076
Locus: Sp-3		0.8665	0.1914	0.8921
Locus: Sp-5		0.7485	0.1812	0.7941
Mean		0.8263	0.2746	0.874

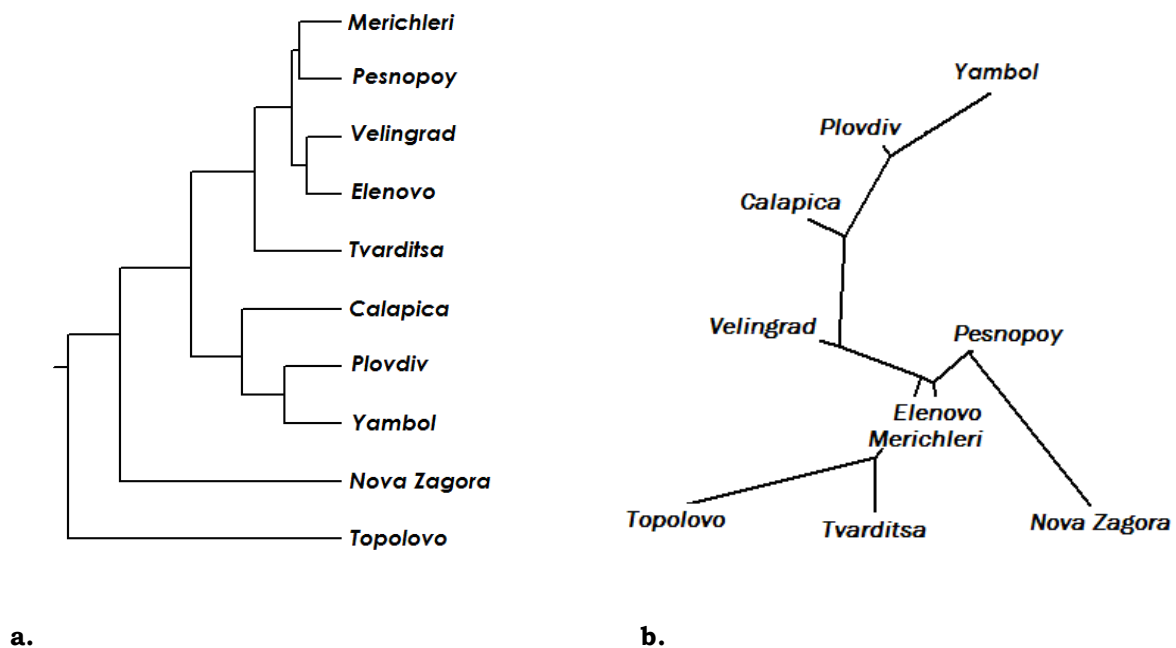


Fig. 3. a. UPGMA dendrogram (Sneath *et al.* 1973); **b.** Neighbour-joining dendrogram (Saitou & Nei 1987).

The expected heterozygosity (H_e) by polymorphic protein loci in Bulgarian populations was higher than the observed one (H_o) in all tested populations and ranged from 0 to 0.170. Calculated mean F_{IS} value over all loci was very high (0.8263), demonstrating high level of inbreeding within populations, (Conner & Hartl 2004) which was in correlation with a low level of observed heterozygosity and disequilibrium. Arthofer *et al.* (2005) noted that deviation from Hardy-Weinberg equilibrium might be partially due to habitat fragmentation.

F_{ST} , which shows the correlation between genes of different individuals within population, (Nei 1977, Weir *et al.* 1984) indicates extend of genetic differentiation among the populations. The mean F_{ST} value of 0.2746 from our study indicates that 27.46% of the overall genetic diversity observed was among populations, which shows that more than 72% of the observed genetic variability resides within populations analysed in our study. This value indicates relatively high level of genetic differentiation among the populations for polymorphic loci studied (Table 3).

The topology of both UPGMA and NJ dendrograms (Fig. 3) confirm the level of genetic diversity within *Messor cf. structor* populations studied and the genetic differentiation among them. In the dendrograms constructed two populations (Topolovo and Nova Zagora) are clearly separated than others which are grouped in two different sub-clusters.

Conclusions

In conclusion, this study provides new information on the genetic variability in populations of harvester ant *M. cf. structor* in Bulgaria on the base of protein electrophoretic analysis. The indicated protein electrophoretic markers could be used appropriately together with other genetic markers for comparisons, discrimination and characterization of *M. structor* populations. Further complex investigations, including different methods and additional genetic markers have to be done, in order to analyze in details genetic structure of *M. structor* populations in Bulgarian and European.

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Evidences for the Lynx recovery in Bulgaria: the Lynx discovered in Western Rhodopes

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Abstract. On photographs taken by a camera trap in the region of Yagodina village, Western Rhodopes, (Bulgaria) the presence of the Eurasian Lynx *Lynx lynx* L. has been registered in the summer of 2014. This is the most reliable to date argument for the presence in Rhodope mountains of this critically endangered species in Bulgaria and for its gradual dispersal from the western mountainous border region of Bulgaria to more appropriate country habitats inland.

Key words: lynx recovery, Bulgaria, Rhodopes.

Introduction

The Lynx, *Lynx lynx* (Linnaeus) was assumed to be extinct in Bulgaria at the beginning of the 40s of the previous century (Spiridonov & Spassov 1985). After this period reports of its appearance appear for the Central Balkan range, Rila mountain, the Western Rhodopes (Dobrostan and Dabrash), the valley of the river Danube and the Ludogorie region, the river Ropotamo, Strandzha, and the Western border mountains (Spassov & Spiridonov 2011). These reports are mentioned and discussed in details in Spassov *et al.* (2006) and Spassov (2007) but they represent unconfirmed data. The presence of the species was proved in the region of the Hunting reserve of Stakevtsi, the Western Balkan in 2004/5 (Spassov *et al.* 2006) where year after, a lynx was found killed illegally. In 2008 it was detected by a camera trap (photo of Zlatanova & Racheva) (Zlatanova *et al.* 2009) in Osogovo mountain and after it was captured on photo twice again in the same mountain till 2011. Obviously, these lynxes are part of the population formed in the last 20-25 years in Eastern Serbia by animals that settled on their own from the Carpathian Mountains (Grubač 2000, Spassov *et al.* 1999, Spassov 2007). Now the lynx is included in the Red book of Bulgaria (second edition) as critically endangered (Spiridonov & Spassov 2011).

It could be considered that the species is entering to more appropriate mountainous biotopes of the country but evidence has not been found so far. Until now, certain evidences were known only for the western mountainous border region of Bulgaria. In the report for *Lynx lynx* in the project of the consortium Natura Bulgaria for mapping and determining the

conservation status of habitats and species (D. Zlatanova) data about the presence of the species inside the country was published. However the presence of lynx in Strandzha seems doubtful and that in Rila and Central Balkan Mountains - uncertain. The pugmarks photographed in Bulgarka Natural Park (Middle Stara Planina) by Al. Dutsov in 2011 (pers. comm.) are very similar to the lynx, but the prints of toes are slightly larger and less oval than in cats. In some cases, hunting dogs have the same size and proportions of traces and the nails are not always printed in the pugmark (personal photo library of authors - NS). For the Rhodopes, although some existing information, there is no credible evidence for lynx presence.

On 09.08.2014, in the region of Western Rhodopes, between Trigrad and Yagodina (the GPS data are at disposal in the authors' data base), two photos were made with embedded camera traps (Fig. 1 and 2) which provide new data on lynx entry into the country. The pictures are unclear and were taken in bad weather conditions, deformed by movement and the slow speed of shooting. Even so, there are some clear identification features that distinguish the animal from the wild cat, the Shepard dog with cut tail (often Asian Shepherd is used in the Rhodopes) and the other mammals: the body has markedly long legs, spotted coloration can be seen on the back, the tail is quite short but not that short as in Central Asian Shepherd, dark end on the tail (especially visible on the second photo). All features of the posture, movement, stride and the general constitution show that this is lynx.



Fig. 1. A night picture of a lynx photographed (V.P.) in August, 2014 by a camera trap in the region between Trigrad and Yagodina.



Fig.2. A second photo of the same animal made by the camera trap an hour after the first one. The dark end of the tail is visible.

After the witness evidence of the Director of the hunting husbandry “Izvora”, Stefan Manolov, he has seen a lynx near the Kastrakli Natural reserve (Devin region) in 1975. In 2007, after other evidence, the hunter Mustafa Tair from Borino noticed a lynx at about 5 km to the west of Borino. About a year earlier, again in this region (Muglenska River) were observed traces similar to the lynx ones. In 2012, according to G. Spiridonov (pers. comm.) a team for study of bear in the above mentioned project of Natura 2000 (G. Spiridonov & L. Asenov) found excrements very similar to the ones of lynx, containing hairs from chamois. The location of the find is about 10 km from the place where the pictures were taken. All these observations are from the region of the camera-trap registration.

The recent pictures are the strongest argument to date for the presence of lynx in the Western Rhodopes. It is difficult to say whether it is a local nucleus or single entry. The species (individual animals) apparently moves to the inland of the country in more appropriate mountain habitats. However, cannot be stated so far that there is a presence of a real lynx population in the country. Cases of reproduction could be registered till now only in the west border area. In 2009, the trail of male and female lynx was detected in the breeding season by Dr. Vassil Ivanov (pers. comm.) in the high mountain zone of Osogovo.

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Случаи на морфологична аномалия на тестиса при бялата риба (*Sander lucioperca* Linnaeus, 1758) в яз. Тополница (Средна гора, България)

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Cases of morphological testis abnormality in pikeperch (*Sander lucioperca* (Linnaeus, 1758) from dam-lake Topolnitsa (Sredna Gora, Bulgaria)

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Abstract. The paper presents cases of abnormal morphology of the pikeperch (*Sander lucioperca*) testis. Three of the investigated 7 males caught in the dam-lake Topolnitsa showed different morphological gonadal alterations. Possible reason for the morphological abnormalities of the male gonads in the pikeperch could be the pollution of this particulare freshwater ecosystem with different industrial wastes such as heavy metals and pesticides.

Key words: *Sander lucioperca*, pikeperch, testis, alterations.

Увод

Атрофията, асиметрията и различни други аномални морфологични изменения на половите органи при различните видове риби е сравнително често явление. Подобни данни са известни за някои сигови риби, езерната пъстърва (*Salvelinus namaycush* Walbaum, 1792), бабушката (*Rutilus rutilus* Linnaeus, 1758) и др. (Bernet *et al.* 2004; Bernet *et al.* 2008; Urbach *et al.* 2008; Wicklund *et al.* 1994). Ratty *et al.* (1990) съобщават за асиметрично развитие на тестисите на белия тон (*Thunnus alalunga* Bonnaterre, 1788), Hayakawa & Kobayash (2012) за гонадите на колизията (*Colisa lalia* Hamilton, 1822), а Kobelkowsky (2012) за яйчниците на някои видове риби от ихтиофауната на Мексико. Случаи с интерсексуални характеристики на гонадите се съобщават от Rzepkowska *et al.* 2014; Jackson *et al.* 2006; Poprek *et al.* 2006 и др. За естествени популации на бялата риба (*Sander lucioperca* Linnaeus, 1758) подобни данни липсват, но съществуват изследвания за влиянието на различни вещества върху морфологията и диференцирането на гонадите им (Jarmołowicz *et al.* 2013; Zakes & Zakes 2006).

Причините за аномалните морфологичните изменения на тестисите могат да се различат (Urbach *et al.* 2008). Понякога те са генетични, а в други случаи се дължат на въздействието на абиотични и антропогенни фактори на околната среда – химикали, тежки метали и др. Често пъти произходът на аномалиите остава неизяснен, но е възможно появата им, когато е свързана с нарушения на сперматогенезата или оплодителната способност на сперматозоидите, в една или друга степен да се отрази на устойчивото развитие на съответната популация.

Язовир Тополница е разположен в Средна гора (42°25'90" N, 23°59'38" E) на площ от 570 ha. Според Янчева и кол. (2011) той е подложен на дългогодишно антропогенно замърсяване, като основните емитери са рудниците, металургичните заводи, комбинатите за цветни метали и хвостохранилищата. Язовирът и едноименната река се замърсяват в продължение на много години с тежки метали (Велчева и Николов 2009; Georgieva *et al.* 2014; Velcheva *et al.* 2012; Yancheva *et al.* 2014a; Yancheva *et al.* 2014b).

Настоящата статия е първото съобщение за случаи на установени аномални морфологични изменения на тестиса на бялата риба у нас, което е много рядко явление за този вид.

Материал и Методи

Изследването е извършено върху 7 мъжки екземпляра. Рибите са уловени с хрилни мрежи в яз. Тополница в близост до язовирната стена през първата половина на месец март 2014 г. Те са доставени живи в Института по рибарство и аквакултури, Пловдив, където са държани във вани при проточен режим в продължение на една седмица без да се хранят, след което са притеглени и сецирани. Теглото на тестисите е измерено в свежо състояние с точност до 100 mg, а гонадосоматичният индекс (GSI, %) е изчислен по формулата:

$$q=W_1.100/W,$$

където q - гонадосоматичният индекс; W₁-теглото на гонадите; W-теглото на рибата.

Резултати и Дискусия

Изследваните бели риби са полово зрели индивиди с тегло на тялото от 0.850 g до 1315 g. Тестисите са с тегло от 2.1 g до 6.3 g, а изчисленият гонадосоматичен индекс е в границите от 0.2 до 0.5 %.

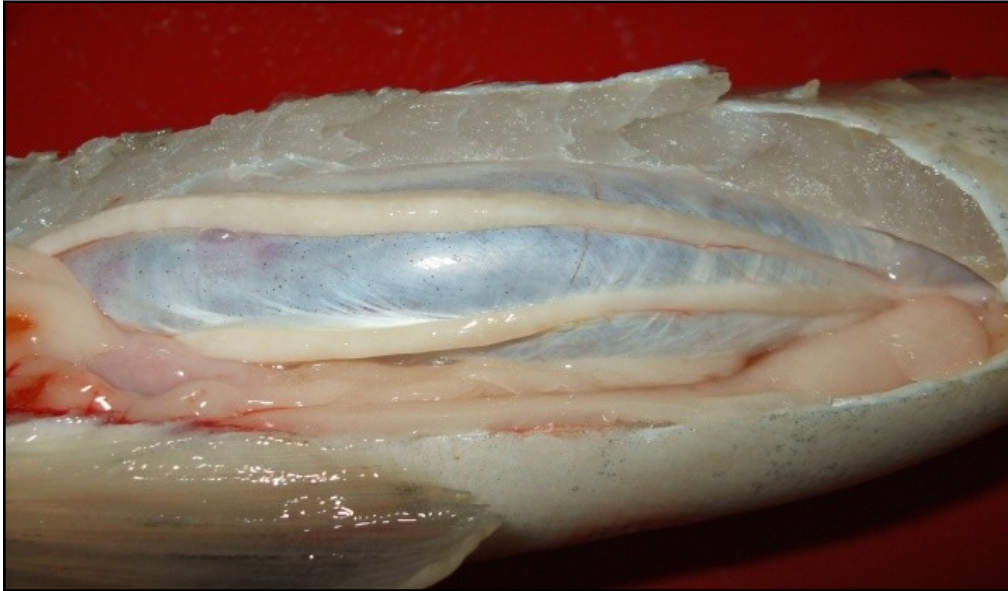
При проведеното изследване са установени аномалии в развитието на мъжките полови органи при 3 бели риби, което представлява 42.8 % от всички изследвани индивиди, т.е. процентът на тези риби е значителен. Отчетените отклонения са при екземпляри с маса 1255 g, 1280 g и 1190 g, при които гонадосоматичният индекс е съответно 0.3%, 0.2% и 0.2% и не се различава от този при другите индивиди.

Тестисите на бялата риба с нормална морфология са чифтен орган с еднакво развити два симетрични дяла, които имат удължена лентовидна форма (Фиг. 1). Отклонение от посочените характеристики са случаите на неравномерно развитие на дяловете, наблюдавано при една от изследваните бели риби (Фиг. 2). Значително по-голяма морфологична аномалия са установените при други две риби морфологични изменения, които засягат формата и дължината на тестисите, както и равномерното развитие на двата им дяла като чифтен орган (Фиг. 3). На този етап причините за това явление не могат да бъдат посочени, като за целта са необходими допълнителни изследвания.

Заклучение

Една от вероятните причини за морфологични аномалии на мъжките полови органи на белите риби в яз. Тополница е посоченото от редица автори замърсяване на

водоема с индустриални отпадъци. Установеният значителен процент на риби с морфологични изменения на тестисите дава основание за провеждането на допълнително изследване на проблема с цел доказване на евентуалното влияние на замърсяването на язовира върху морфологията на половите органи на рибите.



Фиг. 1. Нормално развити тестиси на бяла риба (*Sander lucioperca*) от яз. Тополница.

Fig. 1. Normal testes of pike perch (*Sander lucioperca*) from dam-lake Topolnitsa.



Фиг. 2. Неравномерно развитие на дяловете на тестисите на бяла риба (*Sander lucioperca*) от яз. Тополница.

Fig 2. Abnormal development of both testicular lobes of pikeperch (*Sander lucioperca*) from dam-lake Topolnitsa.



Фиг. 3. Аномалии на тестисите на бели риби (*Sander lucioperca*) от яз. Тополница.

Fig 3. Abnormalities of pikeperch (*Sander lucioperca*) testes from dam-lake Topolnitsa.

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Fossil and subfossil remains of birds and mammals from the Mirizlivka cave (Vidin Region – NW Bulgaria)

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Abstract. The fossil/subfossil record of Mirizlivka cave numbers 17 taxa – 3 species of birds and 14 species of large mammals, 3 of them disappeared from the recent fauna of Bulgaria, and 6 other globally extinct.

Key words: Pleistocene, Quaternary, Fossil birds and mammals, Bulgaria, extinct mammals

Introduction

The Mirizlivka cave is located in the Bashovitsa locality of the Belogradchishki Venets rock massive near the village of Gara Oreshets (Vidin Region) at about 750 m a.s.l. The cave is 40 m long (Popov 1936).

The present short note aims to summarize all available information on the fossil record of the cave and to evaluate its importance to the Bulgarian prehistory and paleozoology.

Results

History of excavations and dating of the finds

First data on the fossil fauna of the cave has been published by Popov (1933). In 1931 this researcher uncovered Paleolithic tools and bones of carnivores and ungulate mammals (mainly of Würm age; Popov 1933). According Popov (1936) the cave was firstly excavated in 1924 and 1929 by two local teachers from Vidin, V. Atanasov and L. Filkov. In 1931 Rafail Popov and V. Atanasov started new excavations and discovered that the cave was inhabited in the Paleolithic by man.

The presence of remains of the fossil vole *Villania* sp. according V. Popov (1983) is an indication of the early-Pleistocene deposits in the cave. On the other hand, the materials collected by R. Popov date part of the deposits as late Pleistocene.

In October 1993 we made a sounding (0.60 m depth) at 12 m from the cave entrance and collected some bone remains of mammals and birds.

Birds

In contrast to mammalian remains, the avian ones according to Popov (1933) were extraordinary scant. In the Pleistocene layers he found “two bones of wings. One is a metacarpus, 51.0 mm long, and the other – a radius of 90.0 mm length.” (p. 62).

In 1993 we also collected bones of three species of birds: the disappeared from the recent Bulgarian avifauna black grouse, Eurasian magpie and an unidentified small passeriform.

Mammals

Popov (1936): All the collected material represented only Pleistocene (Paleolithic) artefacts and animal bones. He lists: “*Ursus spelaeus*, *Ursus arctos*, *Hyaena spelaea*, *Canis lupus*, *Canis vulpes*, *Canis* sp. *Cervus elaphus*, *Bos* sp., *Equus caballus fossilis*, *Equus asinus* (*Equus hydruntinus* after Spassov (1982), *Capra aegagrus*, *Rhinoceros tichorhynus*, Aves.”. In other publication Popov (1936) lists some modified list of large mammals: “*Ursus spelaeus*, *U. arctos*, *Hyaena spelaea*, *Canis lupus*, *Canis vulpes*, *Canis* sp., *E. caballus fossilis*, *Rhinoceros tichorhinus*, *C. elaphus*, *Cervus* sp., *Bos primigenius*, and *Castor fiber*”.

Nikolov (1983) lists remains of seven large mammals: *Coelodonta antiquitatis*, *Crocota spelaea*, *Canis lupus*, *Capra aegagrus* (*Capra ibex* according to N. Spassov), *Equus ferus ferus*, *Ursus spelaeus* and *Cervus elaphus*.

In October 1993 we collected some bones and teeth of brown bear (*U. arctos*), and small ruminants (sheep/goat) (Ovicaprinae indet.).

Conclusions

Thus, for the last 90 years of the excavation of the cave, the established fossil record numbers 17 taxa – 3 species of birds and 14 species of large mammals, 3 of them disappeared from the recent fauna of Bulgaria, and 6 globally extinct, i. e. over 50 per cent of the established fossil/subfossil fauna of birds and mammals is recently either disappeared or extinct (Table 1). This ranks the Mirizlivka cave along with the well known caves Magurata and Kozarnika, as one of the most important nature monuments in the Northwest Bulgaria.

All excavation campaigns so far were accidental and a special archaeological and paleozoological exploration of the cave would be very promising.

Table 1. Species composition of the fossil birds and mammals from the Mirizlivka cave (after modern systematics)

No	Common Name	Scientific name	Disappeared from the recent fauna of Bulgaria	Globally extinct
BIRDS (AVES)				
Galliformes				
1.	Black Grouse	<i>Tetrao tetrix</i> (Linnaeus, 1758)	+	
Passeriformes				
2.	Eurasian Magpie	<i>Pica pica</i> Linnaeus, 1758		
3.	Unidentified small passeriform	Passeres fam. indet.		
MAMMALS (MAMMALIA)				
Carnivora				
4.	Cave bear	<i>Ursus spelaeus</i> Rosenmüller, 1794		+
5.	Brown bear	<i>Ursus arctos</i> Linnaeus, 1758		
6.	Cave hyena	<i>Crocota crocota spelaea</i> (Goldfuss 1823)		+
7.	Grey wolf	<i>Canis lupus</i> Linnaeus, 1758		
8.	Red fox	<i>Vulpes vulpes</i> (Linnaeus, 1758)		
9.	Unidentified canine	<i>Canis</i> sp.		
Artiodactyla				
10.	Alpine ibex	<i>Capra ibex</i> Linnaeus, 1758	+	
11.	Red deer	<i>Cervus elaphus</i> Linnaeus, 1758		
12.	Auroch	<i>Bos primigenius</i> (Bojanus, 1827)		+
13.	Unidentified large bovid	<i>Bos</i> sp.		
Perissodactyla				
14.	Woolly rhinoceros	(<i>Coelodonta antiquitatis</i> (Blumenbach, 1807)		+
15.	Tarpan	<i>Equus ferus ferus</i> Boddaert, 1785		+
16.	European ass	<i>Equus hydruntinus</i> Regalia, 1907		+
Rodentia				
17.	Eurasian beaver	<i>Castor fiber</i> Linnaeus, 1758	+	

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Malacofauna of the catchment area of Rusenski Lom River (North Bulgaria)

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Abstract. Freshwater and terrestrial molluscs were studied at the catchment area of Rusenski Lom River (North Bulgaria), and 121 species were found. Second records of *Bythiospeum iltchokolevi* Georgiev & Glöer, 2015, *Discus rotundatus* (Müller, 1774), and *Oxyloma dunkeri* (L. Pfeiffer, 1865) in Bulgaria were reported.

Key words: Gastropoda, Bivalvia, species diversity, Bulgaria, Rusenski Lom.

Introduction

Büttner (1928) reported the first data for the area, three mollusc species for the Rusenski Lom River at the city of Russe: *Theodoxus transversalis* (C. Pfeiffer, 1828), *Esperiana esperi* (A. Férussac, 1823), and *Esperiana daudebartii acicularis* (A. Férussac, 1823). Later *Gyraulus laevis* (Alder, 1838) was reported for the Danube at Russe (Angelov, 1960). Russev *et al.* (1987) in their hydrobiological study of Rusenski Lom River reported 17 species: *Unio tumidus* Retzius, 1788, *U. crassus* Philipsson, 1788, *Sphaerium* sp., *Pisidium* sp., *Theodoxus danubialis* (C. Pfeiffer, 1828), *Th. fluviatilis* (Linnaeus, 1758), *Th. transversalis* (C. Pfeiffer, 1828), *Esperiana esperi* (A. Férussac, 1823), *E. daudebartii acicularis* (A. Férussac, 1823), *Holandriana holandrii* (C. Pfeiffer, 1828), *Lithoglyphus naticoides* (C. Pfeiffer, 1828), *Valvata piscinalis* (O. F. Müller, 1774), *Galba truncatula* (O. F. Müller, 1774), *Radix auricularia* (Linnaeus, 1758), *Radix* sp., *Planorbis* sp., and *Physella acuta* (Draparnaud, 1805). Information for terrestrial gastropods from the region is scarce and concerning mainly the city of Russe. Petrbok (1941) reported the snail *Arianta arbustorum* (Linnaeus, 1758) from the region of Russe. Later for the same area were published *Aegopinella minor* (Stabile, 1864), *Monacha cartusiana* (O. F. Müller, 1774), *Xerolenta obvia* (Menke 1828) (Pinter & Pinter, 1970), *Bulgarica varnensis* (L. Pfeiffer, 1848) (Damjanov & Likharev 1975), and *Limax maximus* (Linnaeus, 1758) (Wiktor 1983). Nordsieck (2008) reported two more species from Tabachka village – *Alinda biplicata* (Montagu, 1803) and *Laciniaria plicata* (Draparnaud, 1801). Irikov & Georgiev (2008) reported the terrestrial species *Agardhiella truncatella* (L. Pfeiffer, 1841) from deposits of Rusenski Lom River. Subai (2011) revised their materials and reported *Agardhiella armata* (Clessin, 1887) and *Agardhiella parreyssii* (L. Pfeiffer, 1848) from the same area. Neubert (2014) reported *Helix albescens* Rossmässler, 1839 from Tabachka vill. area (I. Dedov Leg.).

Material and Methods

The study was carried out during 12 years period (from 2002 to 2014) at the catchment of Rusenski Lom River (Fig. 1). Snails were collected mostly by the first author. One sample was collected by D. Georgiev (04.07.2006, deposits of Beli Lom River near vill. Ivanovo), and one by I. Dedov (26.5.2007, NE-border of vill. Tabachka, the region of the village). The material was deposited in the collection of the second author. The species are arranged alphabetic. The abbreviation of the conservation status of the species is after Cuttelod et al. (2011). Abbreviation: CS - conservation status (from low to high extinction risk): NA – not applicable, LC – least concern, NT – near threatened, VU – vulnerable, EN – endangered. EM – endemics: BGE – Bulgarian endemic species, BLE – Balkan endemic species.



Fig. 1. Approximate position of the study area: the catchment of Rusenski Lom River.

Results

Class Bivalvia

Anodonta woodiana (Lea, 1834)

26.03.2011, fishponds at vill. Basarbovo; 25.04.2012, vill. Katselovo, fishpond, Yama area; CS – NA.

Corbicula fluminea (O. F. Müller, 1774)

13.07.2011, Russe at Liulyaka base; 11.05.2013, Rusenski Lom River at vill Basarbovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – NA;

Dreissena polymorpha (Pallas, 1771)

13.07.2011, Russe at Liulyaka base; 11.05.2013, Rusenski Lom River at vill Basarbovo; CS – NA;

Musculium lacustre (Müller, 1774)

11.05.2013, Rusenski Lom River at vill Basarbovo; 12.11.2013, fishponds near vill. Stalpishte; 04.06.2014, Cherni Lom River at vill. Krepcha; CS – LC;

Pisidium amnicum (Müller, 1774)

16.03.2011, Rusenski Lom River at vil. Basarbovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – LC;

Pisidium casertanum (Poli, 1791)

01.01.2012, Malko Selishte and Golyamo Selishte water sources near vill. Katselovo; 08.08.2012, water source at Beli Lom River, near vill. Pisanets; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 11.05.2013, Rusenski Lom River at vill. Basarbovo; CS – LC;

Pisidium moitessierianum (Paladilhe, 1866)

08.10.2013, spring on the bank of Cherni Lom River at vill. Pepelina; CS – LC;

Pisidium personatum Malm, 1855

09.03.2012, vill. Katselovo, "Startchi Krak" watersource; 21.04.2012, vill. Garchinovo, watersource; 21.04.2012, vill. Tserovets, watersource; 13.09.2012, water source on the hill near the fishponds of vill. Valenik; CS – LC;

Sphaerium rivicola (Lamarck, 1818)

16.06.2011, Cherni Lom River at vill. Cherven; 05.10.2011, Rusenski Lom River at Russe; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – LC;

Unio crassus Philipsson, 1788

01.11.2010, Cherni Lom river at vill. Katselovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – VU;

Unio tumidus Retzius, 1788

26.03.2011, Rusenski Lom River at vill. Basarbovo; CS – VU;

Class Gastropoda**Freshwater snails*****Acroloxus lacustris*** (Linnaeus, 1758)

12.11.2013, fishponds near vill. Stalpishte; CS – LC;

Anisus leucostoma (Millet, 1813)

13.07.2011, Russe at Liulyaka base; 06.11.2012, Cherni Lom River at vill. Krepcha; CS – LC;

Anisus septemgyratus (Rossmässler, 1835)

13.07.2011, Russe at Liulyaka base; 08.10.2013, spring on the bank of Cherni Lom River at vill. Pepelina;

Anisus spirorbis (Linnaeus, 1758)

02.11.2012, deposits of Cherni Lom River, "Varbovska Vodenitsa" area; 09.07.2014, Cherni Lom River at vill. Katselovo;

Bithynia tentaculata (Linnaeus, 1758)

13.07.2011, Russe at Liulyaka base; CS – LC;

Bythiospeum iltchokolevi Georgiev & Glöer, 2015

21.04.2012, vill. Tserovets, watersource; EM – BGE;

Remark: This is the second record of this species.

***Bythiospeum* sp.**

08.08.2012, water source at Beli Lom River, near vill. Pisanets; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 08.10.2013, spring on the bank of Cherni Lom River at vill. Pepelina;

Borysthenia naticina (Menke, 1845)

13.07.2011, Russe at Liulyaka base; 02.11.2012, Cherni Lom River at vill. Karan Varbovka; CS – LC;

Esperiana daudebartii acicularis (A. Férussac, 1823)

01.11.2010, Cherni Lom river at vill. Katselovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; 13.07.2011, Russe at Liulyaka base; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; 13.10.2013, vill. Katselovo, "Lakanska Yama", lower fishpond of Ivan Kovacha; CS – LC;

Esperiana esperi (A. Férussac, 1823)

01.11.2010, Cherni Lom river at vill. Katselovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; 11.05.2013, Rusenski Lom River at vill. Basarbovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – LC;

Ferrissia fragilis (Tryon 1863)

14.01.2014, Cherni Lom River, Mochura area near vill. Katselovo; 11.05.2013, Rusenski Lom River at vill Basarbovo;

Galba truncatula (O. F. Müller, 1774)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 26.03.2011, fishponds at vill. Basarbovo; 21.04.2012, vill. Garchinovo, watersource; 02.11.2012, deposits of Cherni Lom River, "Varbovska Vodenitsa" area; 11.05.2013, Rusenski Lom River at vill Basarbovo; CS – LC;

cf. *Grossuana* sp.

01.01.2012, 17.09.2012, vill. Katselovo, "Panitchka" watersource; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo;

Gyraulus albus (O. F. Müller, 1774)

13.07.2011, Russe at Liulyaka base; 02.11.2012, Cherni Lom River at vill. Karan Varbovka; 12.11.2013, fishponds near vill. Stalpishte; CS – LC;

Gyraulus crista (Linnaeus, 1758)

04.06.2014, Cherni Lom River at vill. Krepcha; 09.07.2014, Cherni Lom River at vill. Katselovo; CS – LC;

Gyraulus laevis (Alder, 1838)

11.10.2012, Danube at Russe, Matey Island, shells; 08.10.2013, spring on the bank of Cherni Lom River at vill. Pepelina; CS – LC;

Holandriana holandrii (C. Pfeiffer, 1828)

13.07.2011, Russe at Liulyaka base; CS – LC;

Lithoglyphus naticoides (C. Pfeiffer, 1828)

26.03.2011, fishponds at vill. Basarbovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; 13.07.2011, Russe at Liulyaka base; 23.10.2012, water source at vill. Ostritsa; 11.05.2013, Rusenski Lom River at vill Basarbovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – LC;

Lymnaea stagnalis (Linnaeus, 1758)

03.08.2012, Russe, GZ "Liulyaka", estuary of the canal, near the water catchment; 19.10.2012, Stalpishte area, the hills above the fishponds; 12.11.2013, fishponds near vill. Stalpishte; CS – LC;

Physella acuta (Draparnaud, 1805)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 26.03.2011, fishponds at vill. Basarbovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; 02.11.2012, deposits of Cherni Lom River, "Varbovska Vodenitsa" area; 11.05.2013, Rusenski Lom River at vill Basarbovo; 13.07.2011, Russe at Liulyaka base; 13.10.2013, vill. Katselovo, "Lakanska Yama", lower fishpond of Ivan Kovacha; 12.11.2013, fishponds near vill. Stalpishte; CS – LC;

Planorbarius corneus (Linnaeus, 1758)

13.07.2011, Russe at Liulyaka base; 12.11.2013, fishponds at vill. Stalpishte; CS – LC;

Planorbis planorbis (Linnaeus, 1758)

26.03.2011, fishponds at vill. Basarbovo; 13.07.2011, Russe at Liulyaka base; 23.07.2013, Cherni Lom River near Murdova Leska gorge; 02.11.2012, deposits of Cherni Lom River, "Varbovska Vodenitsa" area; 12.11.2013, fishponds near vill. Stalpishte; CS – LC;

Radix auricularia (Linnaeus, 1758)

26.03.2011, fishponds at vill. Basarbovo; 13.10.2013, vill. Katselovo, "Lakanska Yama", lower fishpond of Ivan Kovacha; CS – LC;

Radix cf. balthica (Linnaeus, 1758)

26.03.2011, fishponds at vill. Basarbovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; CS – LC;

Segmentina nitida (O. F. Müller, 1774)

12.11.2013, fishponds near vill. Stalpishte; CS – LC;

Theodoxus danubialis (C. Pfeiffer, 1828)

01.11.2010, Cherni Lom river at vill. Katselovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; 16.06.2011, Cherni Lom River at vill. Cherven; 13.07.2011, Russe at Liulyaka base; 05.10.2011, Rusenski Lom River at Russe; 11.05.2013, Rusenski Lom River at vill. Basarbovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – LC;

Theodoxus fluviatilis (Linnaeus, 1758)

13.07.2011, Russe at Liulyaka base; CS – LC;

Theodoxus transversalis (C. Pfeiffer, 1828)

26.03.2011 and 11.05.2013, Rusenski Lom River at vill. Basarbovo; 23.07.2013, Cherni Lom River near Murdova Leska gorge; CS – EN.

Valvata piscinalis (O. F. Müller, 1774)

13.07.2011, Russe at Liulyaka base; 06.11.2012, water source near vill. Garchinovo; 04.06.2014, Cherni Lom River at vill. Krepcha; CS – LC;

Viviparus acerosus (Bourguignat, 1862)

13.07.2011, Russe at Liulyaka base; CS – LC;

Viviparus viviparus (Linnaeus, 1758)

16.03.2011, Rusenski Lom River at vil. Basarbovo; 26.03.2011, Rusenski Lom River at vill. Basarbovo; 13.07.2011, Russe at Liulyaka base; CS – LC;

Terrestrial snails***Acanthinula aculeata*** (O. F. Müller, 1774)

18.03.2012, vill. Pepelina; 26.04.2012, vill. Karan Varbovka; 20.03.2013, vill. Krepcha; CS – LC;

Agardhiella armata (Clessin, 1887)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 29.08.2011, vill. Katselovo; 29.09.2012, vill. Basarbovo; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area; CS – LC;

Remark: The specimens collected by D. Georgiev on 04.07.2006 were deposited in the coll. of Peter Subai, and identified by him (Subai, 2011);

Agardhiella parreyssii (L. Pfeiffer, 1848)

Reported for the region around Danube by (Subai, 2011); CS – LC , EM – BLE;

Alinda biplicata (Montagu, 1803)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 10.09.2012, vill. Ostritsa, Kanarak dere, after Dzhabaza area;

Aegopinella minor (Stabile, 1864)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 20.04.2011, vill. Katselovo, Gartchinovi terasi area; 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 10.09.2012, vill. Ostritsa, Kanarak dere, after Dzhabaza area;

Arion subfuscus (Draparnaud, 1805)

25.04.2014, vill. Karan Varbovka; 09.05.2014, vill. Pepelina;

Arion silvaticus Lohmander, 1937

10.04.2012, vill. Katselovo; 20.03.2013, vill. Krepcha; 08.10.2013, vill. Pepelina; 09.05.2014, vill. Ostritsa;

Bulgarica varnensis (L. Pfeiffer, 1848)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 24.03.2012, vill. Ivanovo, above "Pismata" (nest of Egyptian vulture); 31.03.2012, sport base Liulyaka, Russe, near canals; 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 19.10.2012, Stalpishte, the gorge going to Trastenik; EM – BLE;

Candidula rhabdotoides (A. J. Wagner, 1927)

13.07.2011, Russe, Liulyaka sport base; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; CS – LC, EM – BLE;

Carpatica stussineri (A. J. Wagner, 1895)

31.03.2012, sport base Liulyaka, Russe, near canals;

Carychium minimum O. F. Müller, 1774

24.04.2011, vill. Katselovo; 19.10.12, vill. Stalpishte; 06.11.2012, vill. Krepcha; 23.07.2013, vill. Cherven;

Carychium tridentatum (Risso, 1826)

24.04.2011, vill. Katselovo; 29.09.2012, vill. Basarbovo; 19.10.12, vill. Stalpishte; 02.07.2013, Russe; 08.10.2013, vill. Pepelina; 12.05.2014, vill. Krepcha;

Cattania balcanica (Kobelt, 1876)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 06.02.2011, Russe, Liulyaka sport base; 16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; EM – BLE;

Cecilioides acicula (O. F. Müller, 1774)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 02.11.2012, deposits of Cherni Lom River, "Varbovska Vodenitsa" area;

Cecilioides jani (De Betta et Martinati, 1855)

31.03.2012, sport base Liulyaka, Russe, near canals;

Cepaea vindobonensis (Férussac, 1821)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 07.11.2010, vill. Basarbovo; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 19.10.2012, Stalpishte, the gorge going to Trastenik; 02.03.2013, Russe; 13.06.2013, vill. Pisanets and vill. Tsar Kaloyan. CS – LC;

Cochlicopa lubrica (O. F. Müller, 1774)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo;

Cochlicopa lubricella (Porro, 1838)

23.10.2011, vill. Ostritsa; 18.03.2012, vill. Pepelina; 08.08.2012, vill. Pisanets; 13.06.2013, vill. Tsar Kaloyan; 23.07.2013, vill. Cherven; 19.11.2013, vill. Stalpishte; 20.12.2013, vill. Nisovo; 12.05.2014, vill. Nisovo;

Cochlicopa nitens (Gallenstein, 1852)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 02.11.2012, deposits of Cherni Lom River, "Varbovska Vodenitsa" area;

Chondrina avenacea (Bruguière, 1792)

24.03.2012, vill. Ivanovo, above "Pismata" (nest of Egyptian vulture); 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; CS – LC;

Chondrula microtragus (Rossmässler, 1839)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 20.04.2011, vill. Katselovo, Gartchinovi terasi

area; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 24.03.2012, vill. Ivanovo, above "Pismata" (nest of Egyptian vulture); 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 19.10.2012, Stalpishte area, the hills above the fishponds; 19.10.2012, Stalpishte, the gorge going to Trastenik; CS – LC, EM – BLE;

Chondrula tridens (O. F. Müller, 1774)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; CS – NT;

Cochlodina laminata (Montagu, 1803)

16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 20.04.2011, vill. Katselovo, Gartchinovi terasi area; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 10.09.2012, vill. Ostritsa, Kanarak dere, after Dzhambaza area;

Daudebardia brevipes (Draparnaud, 1805)

18.03.2012, vill. Pepelina; 06.04.2013, vill. Basarbovo; 04.06.2014, vill. Krepcha;

Daudebardia rufa (Draparnaud, 1805)

14.02.2011, vill. Katselovo; 26.03.2011, vill. Basarbovo; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 26.04.2013, vill. Shirkovo; 23.07.2013, vill. Cherven; 21.10.2013, vill. Karan Varbovka; 27.03.2014, vill. Nisovo; 04.06.2014, vill. Krepcha;

Deroceras laeve (O. F. Müller, 1774)

20.04.2011, vill. Katselovo; 23.07.2013, vill. Cherven; 01.09.2013, vill. Ivanovo; 17.09.2013, Russe; 20.03.2014, vill. Basarbovo;

Deroceras sturanyi (Simroth, 1894)

13.06.2013, vill. Tsar Kaloyan; 10.07.2013, vill. Katselovo;

Deroceras turcicum (Simroth, 1894)

16.03.2011, vill. Basarbovo; 21.03.2011, vill. Katselovo; 21.04.2012, vill. Tserovets; 02.11.2012, vill. Karan Varbovka; 20.03.2013, vill. Krepcha; 08.10.2013, vill. Pepelina; 23.07.2013, vill. Cherven; 10.12.2013, vill. Pisanets; 20.12.2013, vill. Nisovo;

Discus rotundatus (Müller, 1774)

02.07.2013, Russe, Matey Island, deposits of Danube River;

Remark: This is the second report of this species in Bulgaria;

Euconulus fulvus (O. F. Müller, 1774)

05.03.2014, vill. Katselovo;

Euomphalia strigella (Draparnaud, 1801)

07.02.2011, Russe; 18.03.2011, vill. Basarbovo; 08.08.2012, vill. Tsar Kaloyan; 20.03.2013, vill. Krepcha; 14.04.2013, vill. Katselovo; 13.06.2013, vill. Pisanets; 23.07.2013, vill. Cherven; 01.09.2013, vill. Ivanovo; CS – LC;

Fruticicola fruticum (O. F. Müller, 1774)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 26.12.2010, vill. Katselovo; 01.05.2011, vill. Shirokovo; 15.12.2011, vill. Ostritsa; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 19.10.2012, vill. Mechka; 19.10.2012, Stalpishte, the gorge going to Trastenik; 07.11.2012, vill. Karan Varbovka; 27.11.2012, vill. Basarbovo; 20.03.2013, vill. Krepcha; 23.07.2013, vill. Cherven; CS – LC;

Granaria frumentum (Draparnaud, 1801)

19.11.2013, vill. Stalpishte; CS – LC;

Helix lucorum Linnaeus, 1758

12.11.2010, vill. Katselovo; 15.12.2011, vill. Ostritsa; 18.03.2012, vill. Pepelina; 03.03.2013, Russe; 13.06.2013, vill. Pisanets and vill. Tsar Kaloyan; CS – LC;

Helix pomatia Linnaeus, 1758

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 12.11.2010, vill. Katselovo; 15.12.2011, 06.02.2011, Russe; 13.06.2013, vill. Pisanets and vill. Tsar Kaloyan; 23.07.2013, vill. Cherven; 01.09.2013, vill. Ivanovo; CS – LC;

Krynickillus urbanskii (Wiktor, 1971)

27.05.2014, on trunks of cherry trees (*Cerasus* sp.), broad leaf forest near vill. Katselovo;

Laciniaria plicata (Draparnaud, 1801)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries;

Limax cinereoniger Wolf, 1803 – complex

12.03.2014, vill. Pepelina; 14.04.2014, vill. Katselovo; 28.06.2014, vill. Pisanets;

Limax graecus (Simroth, 1889) – complex

13.03.2011 and 15.07.2014, Russe; EM – BLE;

Limax maximus Linnaeus, 1758 - complex

11.11.2011, vill. Katselovo; 21.04.2012, vill. Tserovets; 31.08.2013, vill. Nisovo; 25.04.2014, vill. Karan Varbovka;

Limacus flavus Linnaeus, 1758

28.04.2011, vill. Katselovo; 20.05.2014, Russe;

Lindholmiola girva (Frivaldsky, 1835)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 24.03.2012, vill. Ivanovo, above “Pismata” (nest of Egyptian vulture); 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 10.09.2012, vill. Ostritsa, Kanarak dere, after Dzhambaza area; 19.10.2012, Stalpishte area, the hills above the fishponds; 19.10.2012, Stalpishte, the gorge going to Trastenik; CS - LC, EM – BLE;

Macedonica marginata (Rossmässler, 1835)

18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; EM – BLE;

Mediterranea inopinata (Uličny, 1887)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 01.01.2012, vill. Katselovo, at “Panitchka” watersource; 28.04.2014, vill. Svalenik; 12.05.2014, vill. Krepcha;

Merdigera obscura (O. F. Müller, 1774)

29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; CS – LC;

Monacha carascaloides (Bourguignat, 1855)

26.12.2010, vill. Katselovo; 16.03.2011, vill. Basarbovo; 05.10.2011, Russe; 16.06.2011, vill. Tabachka; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 29.10.2011, vill. Bazovets; 01.05.2012, vill. Shtraklevo; 16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 24.03.2012, vill. Ivanovo, above “Pismata” (near a nest of Egyptian vulture); 20.03.2013, vill. Krepcha; 30.04.2013, vill. Zahari Stoyanovo; CS – LC;

Monacha cartusiana (O. F. Müller, 1774)/ ***claustralis*** – complex

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 26.12.2010, vill. Katselovo; 06.02.2011, Russe; 16.03.2011, vill. Basarbovo; 05.10.2011, Russe, Liulyaka sport base; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 15.12.2011, vill. Ostritsa; 18.03.2012, vill. Pepelina; 31.03.2012, sport base Liulyaka, Russe, near canals; 08.08.2012, vill. Tsar Kaloyan; 19.10.2012, Stalpishte, the gorge going to Trastenik; 23.07.2013, vill. Cherven; 01.09.2013, vill. Ivanovo; 19.11.2013, vill. Stalpishte; 22.04.2014, vill. Bazovets;

Monacha claustralis (Menke 1828)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; CS – LC;

Morlina glabra striaria (Westerlund 1881)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 10.09.2012, vill. Ostritsa, Kanarak dere, after Dzhambaza area;

Oligolimax annularis (Studer, S. 1820)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; CS – LC;

Oxyloma elegans (Risso, 1826)

13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 13.10.2013, vill. Katselovo, “Lakanska Yama”, at lower fishpond of Ivan Kovacha;

Oxyloma dunkeri (L. Pfeiffer, 1865)

03.08.2013, small isle at Cherni Lom River, vill. Katselovo;

Remark: This is the second report of this species in Bulgaria;

Perforatella incarnata (O. F. Müller, 1774)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo; 14.02.2011, vill. Katselovo; 13.07.2011, Russe; 29.08.2012, vill. Nisovo, along Mali Lom River, between Golyam and Malak Nisovski monasteries; 10.09.2012, vill. Ostritsa, Kanarak dere, after Dzhambaza area; 15.12.2011, vill. Ostritsa; 18.03.2012, vill. Pepelina; 24.03.2012, vill. Ivanovo; 29.08.2012, vill. Nisovo; 22.04.2014, vill. Bazovets; 04.06.2014, vill. Krepcha; CS – LC;

Pomatias rivularis (Eichwald, 1829)

16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 20.04.2011, vill. Katselovo, Gartchinovi terasi area; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 10.09.2012, vill. Ostritsa, Kanarak dere, after Dzhambaza area;

Pseudochondrula seductilis (Rossmässler, 1846)

20.04.2011, vill. Katselovo, Gartchinovi terasi area; CS – VU;

Pseudotrichia rubiginosa (Schmidt, 1853)

10.01.2011, vill. Katselovo; 15.09.2011, Matey Island, Danube River, Russe; 19.10.2012, vill. Stalpishte, Danube River bank; CS – LC;

Punctum pygmaeum (Draparnaud, 1801)

16.03.2011, vill. Basarbovo; 14.04.2012, vill. Katselovo; 20.03.2013, vill. Krepcha; 13.06.2013, vill. Tsar Kaloyan; 23.07.2013, vill. Cherven; 27.03.2014, vill. Nisovo;

Pupilla muscorum (Linnaeus, 1758)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 21.04.2012, vill. Garchinovo, near watersource; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area; CS – LC;

Pupilla sterri (Voith, 1838)

02.07.2013, Russe; 09.07.2014, vill. Katselovo; 07.09.2014, vill. Karan Varbovka; CS – LC;

Sphyradium doliolum (Bruguière, 1792)

21.03.2011, vill. Katselovo; 12.05.2011, vill. Pepelina; 23.10.2012, vill. Ostritsa; 20.03.2013, vill. Krepcha; 13.06.2013, vill. Tsar Kaloyan; 23.07.2013, vill. Cherven; 20.12.2013, vill. Nisovo; 22.04.2014, vill. Bazovets; CS – LC;

Succinea oblonga Draparnaud, 1801

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 09.03.2012, vill. Katselovo, at “Startchi Krak” watersource; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area;

Succinea putris (Linnaeus, 1758)

10.08.2013, Liulyaka Island at Danube River near Russe;

Tandonia budapestensis (Hazay, 1881)

07.02.2011, Russe; 13.02.2011, vill. Katselovo; 26.03.2011, vill. Basarbovo; 23.07.2013, vill. Cherven;

Tandonia cristata (Kaleniczenko, 1851)

24.04.2012, vill. Katselovo; 20.03.2013, vill. Krepcha; 08.10.2013, vill. Pepelina; 31.08.2013, vill. Nisovo; 04.10.2013, vill. Basarbovo; 21.10.2013, vill. Karan Varbovka; 09.05.2014, vill. Ostritsa;

Tandonia kusceri (H. Wagner, 1931)

07.02.2011, Russe; 06.03.2011, vill. Basarbovo; 12.12.2012, vill. Katselovo; 08.10.2013, vill. Pepelina; 12.05.2014, vill. Krepcha;

Truncatellina claustralis (Gredler, 1856)

16.03.2011, rocky gorge, near the fishponds of vill. Basarbovo; 23.06.2011, vill. Nisovo; 20.03.2013, vill. Krepcha; CS – LC;

Truncatellina cylindrica (Férussac, 1807)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 31.03.2012, sport base Liulyaka, Russe, near canals; 19.10.2012, Stalpishte, the gorge going to Trastenik; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area; CS – LC;

Vallonia costata (O. F. Müller, 1774)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 09.03.2012, vill. Katselovo, at “Startchi Krak” watersource; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 31.03.2012, sport base Liulyaka, Russe, near canals; 21.04.2012, vill. Garchinovo, near watersource; 21.04.2012, vill. Tserovets, near watersource; 08.08.2012, water source at Beli Lom River, near vill. Pisanets; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 19.10.2012, Stalpishte, the gorge going to Trastenik; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area; CS – LC;

Vallonia enniensis (Gredler, 1856)

09.03.2012, vill. Katselovo, at “Startchi Krak” watersource; 21.04.2012, vill. Garchinovo, near watersource; 08.08.2012, water source at Beli Lom River, near vill. Pisanets; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area; CS – NT;

Vallonia pulchella (O. F. Müller, 1774)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 09.03.2012, vill. Katselovo, at “Startchi Krak” watersource; 18.03.2012, Urum Punar area, under the rocks, near vill. Pepelina; 13.09.2012, vill. Vlenik, Kiusara area, at water source, near the road to vill. Katselovo; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area; 17.09.2012, vill. Katselovo, at “Panitchka” watersource; 19.10.2012, Stalpishte, the gorge going to Trastenik; CS – LC;

Vertigo antivertigo (Draparnaud, 1801)

19.10.12, vill. Stalpishte; 09.07.2014, vill. Katselovo; CS – LC;

Vertigo pygmaea (Draparnaud, 1801)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 20.04.2011, vill. Katselovo; 29.09.2012, vill. Basarbovo; 11.10.2012, Russe; 19.10.2012, vill. Stalpishte, the gorge going to Trastenik; 02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area; 06.11.2012, vill. Krepcha; 11.05.2013, vill. Basarbovo; 13.06.2013, vill. Tsar Kaloyan; 23.07.2013, vill. Cherven; 08.10.2013, vill. Pepelina; 05.03.2014, vill. Katselovo; 27.03.2014, vill. Nisovo; 07.09.2014, vill. Karan Varbovka; CS – LC;

Vitrea diaphana (Studer, 1829)

12.05.2014, vill. Krepcha;

Vitrea pygmaea (Boettger, 1880)

02.11.2012, deposits of Cherni Lom River, “Varbovska Vodenitsa” area;

Vitrea subrimata (Reinhardt, 1871)

05.10.2011, Russe; 01.01.2012, vill. Katselovo; 29.09.2012,, vill. Basarbovo; 02.11.2012, vill. Karan Varbovka; 08.10.2013, vill. Pepelina; 27.03.2014, vill. Nisovo;

Xerolenta obvia (Menke, 1828)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 05.10.2011, under a rock arc, Russe, at the big bridge above Rusenski Lom River; 26.12.2010, vill. Katselovo; 29.10.2011, vill. Bazovets; 29.11.2011, Russe; 15.12.2011, vill. Ostritsa; 21.04.2012, vill. Garchinovo, near watersource; 01.05.2012, vill. Shtraklevo; 08.08.2012, vill. Pisanets; 19.10.2012, Stalpishte area, the hills above the fishponds; 19.10.2012, Stalpishte, the gorge going to Trastenik; 30.04.2013, vill. Zahari Stoyanovo; CS – LC;

Zebrina detrita (O. F. Müller, 1774)

26.5.2007, NE-border of vill. Tabachka, at limestone rocks beside cave; 24.03.2012, vill. Ivanovo, above “Pismata” (nest of Egyptian vulture); 19.10.2012, Stalpishte area, the hills above the fishponds;

Zonitoides nitidus (O. F. Müller, 1774)

04.07.2006, deposits of Beli Lom River near vill. Ivanovo;

At least 120 mollusc species were registered at the area (freshwater clams - 11, freshwater snails - 31, and terrestrial snails - 78 species). Some of them are still with unclear taxonomic statute as for example *Bythiospeum* sp., and the species from the complexes of *M. cartusiana*, *Limax* spp.

Two bivalve species were confirmed for the region (*U. tumidus*, *U. crassus*), 9 are new findings. From fresh-water gastropods 12 previously reported species were confirmed for the region (*E. daudebartii acicularis*, *E. esperi*, *G. truncatula*, *G. laevis*, *H. holandrii*, *L. naticoides*, *R. auricularia*, *Ph. acuta*, *T. danubialis*, *T. fluviatilis*, *T. transversalis*, *V. piscinalis*) and 19 are new findings.

From the terrestrial gastropods 7 previously reported species were confirmed for the region (*A. minor*, *A. biplicata*, *B. varnensis*, *L. plicata*, *L. maximus*, *M. cartusiana*, *X. obvia*) and one was not found (*A. arbustorum*), 71 terrestrial gastropod species are new records for the region.

Second records of *Bythiospeum iltchokolevi* Georgiev & Glöer, 2015, *Discus rotundatus* (Müller, 1774), and *Oxyloma dunkeri* (L. Pfeiffer, 1865) in Bulgaria were reported. New distributional data about some invasive aquatic molluscs as *Anodonta woodiana*, *Corbicula fluminea*, and *Ferissia fragilis* was provided.

According Cuttelod et al. (2011) all clams from the region have conservation status: 3 species are in category NA – not applicable, 6 species – least concern and 2 (*U. crassus* and *U. tumidus*) are VU – vulnerable. From the fresh-water snails 24 species are in category LC – least concern, and one in EN – endangered (*T. transversalis*). One fresh water snails is Bulgarian endemic species (*B. iltchokolevi*). From the terrestrial gastropods 29 species are in category LC – least concern, 2 species are in NT – near threatened, and one (*P. seductilis*) is VU – vulnerable. Eight terrestrial gastropods are Balkan Peninsula endemics.

Two more species *H. lucorum* and *H. pomatia* are objects of the Bulgarian conservation legislation, as they are included in Section III (Regulated use of plant and animal species), Art. 41 (1), Annex 4 of “Biodiversity Act”.

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Contribution to the knowledge of the gastropods (Gastropoda, Pulmonata) of Ilinska and Plakinska Mountains (Republic of Macedonia)

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Abstract: The work presents results of a collecting trip in July 2008 in the mountains Ilinska and Plakinska (Republic of Macedonia). The work summarizes data for distribution and ecology of 19 species from the mountains. The data collected during the expedition provide the first information for terrestrial gastropods in this region of Macedonia.

Key words: terrestrial gastropods, fauna, Ilinska and Plakinska mountains, Republic of Macedonia.

Introduction

Ilinska Planina Mountains is a comparatively high mountain among the medium-high mountains in Macedonia. The highest peak of the mountain is Liska (1908 m). Ilinska Mountain has a complex geology, the bedrock is generally composed of Devonian marbles and phylitic schist which is similar to other medium-high mountains in this broader region. Ilinska Mts. is characterized by moderate continental climate and mountainous climate on its upper parts. Average altitude is 1159 m, total area 293.65 km² (Melovski *et al.* 2013).

Plakenska Planina Mts. represents the middle part of the Ilinska Planina Mts.-Plakenska Planina Mts.-Bigla Range. Unlike Ilinska Planina Mts. on the north, Plakenska Planina Mts. is almost exclusively composed of silicate bedrock. Moderate continental climate is dominant in the region, and mountainous climate prevails on higher elevations. Average altitude is 1288 m, total area 191.57 km² (Melovski *et al.* 2013).

For both mountains there is no information for the gastropods' fauna. In the course of the collecting trip, a new gastropod species was found, described by Dedov & Subai (2012) (*Gyralina nautilopsis*, Dedov & Subai, 2012).

Material and Methods

The materials were collected in 2008 (Tab. 1). The snails were collected by hand and soil-sifting. The material is deposited in the private collection of the author. The localities are presented in tab. 1. Used abbreviations: ad. – adult, juv. – juvenile, d. – dead, l. – alive.

Results and Discussion

The collected specimens are presented in Tab. 2. All findings are new to the region and the first registered gastropods for both mountains. The knowledge for those regions is fragmented and scarce and any summaries will be too preliminary.

Tab. 1. Visited localities in the mountains Ilinska and Plakinska, Republic of Macedonia, VII 2008, leg. I. Dedov (№5, leg. G. Ivanov, №8, leg., S. Hristovski and I. Dedov).

№	locality	habitat	Altitude (m)
Ilinska Mts			
1.	Road-fork to vill. Golemo Ilino, Zli Dol River Gorge	<i>Carpinus</i> , <i>Quercus</i> forest	800 – 820
2.	Strumna area	limestone meadows	1692 – 1846
3.	Strumna area	<i>Fagus</i> forest, in rotten trunks	1700 – 1750
4.	Area of Ilinska Tsurkva	<i>Fagus</i> forest	1550
5.	Plosh area	<i>Fagust</i> forest, wet stones	1200 - 1350
6.	Near cave Yaorets	limestones	1030
7.	Iglar area	limestone rocks, under stones	1550
Plakenska Mts			
8.	Up to village Sloeshtitsa, in and around Zmeyova Cave	Deciduous forest on limestone base	950

Tab. 2. Terrestrial gastropod species from the mountains Ilinska and Plakinska, Republic of Macedonia (in alphabetic order). The numbers of localities correspond with those in Table 1.

№	species	localities (specimens)
1.	<i>Allaegopis skandergianus</i> (Polinski, 1924)	3 (1 juv. / 1.)
2.	<i>Bulgarica vetusta</i> (Rossmässler, 1836)	8 (8 ad. / 1.)
3.	<i>Candidula rhabdotoides</i> (A.J. Wagner, 1928)	4 (1 ad. / 1.)
4.	<i>Chilostoma (Dinarica) serbica</i> (Kobelt, 1872)	3 (3 ad. / 1.)
5.	<i>Chondrulara microtragus</i> (Rossmässler, 1839)	4 (1 ad. / d.)
6.	<i>Daudebardia rufa</i> (Draparnaud, 1805)	3 (3 ad. / 1.)
7.	<i>Deroceras</i> af. <i>turcicum</i> (Simroth, 1894)	3 (2 ad. / 1.)
8.	<i>Gyalina (Gyalina) nautilopsis</i> Dedov & Subai, 2012 (locus typicus)	7 (2 ad. / d., 5 juv. / d.)
9.	<i>Gyalina (Gyalina)</i> cf. <i>velkovrhi</i> Riedel, 1985	8 (3 ad. / d.)
10.	<i>Helix lucorum</i> Linnaeus, 1758	4 (1 ad. / 1.), 6 (2 ad. / 1.)
11.	<i>Jamina quadridens</i> (Müller, 1774)	4 (1 ad. / 1.)
12.	Limacidae	5 (1 juv. / 1.)
13.	<i>Monacha</i> sp.	1 (1 ad. / d.)
14.	<i>Oxychilus</i> cf. <i>glaber</i> . (Rossmässler, 1835)	1 (1 juv. / d.), 8 (3 juv. / 1., 2 ad. / d.)
15.	<i>Tandonia</i> sp.	3 (1 juv. / 1.)
16.	<i>Triloba thaumasia</i> (Sturany, 1907)	3 (7 ad. / 1.)
17.	<i>Vitrina pellucida</i> (Müller, 1774)	5 (1 ad. / 1.)
18.	<i>Xerolenta obvia</i> (Menke, 1828)	2 (9 ad. / 1.), 4 (10 ad. / 1.)
19.	<i>Zebrina detrita</i> (Müller, 1774)	4 (2 ad. / 1.)

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Ground beetles (Coleoptera: Carabidae) from the region of Cape Emine (central Bulgarian Black sea coast). Part III. Spatial distribution and gradient analysis

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Abstract. The ground beetle fauna of the area of Cape Emine was studied. Over the period 2010 – 2012 adult carabid beetles were collected. The investigation was carried out at 13 sampling sites and pitfall traps were used. During the study altogether 12618 specimens were captured. Mathematical processing of the data by gradient analysis with methods for classification and ordination of the communities was performed. Analysis of distribution of the zoogeographical categories and life forms was done. The primary role of the humidity and vegetation as ecological factors affecting the distribution of the communities of ground beetles was proven.

Keywords: Carabidae, Cape Emine, gradient analysis, ordination, classification

Introduction

All species occur in a characteristic, limited range of habitats and within their range they tend to be most abundant around their particular environmental optimum. The composition of biotic communities thus changes along the environmental gradients.

Mathematical methods in ecology aim at carrying out numerical analyses of environmental data, and determination and interpretation of their multidimensional structure. In the gradient analysis the significance of the gradients of the environment (conditions, within which a biological object may exist) is determined by means of complex mathematical methods (Ter Braak 1994). Gradients are useful abstraction for explaining the distribution of organisms in space and time (Austin 1985).

Methods of the classification perform an objective grouping of the species in similar communities. This serves for separation of the data into groups of similar samples and underlies in resource management and implementation of conservation policies. Ordination is the tool for exploratory analysis of community data with no prior information about the environment and is used for arranging of the samples on individual axes, which reflects the general trends or gradients among the data. Ordination is helping in understanding the organism-environment relationships. Direct methods use the data about the species and the environment and indirect methods use only the data about the species (Ter Braak 1994).

Practical application of the ordination is related to the management of ecosystems, for example, a given habitat management with a view to achieving the desired conditions in the future, and also with management experiments and recovery efforts towards disturbed environment.

Cape Emine is included within the protected zones BG0001004 „Emine – Irakli” under Directive 92/43 for the protection of natural habitats and wild flora and fauna, and BG0002043 „Emine” under Directive 79/409 for the protection of birds. The geostrategic location of the studied territory, its diverse topography and the contingent climate contributed to the mixing of the representatives of various ecological and biogeographical complexes, which has led to the formation of peculiar biocoenoses.

This study aims to analyze the effect of the local gradients on the spatial differentiation of the ground beetle communities, life forms and chorotypes within the context of their use in site assessment and conservation ecology.

Material and Methods

In connection with the participation in a project for biological monitoring studies in the area of Cape Emine, a series of observations and samplings were carried out in 2010 – 2012. Ground beetles were collected with terrestrial pitfall traps (Barber 1931; Hertz 1927; Dahl 1896). The traps were made of plastic bottles, buried at the level of the ground surface. As fixation fluid a 4 – 10% solution of formaldehyde was used. In all sampling areas 10 – 14 pitfall traps were set. The total number of the traps was 126.

The investigation was performed at 13 different sampling sites (Table 1). Further on in the text, the abbreviated expressions given in the Table 1 were used. In seven of the sampling areas were conducted full catches in 2010 and 2011 (in spring, summer and autumn), as well as additional samplings in 2012 (in spring and autumn). In the remaining areas samplings did not cover the full period of research, and therefore the analysis of the spatial distribution of the carabids was based on the number of the caught specimens for 100 trapdays.

Specimens were identified according to: Kryzhanovskij (unpublished data), Arndt *et al.* (2011), Lindroth (1974), Hůrka (1996), Reitter (2006), Trautner & Geigenmüller (1987) and are deposited in the Carabidae collection of the Institute of Biodiversity and Ecosystem Research (BAS).

Two methods of the gradient analysis were used – classification and ordination. Classification of the studied communities was done with the TWINSpan (TWo-way INDicator SPecies ANalysis) statistical program for multidimensional factorial analysis (Hill & Šmilauer 2005). As a splitting method, it is released from the disadvantages giving too much weight to the statistical artifacts related to the size of the sample (Minkova 2002).

Ordination is a method for removing the subjectivity in describing and assessing the assemblages. It is a complex, multidimensional statistical analysis, which uses the combination of the species composition in the communities and the distribution of the populations under the environmental conditions (Ter Braak 1994). In this study the software product CANOCO 4.5 was used (Ter Braak & Šmilauer 2002).

Table 1. Abbreviations, description and location (altitude a. s. l. and geographic coordinates) of the sampling sites. **Ir** is used for all samples in the Irakli site; **V** is used for the two samples from the Military base southern from the town of Obzor; **R** is used for the sampling sites near the signal repeaters, located northern from the Irakli site.

Abbr.	Description of the sampling site	Altitude	Coord.
IrL	An abandoned vineyard in the Irakli site, with initial stages of a autochthonous forest recovering succession	26–34 m	N 42°45' E 27°53'
IrD	Oak forest (<i>Quercus</i> spp.) near the Irakli site	45–54 m	N 42°45' E 27°53'
IrB	Shore ecotone, immediately upon the rocky edge above the sea shore in Irakli, near a mixed pine-oak forest	38–47 m	N 42°45' E 27°53'
IrV	Along the shore of the Vaya river, unaffected by the “cleansing” of the river bed (in 2007), with typical wet riparian habitats and rich and abundant vegetation	14–15 m	N 42°45' E 27°52'
IrN1	In the ecotone between farmlands and the shore of the Vaya river, among dense grass-shrub ruderal and weed vegetation, at the edge of the wheat field	14–15 m	N 42°45' E 27°52'
IrN2	In newly formed wheat field coenose beside the Vaya river, near the main road E87	19–20 m	N 42°45' E 27°52'
Ir2e	In the ecotone between IrN2 and the river	19–20 m	N 42°45' E 27°52'
VBd	Domuskolak gully, with traps located on the open path from the oak forest to the beach and in the mouth of a brook drying up in summer	8–13 m	N 42°47' E 27°53'
VBb	Black Pine plantation with accompanying bush-grass vegetation	13–25 m	N 42°47' E 27°53'
Rs	Steppe-like habitat at the crest meadow above site Rd, near the signal repeaters	114–117 m	N 42°46' E 27°53'
Rd	Old oak forest with dense undergrowth of spiny shrubs and moderately xerothermic conditions	112–121 m	N 42°46' E 27°53'
Rn	In the newly formed wheat field agrocoenose at the place of the former steppe-like habitat (Rs)	131–134 m	N 42°46' E 27°52'
Rne	In the ecotone between Rn and the small island with remained natural grass-shrub vegetation	131–134 m	N 42°46' E 27°52'

Results

During the study altogether 12618 specimens were captured. They belong to 134 species, 46 genera, 18 tribes, 3 subfamilies.

The largest number of both species and specimens was established in the steppe-like habitat (Rs), and the lowest – in the pine plantation (VBb). Poor species composition seems to be typical for pine cultures, as similar patterns are found for pine forest assemblages in other studies too (Hengeveld 1980; Niemelä 1993; Rainio 2009; Balog *et al.* 2012), while the richest species composition of the steppe-like habitat is likely resulting from the presence of some extrazonal (i.e. forest) and intrazonal (halophilic) and Mediterranean species (Putchkov 2011). Thirty seven species (28%) were represented by only one individual. Similar percentage does not appear to be unusual, as it is also established by other authors

(Coddington *et al.* 2009; Ferro *et al.* 2012). We found the largest number of such species in the sampling sites IrV (9 species), Rs (8 species) and VBd (7 species).

The most abundant species were *Chlaenius nitidulus* (with 1927 indivs.), *Nebria brevicollis* (1890 indivs.), *Carabus coriaceus* (1240 indivs.), *Brachinus crepitans* (1213 indivs.), *Pterostichus melas* (880 indivs.), *Calathus fuscipes* (623 indivs.) and *Harpalus dimidiatus* (555 indivs.).

For further information about species composition, taxonomic and community structure etc., see Teofilova (2015) and Teofilova *et al.* (2015).

Classification of the assemblages

In the Appendix are given the results of the 3-levels TWINSPAN classification. On the first level of division two groups divide – IrV and IrN1 form the right group, with an indicator species *Carabus cancellatus*, and all the other sampling sites are included in the left group. On the second level of division also two groups are separated, and it can be argued that in the right group predominate forests and mixed biotopes with indicator species *Leistus rufomarginatus*, and in the left – the open biotopes, where *Brachinus crepitans* is an indicator. On the third level four groups divide. The abandoned vineyard (IrL) detach in a private group, and IrN2 and the sampling sites near the signal repeaters (Rd, Rs) are united in a common group with the indicator *Notiophilus interstitialis*. The final groups of assemblages are classified into five end groups, arranged from open and dry (the left part of the table) to humid and accompanied by high vegetation (the right part), as it was also found for other areas of the Black Sea coast (Popov & Krusteva 1999). The number of the assemblages in the TWINSPAN groups varies between one and four. Eight distributional species groups are formed at the third level of the TWINSPAN division.

The grouping of the species indicates that the type of the vegetation is not of that importance, although there is some separation of the species: from associated with open habitats, throughout eurybionts, to forest dwellers. Determinative, however, is the importance of the humidity, as far as at the top of the table are concentrated the inhabitants of dry areas, and at the bottom – the ones attached to higher humidity. In the last TWINSPAN species group are focused the most rare riverine species caught near the banks of the Vaya River. Some species or groups of species with a relatively wide ecological tolerance are more or less evenly spread across the table. The same pattern is found by Popov & Krusteva (1999) too.

Ordination of the data

The data from the ordination of the sampling sites show strong dispersion of the studied communities and ecological groups of carabids, which spoke of the relative heterogeneity of the landscapes. We found a grouping of the sampling sites near the signal repeaters (R). The biotope in the abandoned vineyard (IrL) clearly distinguishes from the rest in the full two-year catches (Fig. 1).

When analyzing the data from the trapdays, we found a peculiar separation of the riverside biotope (IrV) (Fig. 2). Ordination data indicates that humid sites were tending to the left site of the graphic.

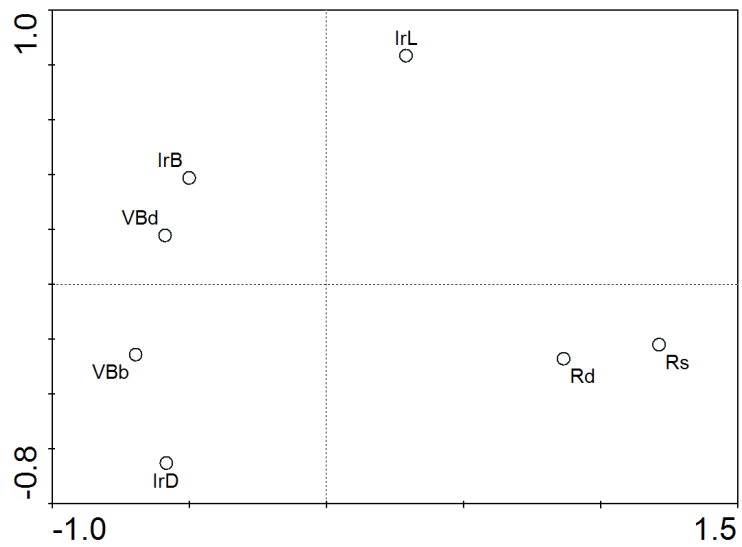


Fig. 1. PCA distribution of the sampling sites with full two-year catches.

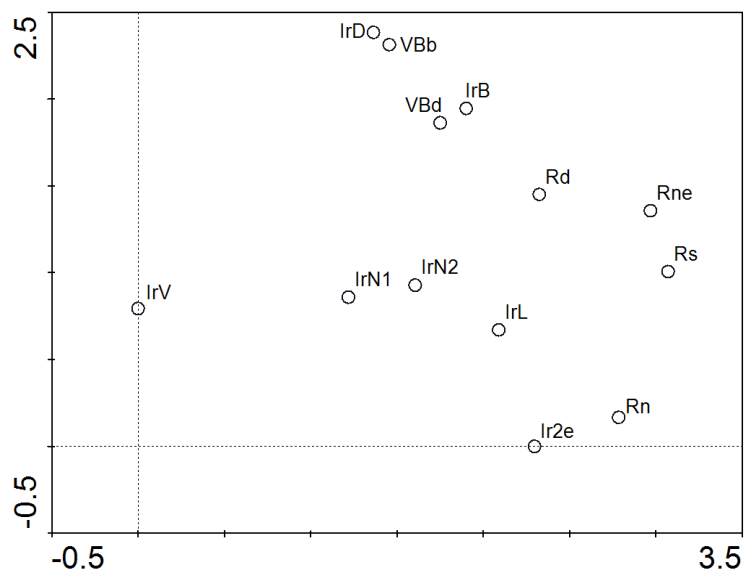


Fig. 2. DCA analysis of the trapdays data for all of the sampling sites.

We performed an analysis of the distribution of the data in relation to the two of the most important environmental factors influencing the spatial distribution of the ground beetles – humidity and vegetation. Fig. 3 shows the relations of the species to the grades of humidity and vegetative cover.

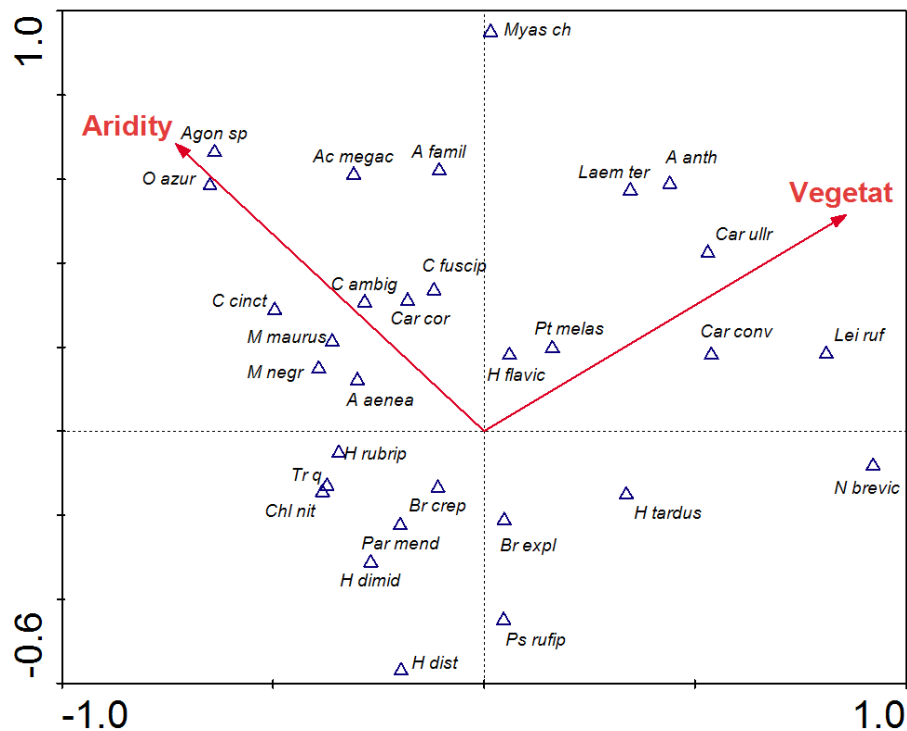


Fig. 3. Distribution of the permanent species in relation to the humidity and vegetation. The analysis included only the permanent species – those with a frequency above 50% (see TEOFILOVA 2015): A aenea – *Amara aenea*; A anth – *Amara anthobia*; A famil – *Amara familiaris*; Ac megac – *Acinopus megacephalus*; Agon sp – *Agonum (Europhilus)* sp.; Br crep – *Brachinus crepitans*; Br expl – *Brachinus explodens*; C ambig – *Calathus ambiguus*; C cinct – *Calathus cinctus*; C fuscip – *Calathus fuscipes*; Car conv – *Carabus convexus*; Car cor – *Carabus coriaceus*; Car ullr – *Carabus ullrichi*; Chl nit – *Chlaenius nitidulus*; H dimid – *Harpalus dimidiatus*; H dist – *Harpalus distinguendus*; H flavic – *Harpalus flavicornis*; H rubrip – *Harpalus rubripes*; H tardus – *Harpalus tardus*; Laem ter – *Laemostenus terricola*; Lei ruf – *Leistus rufomarginatus*; M maurus – *Microlestes maurus*; M minut – *Microlestes minutulus*; Myas ch – *Myas chalybaeus*; N brevic – *Nebria brevicollis*; O azur – *Ophonus azureus*; Par mend – *Parophonus mendax*; Ps rufip – *Pseudoophonus rufipes*; Pt melas – *Pterostichus melas*; Tr q – *Trechus quadristriatus*.

Fig. 4 shows the distribution of the sampling sites in relation to the same environmental gradients. The assemblages from the steppe-like habitat (Rs), the abandoned vineyard (IrL) and the shore ecotone (IrB) seemed to tend to more arid environmental conditions. The agrocoenoses and their adjacent ecotone areas, in turn, were forming an autonomous group of open habitats. In that group we also found the VBd site, which was probably due to the mixing of species, resulting from the specific border location of that sampling site. The habitats with the greatest affection to higher (forest) vegetation and humidity were the shore of the Vaya River (IrV) and one of the oak forests (Rd).

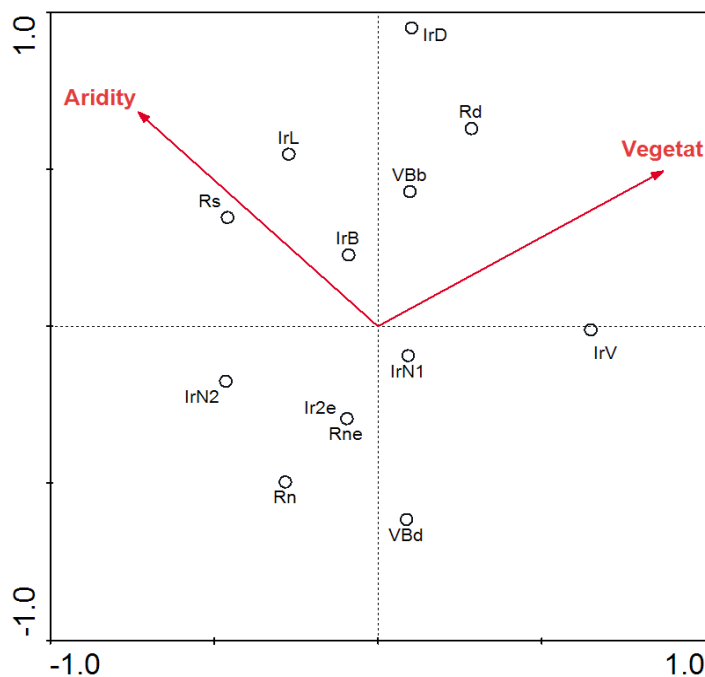


Fig. 4. Ordination of the sampling sites in relation to the humidity and vegetation. The calculations were performed by the use of the results from all of the sampling sites and all of the catches, standardized through the recalculation of the data as number of specimens per 100 trapdays.

We found that there is a relation between the zoogeographical categories and the environmental conditions in the individual sampling sites (Fig. 5). Clearly visible is the attachment of the Mediterranean species to the arid areas with low vegetative cover. Mostly forest-dwelling representatives from the European and Euro-Siberian categories show affection to the sampling sites with predominantly ligneous forest or forest-shrub vegetation.

From the analysis of the distribution of the life form subclasses (Fig. 6) we concluded that the mixophytophagous geobionts and stratohortobionts prefer open and anthropogenically impacted landscapes – the steppe-like habitat, the abandoned vineyard, wheat fields. Zoophagous beetles, in turn, are dependent on the forest habitats. The exception is the situation of IrN1, probably due to the proximity of the bank of the Vaya River and the possible resulting from that mixing of the data.

PCA ordination analysis of the distribution of the life form groups (Fig. 7) showed the connection between the steppe-like habitat and the representatives of the class Mixophytophagous. The high species diversity of the genera *Harpalus* and *Ophonus* contribute to the grouping of the categories stratohortobionts and harpaloid geohortobionts with this habitat. The abundance of the representatives of the genera *Cymindis* and *Brachinus*, in turn, attach this sampling site with the zoophagous litter & crevice-dwelling stratobionts. With VBd are connected the flying epigeobionts of the genus *Cicindela*, and with IrL – the living in crevices litter & bark-dwelling stratobiontis of the genus *Paradromius*, single specimen of which was found only in this sampling site. We established the greatest variety of life forms connected with the Vaya River, where a large number of rare species was found too: small geobiontis, with only representative *Dyschirius rufipes*; running

epigeobionts *Asaphidion flavipes*; endogeobionts living in crevices of the *Tachys* sp. type; surface & litter-dwelling stratobionts living in crevices, represented by the genera *Nebria*, *Notiophilus*, *Bembidion*, *Anchomenus*, *Chlaenius* and others.

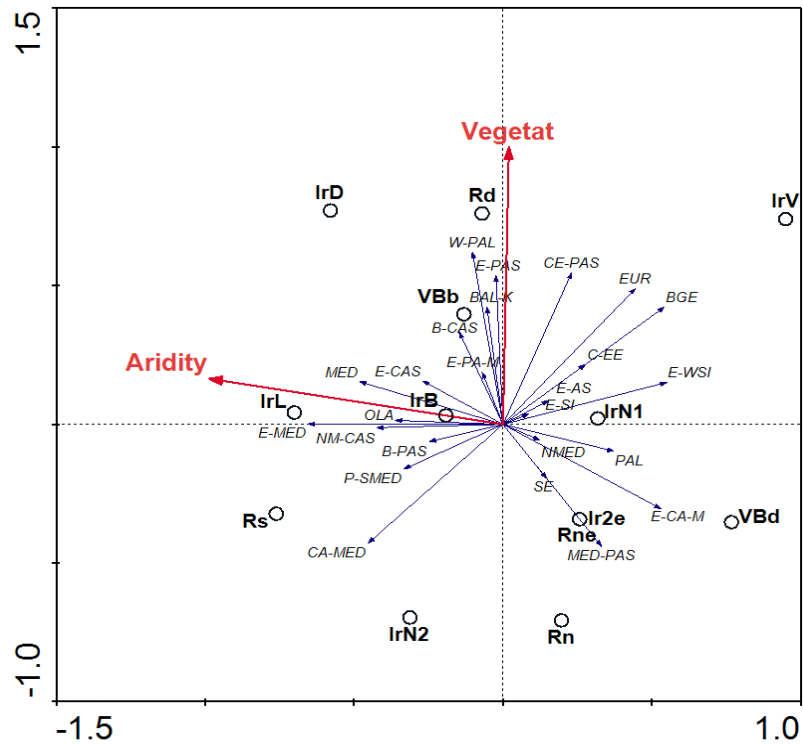


Fig. 5. Distribution of the chorotypes: OLA – Holarctic; PAL – Palearctic; W-PAL – Western Palearctic; E-SI – Eurosiberian; E-WSI – European-Westsiberian; EUR – European; E-PAS – European-Neareastern; CE-PAS – Central European and Neareastern; C-EE – Central and Eastern European; BAL-K – Balkan-Carpathian; E-AS – Euroasiatic steppe complex; E-CAS – European and Central Asian; B-CAS – Balkan and Central Asian; B-PAS – Balkan-Neareastern (+ Balkan-Anatolian); E-CA-M – European-Centralasian-Mediterranean; E-PA-M – European-Neareastern-Mediterranean; CA-MED – Mediterranean-Centralasian; MED-PAS – Mediterranean-Neareastern; MED – Mediterranean; E-MED – Eastmediterranean; P-SMED – Pontic-Submediterranean; SE – South European; NMED – Northmediterranean; NM-CAS – Northmediterranean-Centralasian; BGE – Bulgarian endemic.

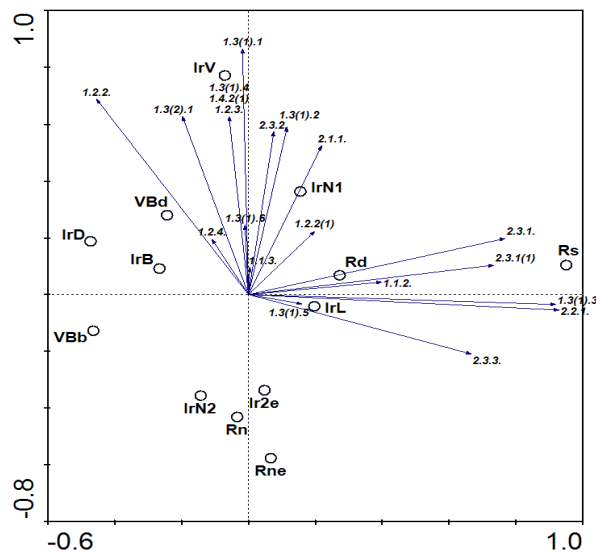


Fig. 6. PCA distribution of the sampling sites and the subclasses of life forms (according to Sharova 1981): Z_Phytob – Zoophagous phytobionts; Z_Strat – Zoophagous stratobionts; M_Strat – Mixophytophagous stratobionts; M_Short – Mixophytophagous stratohortobionts; M_Geoh – Mixophytophagous geobionts.

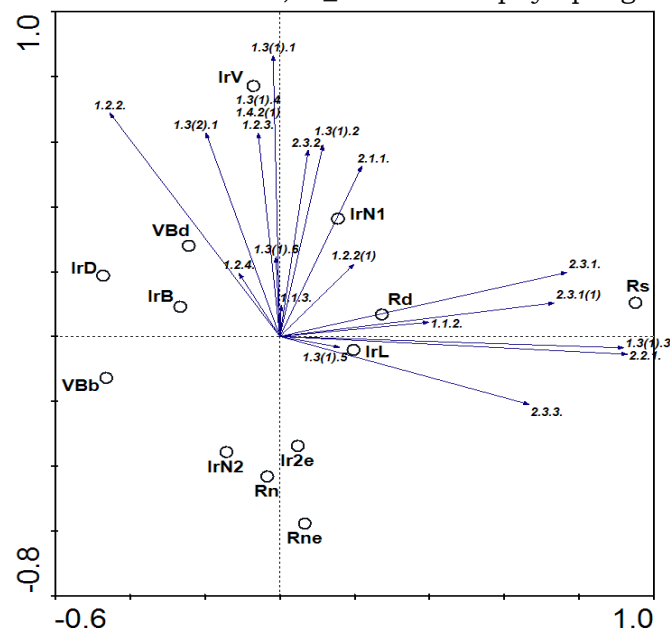


Fig. 7. PCA distribution of the sampling sites and categories of life forms (according to SHAROVA 1981): *Life form class 1. Zoophagous.* Life form subclass: 1.1 – Phytobios; 1.2 – Epigeobios; 1.3 – Stratobios; 1.4 – Geobios. Life form groups: 1.1.2 – stem-dwelling hortobionts; 1.1.3 – leaf-dwelling dendrohortobionts; 1.2.2 – large walking epigeobionts; 1.2.2(1) – large walking dendroepigeobionts; 1.2.3 – running epigeobionts; 1.2.4 – flying epigeobionts; 1.3(1) – series crevice-dwelling stratobionts; 1.3(1).1 – surface & litter-dwelling; 1.3(1).2 – litter-dwelling; 1.3(1).3 – litter & crevice-dwelling; 1.3(1).4 – endogeobionts; 1.3(1).5 – litter & bark-dwelling; 1.3(1).6 – bothrobionts; 1.3(2).1 – litter & soil-dwelling; 1.4.2(1) – large digging geobionts. *Life form class 2. Mixophytophagous.* Life form subclass: 2.1 – Stratobios; 2.2 – Stratohortobios; 2.3 – Geohortobios. Life form groups: 2.1.1 – crevice-dwelling stratobionts; 2.2.1 – stratohortobionts; 2.3.1 – harpaloid geohortobionts; 2.3.1(1) – crevice-dwelling harpaloid geohortobionts; 2.3.2 – zabroid geohortobionts; 2.3.3 – dytomeoid geohortobionts.

Discussion

During the study we found the presence of rich carabid fauna in the region of Cape Emine. Each of the studied sampling sites showed peculiar characteristics in the species composition of the complexes. The differences are probably a result of the interaction between the climatic characteristics, humidity and edaphic conditions, and the structure of the vegetation – key factors for the formation of the species composition of the carabid communities (Thiele 1977; Kryzhanovskij 1983; Antvogel & Bonn 2001; Eyre *et al.* 2005, etc.).

TWINSPAN analysis demonstrated the classification of the habitat groups from open and dry to forest and humid. Cluster analysis of the taxonomic similarity between the ground beetle communities also showed that the grouping of the clusters somewhat follows the humidity gradient (Teofilova 2015). Such pattern is also found for two areas of the northern and southern Black Sea coast by Popov & Krusteva (1999), while the stronger influence of anthropogenic impacts is characteristic for the agrocoenoses near the city of Sofia (Kostova 2004).

Ordination graphics showed the grouping of the sampling areas in the military base (VBb and VBd) with the shore ecotone (IrB) and the oak forest near Irakli (IrD), speaking of similar environmental conditions in these habitats. Such a grouping is also proved by the analysis of the taxonomic similarity of the assemblages (Teofilova 2015). Thus, some separation of the open habitats from the mixed and those with forest and forest-shrub vegetation is presented. This way, the ordination groupings confirm the definite importance of the vegetation as an ecological factor, also established by the classification methods of analysis by TWINSPAN.

The potentially crucial role of the type of the vegetation and the humidity conditions on the distribution of ground beetles is also confirmed by ordination analysis of Popov & Krusteva (1999).

We established great species diversity near the bank of the Vaya River, which matched with the assumption of the primary hygromesophilous preferences of the ground beetles (Kryzhanovskij 1983; Sharova 1981). Some typical forest dwellers like *Myas chalybaeus* were also found here, probably due to the fact, that the species was caught in the part of the river, which was not affected by the correction of the river bed and vegetation “cleansing” conducted in 2007, and the original forest was preserved. This contributed to the greater heterogeneity of this habitat, which increases the species richness of the carabid communities, probably because of the greater variety of microhabitats and the related increase in the number of ecological niches (Baiocchi *et al.* 2007).

Coastal vegetation appeared to be a natural bio-corridor and refugium for the hygrophilous mountainous and riverine component. The fauna of these habitats is considered not affected or slightly influenced by the anthropogenic activity. It includes hygrophilous and mesohygrophilous species, some of which with a limited range of habitation (*Tachys fulvicollis*, *Carabus cancellatus*, *Carabus ullrichi*).

Given the fact that the natural river valleys and periodically inundated coastal forests are becoming increasingly rare in whole Europe (Tomiałojć & Dyrzc 1993), the efforts for the conservation of the biological diversity should be directed towards the conservation and restoration of this type of habitats and the restriction of their anthropogenization (Teofilova *et al.* 2012).

Conclusions

The data from the ordination of the sampling areas show strong dispersion of the studied communities and ecological groups of ground beetles, which reflects the relative heterogeneity of the landscapes.

There is some separation of the open biotopes from the mixed and those with forest and forest-shrub vegetation, confirming the determining significance of the vegetation as an ecological factor.

The correspondence analysis in relation to the gradients of humidity and vegetation demonstrates the separation of the sampling sites to: attached to arid conditions, associated with open biotopes, and dependent on the structure of the vegetation cover.

Distribution of the zoogeographical categories shows the attachment of the Mediterranean species to dry areas with low vegetative cover, while European and European-Siberian categories show predilection to the areas with predominantly forest or forest-shrub vegetation.

The ordination of the life forms proves that mixophytophagous geobionts and stratohortobionts are attached to the open and anthropogenically impacted terrains, while zoophages are dependent on the forest habitats. The greatest variety of life forms is connected with the banks of the Vaya River.

Gradient analysis with methods for classification and ordination proves the primary role of humidity and vegetation as major environmental gradients, testifies to the relative heterogeneity of the landscapes in the region, and gives clarity for the affections of the zoogeographical categories and life forms to specific environmental conditions.

The proper tracking of the gradient of the humidity may give correct information also on the distribution of the vegetation, which would be beneficial for the imposition of measures for preservation of the species of conservation significance. Particular attention should be paid to “mixed” habitats, where a combination of different environmental conditions is seen, as well as the riverside habitats.

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Appendix. TWINSpan classification of ground beetle assemblages from the region of Cape Emine, according to their species composition. The order of the groups of species follows the TWINSpan analysis, and within the groups the species are in taxonomic order, following the system of Kryzhanovskij et al. 1995. Vertical lines separate TWINSpan assemblage groups, horizontal lines separate carabid species distributional groups (I – VIII). Thick lines represent first level of division, broken lines – second level, and points – third level. 1: 0 – 0.9%; 2: 1.0 – 3.9%; 3: 4.0 – 9.9%; 4: 10.0 – 19.9%; 5: 20.0 – 100.0%.

Species	Classification of ground beetle assemblages													Sp. totals	Sp. group
	Ir2e	Rn	Rne	IrN 2	Rs	Rd	IrD	IrB	VBd	VBb	IrL	IrV	IrN 1		
<i>Notiophilus interstitialis</i>				1	1	1								3	I
<i>Notiophilus danieli</i>						1								1	
<i>Bembidion castaneipenne</i>	1	1												2	
<i>Calathus melanocephalus</i>	1	-	-	1	1	1	-	1	1	-	-	-	-	64	
<i>Gynandromorphus etruscus</i>	-	1	1	-	1	-	-	-	-	-	-	-	-	5	
<i>Harpalus hospes</i>	1	1	-	-	1	-	-	-	-	1	-	-	-	33	
<i>Ophonus puncticeps</i>	-	-	-	-	1	1	-	-	-	-	1	-	-	10	
<i>Ophonus azureus</i>	1	1	1	-	1	1	-	1	1	-	1	-	1	118	
<i>Ophonus sabulicola</i>	1	-	1	-	1	1	-	-	-	-	1	-	-	40	
<i>Ophonus oblongus</i>	-	1	-	-	1	-	-	-	-	-	-	-	-	3	
<i>Carterus rufipes</i>	-	-	1	-	1	-	-	-	-	-	-	-	-	5	
<i>Ditomus calydonius</i>	-	1	1	-	1	1	-	1	-	-	-	-	-	16	
<i>Chlaenius nitidulus</i>	1	1	5	1	4	1	1	1	-	1	1	-	2	1927	
<i>Philorhizus notatus</i>	-	-	1	1	1	1	-	-	-	-	-	-	-	5	
<i>Microlestes fissuralis</i>	1	-	-	1	1	-	-	-	-	-	1	-	-	12	
<i>Microlestes fulvibasis</i>	-	-	-	1	1	-	-	-	-	-	-	-	-	3	
<i>Microlestes minutulus</i>	1	-	-	1	1	1	-	1	-	-	1	-	-	64	
<i>Cymindis ornata</i>	-	-	-	-	1	1	-	1	-	-	-	-	-	11	
<i>Brachinus alexandri</i>	-	-	1	-	1	-	-	-	-	-	-	-	-	15	
<i>Brachinus brevicollis</i>	-	-	-	-	1	1	-	-	-	-	-	-	-	36	
<i>Brachinus ejaculans</i>	-	-	-	-	1	1	-	-	-	-	-	-	-	4	
<i>Cicindela germanica</i>	-	-	-	-	-	-	-	-	1	-	-	-	-	1	
<i>Cicindela campestris</i>	-	-	-	-	-	-	-	-	1	-	1	-	-	7	
<i>Calsoma sycophanta</i>	-	-	-	-	1	1	-	1	1	-	-	-	-	126	
<i>Calsoma inquisitor</i>	-	-	-	1	1	1	1	1	1	-	-	-	-	65	
<i>Carabus scabriusculus</i>	-	-	-	-	-	1	1	1	1	-	-	-	-	67	
<i>Carabus montivagus</i>	-	-	-	-	-	-	1	-	-	-	-	-	-	3	
<i>Carabus intricatus</i>	-	-	-	-	-	-	-	-	1	-	-	-	-	1	
<i>Cychrus semigranosus</i>	-	-	-	-	-	-	-	-	1	1	-	-	-	11	
<i>Trechus quadristriatus</i>	2	1	1	1	1	1	1	1	1	1	1	1	-	319	
<i>Bembidion lunulatum</i>	-	-	-	-	-	-	-	1	1	1	-	-	-	6	
<i>Bembidion subfasciatum</i>	-	-	-	-	-	-	-	-	1	-	-	-	-	1	
<i>Bembidion articulatum</i>	-	-	-	-	-	-	-	-	1	-	-	-	-	1	
<i>Calathus ambiguus</i>	1	-	-	-	1	1	-	1	1	1	1	-	-	29	
<i>Calathus cinctus</i>	-	-	-	1	1	1	1	1	1	1	1	-	-	140	
<i>Laemostenus terricola</i>	-	-	-	-	1	1	1	1	1	1	1	-	-	86	
<i>Amara aenea</i>	1	1	1	1	1	1	-	1	1	1	1	-	1	48	
<i>Amara lucida</i>	-	-	-	-	1	-	-	-	-	-	1	-	-	2	
<i>Harpalus attenuatus</i>	-	-	-	-	1	1	1	1	-	-	1	-	-	39	
<i>Ophonus ardosiacus</i>	-	-	-	-	1	-	-	-	-	-	1	-	-	9	
<i>Dixus obscurus</i>	-	-	-	-	-	1	-	-	1	-	-	1	-	3	
<i>Dinodes decipiens</i>	-	-	-	-	1	-	-	-	-	-	1	-	-	3	
<i>Lebia cyanocephala</i>	-	-	-	-	-	1	-	1	-	-	-	-	-	2	
<i>Carabus coriaceus</i>	1	1	2	2	2	1	1	2	1	1	1	1	2	1240	
<i>Myas chalybaeus</i>	-	-	-	1	1	1	1	1	-	1	-	1	-	66	
<i>Pterostichus melas</i>	1	1	3	1	-	1	2	1	1	1	1	2	2	880	
<i>Calathus distinguendus</i>	-	-	-	-	1	1	-	-	-	1	-	-	1	62	
<i>Calathus fuscipes</i>	1	-	1	1	2	2	1	1	1	1	1	1	1	623	
<i>Calathus longicollis</i>	-	-	-	-	1	1	-	-	-	-	1	1	-	36	

<i>Agonum (Europhilus) sp.</i>	-	1	1	-	1	1	-	-	1	-	1	-	1	147		
<i>Parophonus mendax</i>	1	1	1	1	1	1	-	-	-	-	1	1	1	48		
<i>Pseudoophonus rufipes</i>	1	-	1	-	-	1	-	1	1	-	-	-	1	17		
<i>Harpalus rubripes</i>	1	1	-	-	1	1	1	-	1	-	1	-	1	21		
<i>Harpalus serripes</i>	1	-	-	1	-	-	-	-	-	-	-	1	-	3		
<i>Harpalus tardus</i>	1	-	-	1	-	1	1	-	1	1	1	1	1	49		
<i>Harpalus dimidiatus</i>	3	2	1	1	2	1	1	1	1	1	1	1	1	555		
<i>Harpalus distinguendus</i>	1	1	1	-	1	1	-	1	-	-	1	1	-	22		
<i>Acinopus megacephalus</i>	1	-	-	1	1	1	1	1	1	-	1	1	1	277		
<i>Microlestes maurus</i>	1	1	-	-	1	1	-	1	-	-	1	1	1	51		
<i>Microlestes negrita</i>	1	1	1	1	1	1	-	1	1	1	1	1	1	96		
<i>Brachinus berytensis</i>	-	1	1	-	1	1	-	-	-	-	1	-	1	22		
<i>Brachinus crepitans</i>	1	1	4	2	2	2	-	-	-	-	-	1	4	1213		
<i>Brachinus explodens</i>	1	-	1	1	1	1	-	-	-	-	-	1	1	79		
<i>Leistus rufomarginatus</i>	-	-	-	-	-	1	1	1	1	1	-	1	1	49		
<i>Notiophilus rufipes</i>	-	-	-	-	-	1	1	1	-	1	-	1	1	79		
<i>Carabus ullrichi</i>	1	-	-	1	-	-	1	1	1	-	1	1	1	147		
<i>Carabus convexus</i>	1	-	-	1	1	1	1	1	1	1	1	2	1	288	IV	
<i>Bembidion dalmatinum</i>	-	1	-	-	-	-	1	1	-	-	-	-	1	6		
<i>Amara anthobia</i>	-	-	-	-	1	1	1	1	-	1	1	1	-	28		
<i>Amara familiaris</i>	-	-	-	-	1	1	-	1	1	1	1	-	1	19		
<i>Harpalus cupreus</i>	1	1	1	-	-	-	1	-	-	-	-	1	1	26	V	
<i>Amara ingenua</i>	-	-	-	1	-	-	-	-	-	-	-	-	1	2		
<i>Scybalicus oblongiusculus</i>	-	-	-	-	1	-	-	-	-	-	-	-	1	2	VI	
<i>Harpalus albanicus</i>	-	-	-	-	1	-	-	-	-	-	-	1	-	4		
<i>Brachinus psophia</i>	-	-	3	1	1	1	-	-	-	-	-	1	3	458		
<i>Nebria brevicollis</i>	1	-	-	1	-	-	1	1	1	1	1	5	4	1890		
<i>Trechus crucifer</i>	-	-	-	-	-	-	1	-	-	-	-	-	1	1		
<i>Amara communis</i>	-	-	-	-	1	-	-	-	1	-	-	-	1	6	VII	
<i>Harpalus flavicornis</i>	-	1	1	-	-	-	1	-	1	-	1	1	1	108		
<i>Ophonus similis</i>	-	-	-	-	1	-	-	-	-	1	-	-	1	5		
<i>Carabus cancellatus</i>	-	-	-	-	-	-	-	-	-	-	-	1	1	30		
<i>Dyschirius rufipes</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1		
<i>Tachys fulvicollis</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1		
<i>Porotachys bisulcatus</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	2		
<i>Asaphidion flavipes</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	57		
<i>Bembidion lampros</i>	-	-	-	-	-	-	1	-	-	-	-	1	1	42		
<i>Bembidion properans</i>	-	-	1	-	-	-	-	-	-	-	-	1	1	5		
<i>Bembidion inoptatum</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1		
<i>Bembidion combustum</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1		
<i>Stomis pumicatus</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	3		
<i>Poecilus cupreus</i>	-	-	1	-	-	-	-	-	-	-	-	1	1	3	VIII	
<i>Pterostichus nigrita</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	1		
<i>Pterostichus merkli</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	4		
<i>Abax carinatus</i>	-	-	-	-	-	-	-	-	-	-	-	1	-	1		
<i>Anchomenus dorsalis</i>	-	-	-	-	-	-	-	-	1	-	-	3	2	399		
<i>Parophonus laeviceps</i>	-	1	-	-	-	-	-	-	-	-	-	1	1	4		
<i>Ophonus nitidulus</i>	-	-	-	-	1	-	-	-	-	-	-	1	1	6		
<i>Chlaenius festinus</i>	-	-	-	-	-	-	-	-	-	-	-	1	1	12		
<i>Syntomus obscuroguttatus</i>	-	-	1	-	-	1	-	-	-	-	1	1	1	10		
<i>Syntomus pallipes</i>	-	-	-	-	-	-	-	-	1	-	-	1	1	5		
Indicator species	Br crep												Car canc			
							Not int									
							Lei ruf									

Application of allozyme markers for screening of turbot populations along Western Black Sea coast

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Abstract: Data for electrophoretic pattern of 26 loci, resolved for the 8 protein systems (7-enzyme and 1-non-enzyme) assayed were used to investigate population structure of turbot along the Bulgarian and Romanian Black Sea coasts using genetic diversity measures. Seventeen loci were polymorphic in all populations and a total of 34 alleles were identified. Four types of tissue: muscle, retina, plasma and haemoglobin were analyzed. The percentage of polymorphic loci was high (65.38%) within populations. A low level of genetic differentiation among populations was detected, based on the Shannon's information index (0.446-0.448) and the coefficient of genetic differentiation between populations ($F_{ST} = 0.014$). The overall mean of within-population inbreeding estimate (F_{IS}) was (-0.209) and demonstrated low level of inbreeding. The genetic distance (D_{Nei}) between the populations was low and vary between 0.003 and 0.014. Genetic distances among turbot populations were positively correlated with geographic distances ($r = 0.474$), but the association was not significant according to the Mantel test ($p=0.651$) and showed a lack of correlation between genetic distance and the geographic location of populations. Results identified one genetic stock with sufficient gene flow between all the three sites to prevent genetic differentiation from occurring. Only 1.4% of the genetic variation was observed among populations. Results revealed that adopting a single stock model and regional shared management could probably be appropriate for sustainable long-term use of turbot along western Black Sea coast. Determination of the contemporary state of the population distribution will be the prerequisite for determination of adequate measures for exploitation and protection of the existing turbot populations along western Black Sea coast.

Key words: allozymes, turbot, populations, genetic distance, western Black Sea.

Introduction

The turbot *Psetta maxima* is naturally distributed in European waters, from Northeast Atlantic to the Arctic Circle. It occurs in the Baltic and in the Mediterranean, as well as in the Black Sea, where a subspecies *Psetta maxima maeotica* has been described. Two generic names are available for the species – *Scophthalmus maximus* (Linnaeus 1758) and *Psetta maxima* (Linnaeus 1758). Recent studies of Bailly & Chanet (2010) strongly recommended using *Scophthalmus* as the valid generic name for the turbot.

The Black Sea turbot is one of the most valuable commercial species in all countries of the Black Sea basin. It is subject of intensive exploitation and endangered from extinction. Current status of the turbot population in the Black Sea characterized the stock as exploited unsustainably and at risk of collapse (STECF 2014) and “overexploited” and “in overexploitation” (GFCM 2014).

The preservation of the turbot population requires knowledge for the population genetic structure and constant monitoring of its biodiversity.

Different opinions exist regarding the availability of turbot local populations (ecotypes) in the Black Sea. Shlyakhov (2014) considered that turbot in the Black Sea is presented by several local populations, which mix in the adjacent areas. The strongest one of them - “Western” - is distributed in the waters of Ukraine, Romania and possibly in Bulgaria, where it mixes partially with the local population and the “North-Eastern” population is distributed in the waters of the Russian Federation, Ukraine and partially in Georgia (Shlyakhov 2014). Stock identification and stock boundaries are still not well defined and for the time being the turbot population in the Black Sea is assessed as a single stock (STECF 2014).

The population structure of turbot was subject of several allozyme studies (Blanquer *et al.* 1992, Bouza *et al.* 1994, 1997, 2002, Exadactylos & Thorpe 2001, Exadactylos *et al.* 2001, Imsland *et al.* 2003, Nielsen *et al.* 2004, Ivanova *et al.* 2006, Tsekov *et al.* 2008). According to Danancher & Garcia-Vazquez (2006) very little is known about population structure in wild turbot.

Recently the molecular marker technologies become an essential tool for analysis of genetic diversity applied in fish systematics, population genetics and conservation biology. Genetic methods are the most important tools for defining stock structure and evaluating levels and patterns of genetic diversity in fishes Liu & Cordes (2004).

The molecular markers were applied only to the closely related Mediterranean turbot. Suzuki *et al.* (2004), Prado *et al.* (2005), Bouza *et al.* (2002), Vera *et al.* (2011) used mitochondrial and nuclear DNA markers for taxonomic studies of the Mediterranean population. Atanassov *et al.* (2011) using mitochondrial control region of DNA variation to analyze the turbot populations from Bulgarian and Romanian Black Sea coasts.

The goal of the study is to evaluate genetic structure in natural turbot stocks (populations) along the Bulgarian and Romanian coast based on allozyme data.

Material and Methods

Allozyme analyses

65 turbot samples from the western Black Sea coast (Fig.1) caught between 2010-2012 were analyzed. Data for electrophoretic pattern of 26 loci were used to investigate population structure of turbot along the Bulgarian and Romanian Black Sea coasts, analyzing four types of tissue: muscle, eye, haemoglobin and plasma.

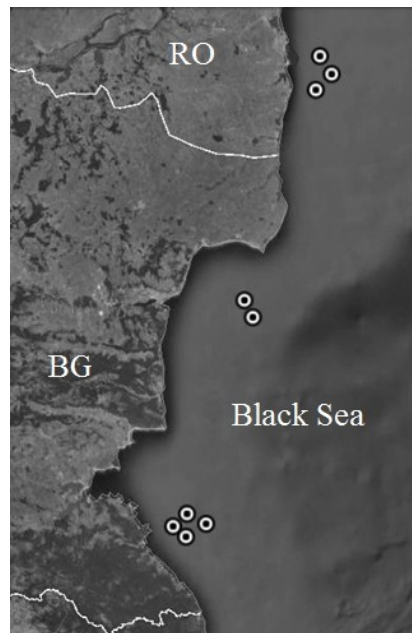


Fig. 1. Sampling localities of turbot samples

For the analysis of the enzymes and non-enzyme protein systems, a homogenate of white dorsal muscle was used. Proteins were separated by horizontal starch gel electrophoresis according to Smithies (1955) methods, modified by Dobrovolov (1973).

The following 7 enzymatic and one nonenzymatic systems were studied: General muscle proteins (PROT), esterase (EC 3.1.1.1 - EST), lactate dehydrogenase (EC 1.1.1.27 - LDH), malate dehydrogenase (EC 1.1.1.37 - MDH), malic enzyme (EC 1.1.1.40 - MEP), superoxide dismutase (EC 1.15.1.1 - SOD), fumarase (FH) and phosphoglucosomerase PGI.

The proteins were stained with Commassie Brilliant Blue R-250. Staining of different enzymes was performed according to Shaw & Prasad (1970). Buffer systems of Dobrovolov (1976) and Clayton & Gee (1969) were used for the electrophoresis.

The nomenclature of mentioned loci and alleles followed essentially the recommendation of Shaklee *et al.* (1990).

Genetic diversity analyses

Gene frequencies of the polymorphic loci were calculated using the Hardy-Weinberg equilibrium. Calculation of indices of genetic similarity and genetic distance was performed according to Nei (1972).

Genetic diversity was determined as allele frequencies, effective number of alleles (N_e), test of Hardy-Weinberg equilibrium (HWE), observed (H_o) and expected (H_e) heterosigosity F-statistics and Nei's genetic distance (D) Nei (1972) using GENALEX 6 (Peakall & Smouse 2006). Percentage of polymorphic loci (PPL), number of different alleles (N_a), average effective number of alleles per locus (N_e), average gene diversity (H_e), Shannon's information index (SI) as well as hierarchical analysis of molecular variance (AMOVA) were done using GENALEX 6 software package.

To visualize the genetic relationship among populations, a dendrogram was constructed based on Nei's genetic distance (D) Nei (1972), by an unweighted pair-group method of cluster analysis using arithmetic averages (PAUP), version 4.0 (Swofford 1998) and Treeview. To test the correlation between Nei's genetic distance (D) between populations and geographic distances (in km) among populations, a Mantel test was performed using IBDWS program.

The program BOTTLENECK (Piry *et al.* 1999) was used to test whether populations have recently passed through a bottleneck. Both the stepwise mutation model (SMM) and the infinite allele model (IAM) were run. The sing test was conducted to determine the significance of heterozygosity excess (Cornuet & Luikart 1996).

Results and Discussion

Allelic variability in turbot populations

Common electrophoretal mobility of LDH-B* (eye-retina), mMDH, sMDH-1*, sMDH-2*, GPI-1*,GPI-2* and FU* (muscle tissue), SOD-1* and SOD-2* (haemoglobin) were observed.

The allelic frequencies of polymorphic loci EST-2*, EST-3*, PROT-1* and PROT-2* (haemoglobin), PROT-1*, PROT-2* and EST-2* (plasma, retina), EST-2*, EST-3*, MEP-1* and MEP-2* (muscle and retina), LDH-A* and LDH-C* (retina) are presented on the Table 1.

General muscle proteins (PROT) - Electrophoretal spectra on general muscle proteins were different on three tissue analyzed (Fig.2 A.). Polymorphism was found on haemoglobins (PROT-1* and PROT-2*), (Fig.2 B) and plasma tissues. The data received for gene frequencies were presented on Table 1.

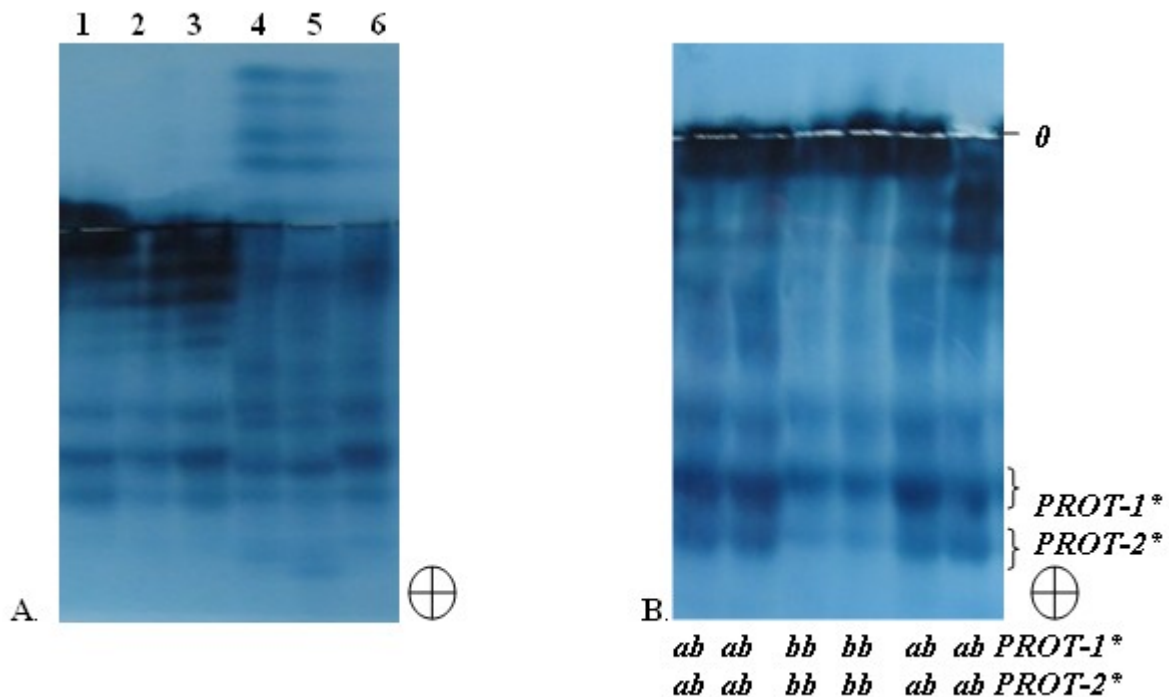


Fig. 2. A. Electrophoregrams on PROT from turbot (Bulgarian and Romanian coast), using different tissues:1-3 – haemoglobins, 4-5- eye (retina) and 6 – muscle, 0 – origin. B. Electrophoregrams on PROT from turbot Bulgarian and Rumanian coast haemoglobin tissue. PROT-1* and PROT-2* were polymorphic, 0 – origin.

The esterases are highly polymorphic. Two esterases loci (EST-2* and EST-3*) were polymorphic in all tissues analyzed and could be used as a marker enzyme system for distinguishing of turbot populations (Fig.3, Table1). The allele frequencies of EST-2* and EST-3* (haemoglobins), PROT-2* and EST-2* (plasma), EST-3*, MEP-1* and MEP-2* (muscle), LDH-C* and PROT-2* (retina) (Table 1) on north Bulgarian and Romanian populations are closely related.

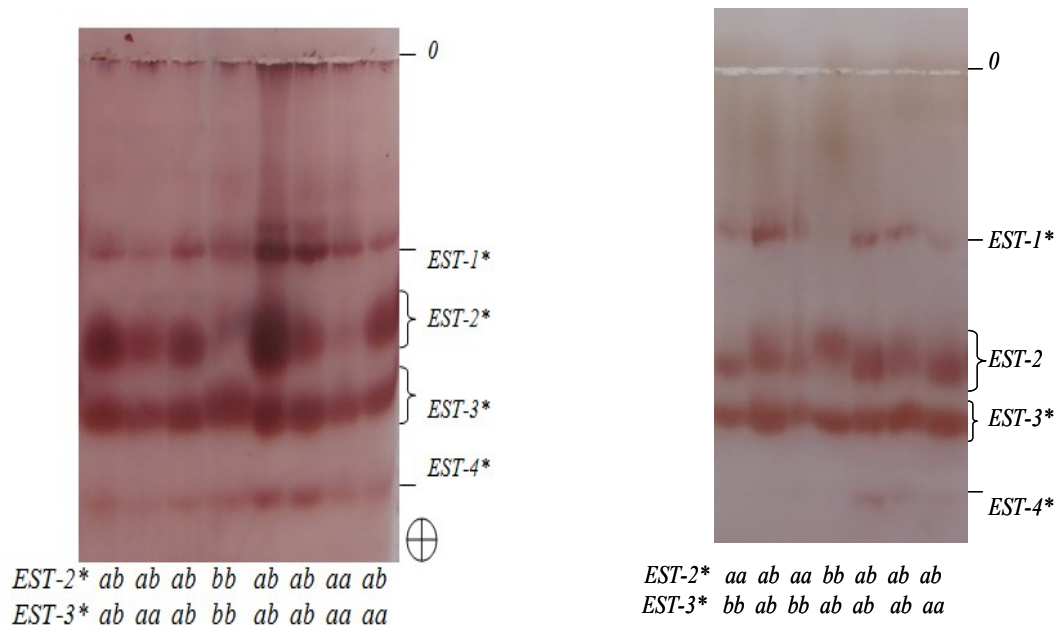


Fig. 3. Enzymograms of general unspecified esterases (EST) from muscle tissue of turbot (1) and haemoglobin (2), EST-2* and EST-3* - polymorphic loci, 0 – origin.

A new polymorphic LDH-C*, sMEP-1* and sMEP-2* loci, useful for identification of turbot stocks along Bulgarian and Romanian coast were found after analyses of retina and muscle tissue (Table 1).

Genetic diversity and genetic structure of western Black Sea populations

Summary statistics i.e. number of individuals screened (n), number of alleles found for each sample at a locus (N_a), observed (H_o) and expected (H_e) heterozygosity for each locus and FIS are shown in Table 1.

The percentage of polymorphic loci (PPL) for a single population was 65.38 %, (Table 2). The number of different alleles (N_a) was 1.654. The average effective number of alleles per locus (N_e) at the population level was 1.63 ranged from 1.626 to 1.633. The average gene diversity (H_e) for all 26 loci was estimated 0.321. The Shannon's information index (SI) ranged from 0.446 to 0.448 at the population level (Table 2). No private alleles were found.

Table 1. Allelic frequencies, genetic variation and heterozygosity statistics of 17 polymorphic allozyme loci in turbot populations. n- number of individuals screened; a and b allele frequencies, H_o – observed heterosigosity, H_e -expected heterosigosity and F_{IS} .

Tissue	Locus	Allele/ Parameter	Romania n=21	BG North n=23	BG South n=21
haemoglobin	<i>EST-2*</i>	a	0.571	0.522	0.452
		b	0.429	0.478	0.548
		H_o	0.571	0.609	0.714
		H_e	0.490	0.499	0.495
		F_{IS}	-0.167	-0.220	-0.442
haemoglobin	<i>EST-3*</i>	a	0.476	0.435	0.595
		b	0.524	0.565	0.405
		H_o	0.667	0.609	0.619
		H_e	0.499	0.491	0.482
		F_{IS}	-0.336	-0.238	-0.285
haemoglobin	<i>PROT -1*</i>	a	0.476	0.565	0.571
		b	0.524	0.435	0.429
		H_o	0.571	0.609	0.667
		H_e	0.499	0.491	0.490
		F_{IS}	-0.145	-0.238	-0.361
haemoglobin	<i>PROT-2*</i>	a	0.548	0.500	0.524
		b	0.452	0.500	0.476
		H_o	0.619	0.739	0.762
		H_e	0.495	0.500	0.499
		F_{IS}	-0.249	-0.478	-0.527
plasma	<i>PROT-1*</i>	a	0.643	0.522	0.524
		b	0.357	0.478	0.476
		H_o	0.524	0.609	0.571
		H_e	0.459	0.499	0.499
		F_{IS}	-0.141	-0.220	-0.145
plasma	<i>PROT-2*</i>	a	0.476	0.478	0.452
		b	0.524	0.522	0.548
		H_o	0.476	0.499	0.714
		H_e	0.499	0.510	0.500
		F_{IS}	0.045	-0.045	-0.429
plasma	<i>EST-2*</i>	a	0.595	0.587	0.500
		b	0.405	0.413	0.500
		H_o	0.524	0.565	0.619
		H_e	0.482	0.485	0.500
		F_{IS}	-0.087	-0.166	-0.238
muscle	<i>EST-2*</i>	a	0.524	0.543	0.524
		b	0.476	0.457	0.476
		H_o	0.667	0.739	0.476
		H_e	0.499	0.496	0.499
		F_{IS}	-0.336	-0.490	0.045

Continued ▼

muscle	<i>EST-3*</i>	a	0.524	0.522	0.476
		b	0.476	0.478	0.524
		H _o	0.571	0.696	0.762
		H _e	0.499	0.499	0.499
		F _{IS}	-0.145	-0.394	-0.527
muscle	<i>MEP-1*</i>	a	0.571	0.630	0.452
		b	0.429	0.370	0.548
		H _o	0.667	0.565	0.524
		H _e	0.490	0.466	0.495
		F _{IS}	-0.361	-0.213	-0.057
muscle	<i>MEP-2*</i>	a	0.619	0.587	0.476
		b	0.381	0.413	0.524
		H _o	0.571	0.652	0.571
		H _e	0.472	0.485	0.499
		F _{IS}	-0.212	-0.345	-0.145
retina (eye tissue)	<i>LDH-A*</i>	a	0.476	0.565	0.571
		b	0.524	0.435	0.429
		H _o	0.571	0.609	0.571
		H _e	0.499	0.491	0.490
		F _{IS}	-0.145	-0.238	-0.167
retina (eye tissue)	<i>LDH-C*</i>	a	0.405	0.391	0.667
		b	0.595	0.609	0.333
		H _o	0.429	0.435	0.571
		H _e	0.482	0.476	0.444
		F _{IS}	0.111	0.087	-0.286
retina (eye tissue)	<i>EST-2*</i>	a	0.476	0.587	0.595
		b	0.524	0.413	0.405
		H _o	0.667	0.565	0.619
		H _e	0.499	0.485	0.482
		F _{IS}	-0.336	-0.166	-0.285
retina (eye tissue)	<i>EST-3*</i>	a	0.524	0.500	0.500
		b	0.476	0.500	0.500
		H _o	0.571	0.565	0.524
		H _e	0.499	0.500	0.500
		F _{IS}	-0.145	-0.130	-0.048
retina (eye tissue)	<i>PROT-1*</i>	a	0.500	0.457	0.429
		b	0.500	0.543	0.571
		H _o	0.524	0.565	0.667
		H _e	0.500	0.496	0.490
		F _{IS}	-0.048	-0.139	-0.361
retina (eye tissue)	<i>PROT-2*</i>	a	0.548	0.587	0.333
		b	0.452	0.478	0.667
		H _o	0.524	0.485	0.476
		Mean H _o	0.571± 0,017	0.596± 0,020	0.613± 0,022
		Mean H _e	0,492± 0,003	0,491± 0,002	0,489± 0,004
		Mean F _{IS}	-0.162±0.033	-0.213±0.038	- 0.255±0.04 2
Total		Mean H _o	0,594± 0,011		
		Mean H _e	0,490± 0,002		
		Mean F _{IS}	-0.210 ± 0.022		

Table 2. Genetic variability within populations of turbot detected by allozyme analysis. PPL - percentage of polymorphic loci; No-number of different alleles; Ne-effective number of alleles per locus; He - expected heterosigosity for 26 loci; SI -Shannon's information index.

Population	PPL (%)	No	Ne	He	SI
Pop R	65.38	1,654±0.095	1,633 ±0,092	0,321±0,047	0,448 ±0,065
Pop N	65.38	1,654±0.095	1,631±0,092	0,321±0,047	0,447±0,065
Pop S	65.38	1,654±0.095	1,626±0,092	0,320±0,047	0,446±0,065

Mean expected heterozygosity (He) per population was relatively high (0.321; Table 2) as compared to genetic diversity values found for turbot wild samples in Liverpool, UK, (0.027 in Exadactylos *et al.* 2001; 0.0295 in Bouza *et al.* 1997, 0.02 - Blanquer *et al.* 1992 and 0.02 - Bouza *et al.* 2002). Our data for the mean heterozygosity He values were higher than previous results, and more close to the data of Florin & Höglund (2007) (He =0.580), based on microsatellites. The group of flatfish has even shown higher heterozygosity than the average values in marine fish (Smith & Fujio 1982, Brulé 1989). Populations of widespread fish species often show significantly higher heterozygosity estimates than for population of species with more restricted distribution (Gopalakrishnan *et al.* 2009).

Wright's fixation index (FIT) was estimated as for each locus in each sample and represent the deviations from expected heterozygosity in overall and within populations. Negative mean value of FIT (-0.194±0.028) were found at almost all loci across all three populations of turbot, except only rPROT-2 and rLDH-C* loci (Table 3), indicating an excess of heterozygotes. A possible explanation for this pattern is that natural selection might favour heterozygotes that can cope with environment changes in highly fragmented populations.

A negative value of FIS was found for all seventeen loci, with a mean value of -0.209 (Table 3). FIS values indicating that there is heterozygote excess compared with HWE expectations and no inbreeding. Deviation from Hardy-Weinberg proportions indicates selection, population mixing or nonrandom mating. This is mentioned from other authors for natural turbot populations (Exadactylos *et al.* 2001, Florin & Höglund 2007). Our work revealed a high level of heterozygosity in all populations, however, in most cases F was not statistically significant.

Britten (1996) suggested that isolated populations might exhibit high heterozygosity due to strong selection pressures. Size selective fishing gear, destruction of habitat, alteration of prey availability, pollution stress and other such activities can impose new selection pressures on a stock or may alter the existing selection forces (Çiftici & Okomuş 2002)

According to Kang *et al.* (2005) two factors are usually involved in driving selection for heterozygotes, environmental stress and inbreeding depression.

The value of FST, a measure of the degree of differentiation among turbot populations analyzed was 0.014 (Table 3), revealing that about 98.60% of the total genetic diversity resides within populations. This indicated that 1.4% of the variation was because of allozyme differences among the three populations and showed low genetic differentiation. AMOVA analyses showed 100% of molecular variance within individuals of each populations and absence of percentage variance between individuals and populations.

Table 3. F-statistics based on 17 polymorphic loci for three turbot populations.

Locus	No of alleles	F _{IS}	F _{ST}	F _{IT}
<i>Hb EST-2*</i>	2	-0.276	0.010	-0.264
<i>Hb EST-3*</i>	2	-0.287	0.019	-0.263
<i>Hb PROT-1*</i>	2	-0.248	0.008	-0.238
<i>Hb PROT-2*</i>	2	-0.419	0.002	-0.417
<i>Plm PROT-1*</i>	2	-0.169	0.013	-0.154
<i>Plm PROT-2*</i>	2	-0.143	0.000	-0.143
<i>mEST-2*</i>	2	-0.260	0.000	-0.259
<i>mEST-3*</i>	2	-0.356	0.002	-0.353
<i>Plm EST-2*</i>	2	-0.165	0.008	- 0.156
<i>mMEP-2*</i>	2	-0.233	0.015	- 0.215
<i>mMEP-1*</i>	2	-0.210	0.022	- 0.183
<i>rLDH-A*</i>	2	-0.183	0.008	-0.174
<i>rLDH-C*</i>	2	-0.023	0.064	0.043
<i>rEST-2</i>	2	-0.263	0.012	- 0.248
<i>rEST-3</i>	2	-0.108	0.001	-0.107
<i>rPROT-2</i>	2	-0.038	0.050	0.014
<i>rPROT-1</i>	2	-0.181	0.003	-0.177
Mean		-0.209	0,014	-0.194
SE		0.025	0.004	0.028

Low mean level of genetic differentiation between populations within species ($F_{ST}=0.029$) was found for seven flatfish using allozymes (Exadactylos & Thorpe 2001). Similar results have been reported by Blanquer *et al.* (1992) and Bouza *et al.* (1997).

Turbot along western Black Sea coast had relatively high diversity $H_e=0.321$ within all of three populations in the present study. In all populations lack of rare alleles was presented. This may be explained as a result of random genetic drift caused by the recent reduction in population size. According to Kang *et al.* (2005) habitat fragmentation in natural populations could result in an immediate loss of rare alleles and a reduction of allele richness rather than a reduction of overall genetic heterozygosity.

Geographic pattern of genetic diversity

The low genetic divergence ($D_{Nei}=0,003\div 0,014$) among western Black Sea turbot populations (Table 4) was found and confirmed with topology of PAUP dendrogram (Fig.4).

The dendrogram constructed showed one cluster, which pointed that in the investigated areas the populations are not good differentiated.

Table 4. Genetic identity (I Nei) above diagonal and Genetic distance (D Nei) between turbot populations analyzed, calculated on 17 polymorphic loci.

Population	RO	BG N	BG S
RO	-	0.990	0.090
BG N	0.003	-	0.080
BG S	0.0014	0.013	-

Maximum genetic differentiation among different populations pairs was observed between Romanian and southern Black Sea region, as these areas are distantly located and have negligible gene exchange between them. Romanian population is more close to the northern Bulgarian population, which may result from gene flow during the pelagic phase. (Table 4, Fig. 4).

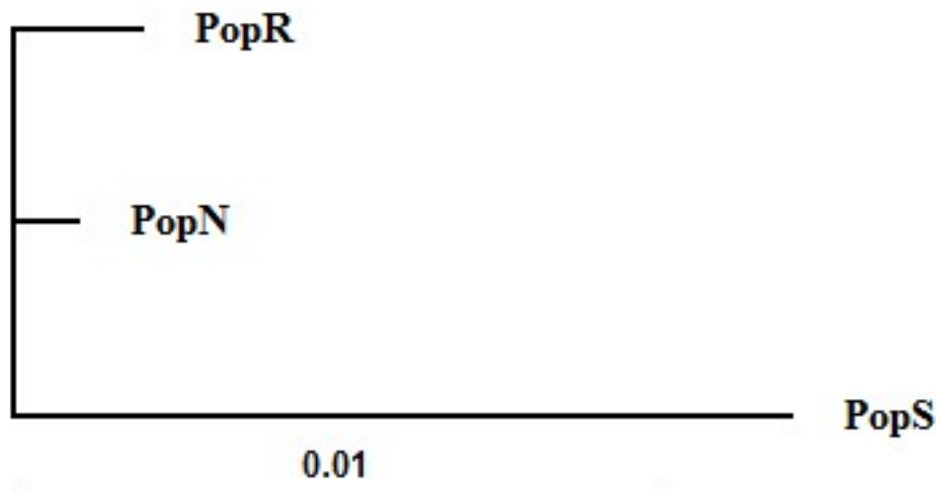


Fig. 4. Dendrogram of three turbot populations using PAUP cluster analysis of allozyme data.

The observed lack of private or locality specific allele at any of allozyme loci argues in favour of effective ongoing gene flow. Therefore, common ancestry in the past and possible continuous exchange of individuals among different areas may explain the observed low levels of genetic differentiation among turbot populations.

The results from Mantel test (Fig. 5), show no statistically significant relationship between genetic distances and geographical distances (km) of individual populations ($Z = 4.593$, $r = 0.474$, $P = 0.651$). No significant correlation between geographic and genetic distance for turbot from Baltic Sea was described also from Florin & Höglung (2007).

The Mantel test indicates that genetic differentiation among turbot populations does not seem to be correlated with geographic distance among populations, which provides further evidence of genetic drift (Shah *et al.* 2008).

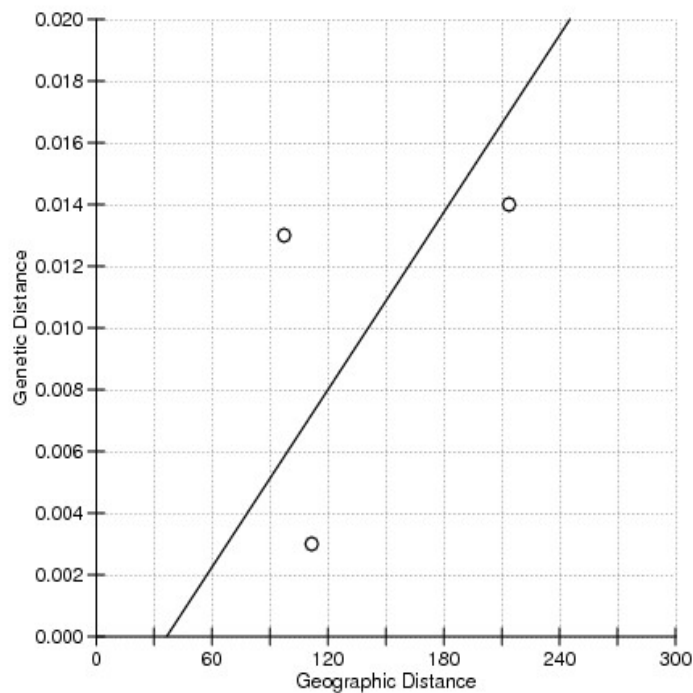


Fig. 5. The Mantel test compares a genetic distance (Y matrix) with a geographical distance (X matrix) in kilometers to test correlation between genetics and geographical location.

The very low genetic variability (F_{ST} 1.4%) found by Black Sea turbot populations could be explained in terms of historical bottlenecks of different evolutionary rates as this is mentioned from Blanquer *et al.* (1992).

It is crucial to identify populations that have undergone ancient or recent bottlenecks, because they may have been affected by the small population size through demographic stochasticity, inbreeding or fixation of deleterious alleles, possibly leading to a reduced evolutionary potential and increased probability of extinction (So *et al.* 2006).

According to Gopalakrishnan *et al.* (2009), allozyme and microsatellite markers were useful in identifying recent genetic bottlenecks in many marine fishes.

When a population is reduced in size, the allelic diversity is reduced faster than heterozygosity leading to heterozygosity excess (Nei *et al.* 1975). All 17 loci, investigated in Black Sea turbot populations along western coast showed under Sign test a significant heterozygote excess (Table 5) and would be considered as having experienced a recent genetic bottleneck (Cornuet & Luikart 1996). This supports the existing hypothesis (Atanassov *et al.* 2011) suggested Black Sea fish population bottleneck during the height of the last glacial period.

Table 5. Results of bottleneck Sign test in the three turbot populations sampled. H_o/H_e , observed and expected number of loci with heterozygosity excess under the infinite allele model (IAM) and the stepwise mutation model (SMM); P - probability.

Model	IAM		SMM	
Population	H_o/H_e	P	H_o/H_e	P
Pop R	7.04/17	0.000	8.15/17	0.000
Pop N	7.45/17	0.000	8.25/17	0.000
Pop S	7.23/17	0.000	8.22/17	0.000

The genetic differentiation of many marine fish species is low. They are less differentiated into populations (Ward 1994).

Low levels of genetic differentiation was found in wild turbot based on previous allozyme studies (Exadactylos *et al.* 2001, Blanquer *et al.* 1992, Bouza *et al.* 1997). Low genetic differentiation of turbot samples from the south and north Bulgarian and north Romanian regions of the west Black Sea coast based on mitochondrial control region /CR/ analyses (Atanassov *et al.* 2011) are comparable with the data results of this allozyme study.

The low differentiation observed, could be the result of persistent gene flow during the turbot pelagic phase or from post-glacial colonization from a single refuge, without enough time having elapsed for differentiation (Exadactylos *et al.* 2001, Florin & Høglung 2007). This could be the reason for low level of inbreeding (FIS) obtained after our data analyses.

It is concluded that there was no genetic differentiation among western Black Sea populations and that these three stocks could be considered as a single stock.

Reduction in the genetic resources of natural fish populations has become an important fisheries management problem. Much of the reduction is due to various human activities. Not only has the genetic diversity of many fish populations been altered, but many thousands of populations and species have been extirpated by pollution, overfishing exploitation, destruction of habitat, blockage of migration routes and other human developments (Ferguson 1995).

Allozyme markers were used to assess the genetic diversity and population structure in three turbot populations, a critically endangered commercial species in view of overexploitation. Ever-increasing pressures on fisheries resources intensify the need to identify stock structure on turbot populations. Understanding fish stock structure is an important component of successful and sustainable long-term management of turbot along western Black Sea coast.

Conclusions

Genetic diversity and population divergence were estimated using 26 allozyme loci and samples from 3 natural turbot populations along western Black Sea coast to analyzed population structure. The distribution of genetic variation evidenced from allozyme data clearly indicate low genetic differentiation among turbot populations along western Black Sea coast and showed no evidence for population subdivision. This lead to the conclusion that along western Black Sea coast one population (stock) existed.

For management of wild turbot stocks, an important challenge will be to maintain high levels of genetic variation over time. Over exploitation of this species will be crucial to maintain necessary large effective breeding population size.

The high level of genetic diversity, low genetic differentiation and the population structure imply that the fragmented habitats of turbot along western Black Sea coast may be due to recent over-exploitation.

Development of additional allozyme markers as well as of highly polymorphic microsatellites could be used for genetic identification of turbot stocks and will have substantial impact for further monitoring of turbot populations and is of primary importance for developing an optimal strategy for their effective management and rational exploitation.

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Изписване на български географски имена в статии на английски език

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Transliteration of Bulgarian geographic names in English papers

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Abstract. Some recommendations on English transliterations of Bulgarian geographic names were given which can be usable in preparing scientific papers. Examples of the following types of names were presented: I. names well known in English; II. transliterated Bulgarian names: complex names, names of mountains and mountains consisted only by a single massif.

Key words: transliteration, Bulgarian, English.

Според Наредбата за транслитерация на българските географски имена на латиница (виж Приложението) *Стара планина* се транслитерира като *Stara planina*. Според правилата на английския правопис, правилното изписване на английски е *Stara Planina Mountains*.

Поради затрудненията, които срещат зоолозите при изписване на български географски имена в статии на английски език, тук даваме някои препоръки.

Някои препоръки за изписване на български географски имена на английски (транслитерираната част е показана с червено)

1. известни с английското си название

Black Sea Coast, Northern Black Sea Coast
Danubian Plain, Northern Danubian Plain
Thracean Lowland
Rhodope Mts., East Rhodope Mts., Eastern Rhodopes

2. транслитерирани български имена

Arda River
Temnata Dupka Cave
Podbalkan Basin
Rusenski Lom Valley
Kresna Gorge

съставни имена

Zlatni Pyasatsi, Shiroko Pole Village
Belite Brezi Hut
Atanasovsko Ezero Lake
Studen Kladenets Dam
Vrachanski Balkan Nature Park

за планини

Stara Planina Mountains или Mts., West Stara Planina Mountains или Mts.
Vrachanska Planina Mountains или Mts.
Sarnena Sredna Gora Mountains или Mts.
Rhodope Mountains или Mts., East Rhodope Mountains или Mts.
Strandzha Mountains или Mts.
Slavyanka Mountains или Mts.
Vitosha Mountains или Mts.
Pirin Mountains или Mts.
Rila Mountains или Mts.

за планини състоящи се само от един връх

Ruy Mountain или Mt.

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Приложение

Извлечение от НАРЕДБА № 3 ОТ 26 ОКТОМВРИ 2006 Г. ЗА ТРАНСЛИТЕРАЦИЯ НА БЪЛГАРСКИТЕ ГЕОГРАФСКИ ИМЕНА НА ЛАТИНИЦА

В сила от 21.11.2006 г. Издадена от Министерство на регионалното развитие и благоустройството, обн. в ДВ, бр. 94 от 21 ноември 2006 г.

Чл. 4. Системата за транслитерацията на българските букви с латински букви е, както следва:

Българска азбука Латинизирана версия

А, а	A, a
Б, б	B, b
В, в	V, v
Г, г	G, g
Д, д	D, d
Е, е	E, e
Ж, ж	Zh, zh
З, з	Z, z
И, и	I, i
Й, й	Y, y
К, к	K, k
Л, л	L, l
М, м	M, m
Н, н	N, n
О, о	O, o
П, п	P, p
Р, р	R, r
С, с	S, s
Т, т	T, t
У, у	U, u
Ф, ф	F, f
Х, х	H, h
Ц, ц	Ts, ts
Ч, ч	Ch, ch
Ш, ш	Sh, sh
Щ, щ	Sht, sht
Ъ, ъ	A, a
Ь, ь	Y, y
Ю, ю	Yu, yu
Я, я	Ya, ya

Чл. 5. (1) Транслитерацията на българските буквени съчетания с латински буквени съчетания е, както следва:

1. буквеното съчетание "дж" се изписва и предава на латиница като "dzh":

Добруджа Dobrudzha

Пазарджик Pazardzhik

2. буквеното съчетание "ьо" се изписва и предава на латиница като "yo":

Васильово Vasilyovo

Синьо бърдо Sinyo bardo

3. буквеното съчетание "йо" се изписва и предава на латиница като "yo":

Змейово Zmeyovo

Йовковци Yovkovtsi

(2) Буквеното съчетание "-ия" в края на думите се изписва и предава чрез "-ia":

София Sofia

Провадия Provadia

Чл. 6. Името на българската държава се изписва и предава на латиница в съответствие с установената традиция:

България Bulgaria

Чл. 7. (1) Номенклатурните географски термини: планина, равнина, низина, плато, град, село, река, езеро, залив и др., които са част от географско име, се транслитерират:

Стара планина Stara planina

Атанасовско езеро Atanasovsko ezero

(2) Номенклатурните географски термини, които не са част от географското име, се превеждат според правилата на съответния език:

нос Емине Cape Emine Cap Emine

(3) Прилагателните "северен", "южен", "източен", "западен", "централен" и др. подобни, когато влизат в състава на географското име, се транслитерират:

Централен Балкан Tsentralen Balkan

Чл. 8. Транслитерацията на съставни географски имена се съобразява с правилата на българския правопис:

Златни пясъци Zlatni pyasatsi

Горна Оряховица Gorna Oryahovitsa

Елено-Твърдишка планина Eleno-Tvardishka planina

Чл. 9. (1) Небългарските собствени имена с латински произход, представляващи географски имена, се изписват с оригиналното им име на латиница:

Томпсън Thompson

(2) Небългарските собствени имена с друг произход, представляващи географски имена, се изписват съгласно системата за транслитерация на съответния език:

Тотлебен Totleben

Biology of the blind geobiont scarab beetle genus *Chaetonyx* Schaum, 1862 (Scarabaeidae: Orphninae) with new distribution records of *Ch. robustus* Schaum, 1862 from Bulgaria

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Abstract: Life cycle of the orphnine scarab beetles genus *Chaetonyx* was recorded for the first time based on the multiyear observations on the populations of its type species, *Ch. robustus* Schaum, 1862, in riverside habitats at Zemen Gorge region, South-Western Bulgaria. Our observations showed that this geobiont species is common in alluvial soils near Struma River, where it forms large colonies. At the Zemen Gorge, its preferable habitats are sparse riverside forests of *Alnus glutinosa* L. and *Populus nigra* L. neighboring grass communities. Larvae, adults and pupae were found up to 50 cm deep with most specimens inhabiting the upper soil layer from 0 to 20 cm deep. The highest recorded density was 89 adults and 581 larvae per 0.25 m². Oviposition, hatching of the eggs, pupation and emergence of the new adults occur during the summer. Eggs have been found in surface soil layer in July and August, the pupae – mainly in July and August. Larvae of different instars were found in soil during all months of the study period. Adults from the new generation first occur in the beginning of August. In August, September and October adults from different generations can be found together. The main part of the adults seems to die after the reproduction period, and the common lifespan of adults seems to be about a year only. The life cycle of *Ch. robustus* in investigated habitat seems to be perennial and includes two years as larval stage and one as adult. Tropical Orphninae occupy a number of ecological niches but lack colonial geobiont specialists similar to *Chaetonyx*.

Key words: Coleoptera, Orphninae, *Chaetonyx robustus*, biology, life cycle, insect ecology.

Introduction

Subfamily Orphninae is one of the poorly known groups within the hyper-diverse family Scarabaeidae. It includes 15 genera and 195 species, distributed mainly in the Afrotropical and Neotropical biogeographic regions (Paulian 1948, 1984; Frolov 2012). Habitat preferences, feeding and nesting behavior, and the life cycles are unknown for the

majority of the orphnine taxa, and the data available are scarce and largely incomplete. The relatively complete account on the biology, including life cycles and biotope preferences is only available for *Hybalus cornifrons* (Brullé, 1832) (Palmà 1938).

In the Palearctic Region, the subfamily Orphninae is represented by two genera – *Hybalus* Brullé, 1834, and *Chaetonyx* Schaum, 1862, occurring in the Mediterranean. Of them, only *Chaetonyx* is relatively widely distributed in Europe including Bulgaria. *Chaetonyx* is a small genus comprised of three nominal species with one being divided into three subspecies (Mariani 1946). The differences between the species and subspecies are feeble and characters provided by Mariani (1946) are difficult to interpret unequivocally. Variability of these characters was not assessed and may call for the revision of the genus but the taxonomy of *Chaetonyx* is outside the scope of the present work. We consider all the specimens collected by us as conspecific and belonging to *Ch. robustus* Schaum, 1862.

Two species of *Chaetonyx* – *Ch. robustus*, and *Ch. schatzmayri* Mariani, 1949, – have been reported from Bulgaria (Král & Malý 1993). *Ch. robustus* was cited in several publications (Joakimov 1904, Nedelkov 1905, Zacharieva-Stoilova 1974, Mikšić 1957, Král & Malý 1993), while *C. schatzmayri* was reported without precise locality by Mikšić (1959), Král & Malý (1993) and Bunalski (2001) from the Eastern part of Bulgaria. The records made by the mentioned authors apparently resulted from occasional findings of the adult specimens without biological and ecological observations. *Ch. robustus* was reported from the region of Zemen Gorge (Gradinarov & Petrova 2012, Gradinarov 2014), mainly in respect to its suitability as a host of the entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae).

The purpose of the present study is to analyze the results of the multiyear observations on the *Ch. robustus* populations in the Zemen Gorge region of SW Bulgaria, to provide the first comprehensive account on the habitat preferences and life cycle of a member of the genus *Chaetonyx*, and to summarize the available data about the biology of the Orphninae.

Material and Methods

Material for the present study was collected from different localities at the Zemen Gorge. The investigated region is a part of the Struma River Valley, situated between Risha and Zemenska Planina Mts., SW Bulgaria. The climate of the region is subcontinental, with an average annual air temperature of about 8.8°C and average annual precipitation of about 600 mm. All the localities where *Ch. robustus* were found are situated on the lower river banks with sandy loam alluvial soils, at altitude of about 500–600 m a.s.l.

Data on the distribution are based on hand collection of adults from soil litter and soil upper layer up to 10–20 cm depth, mainly in the period from 2009–2014. Data from a single sampling in 2002 were also included into the list of distribution. The abbreviations of the names of collectors used in the list are as follow: DG – Denis Gradinarov, YP – Yana Petrova.

Density, vertical distribution in the soil and the biology of *Ch. robustus* were studied on a sampling site where the species have been detected several times in 2011 (Gradinarov & Petrova 2012). The site is situated on the first river terrace of the Struma River near the town of Zemen (42°27.80'N, 22°42.85'E). The overall dimensions of the studied area are 15 x 2 m, the long side being parallel to the river. The distances of the sampling points to the river were 3 to 5 m. The investigated habitat is an ecotone with moderately developed herbaceous vegetation between the sparse riverside forest with black alder (*Alnus glutinosa* (L.)), black poplar (*Populus nigra* L.), black locust (*Robinia pseudoacacia* L.), dogwood (*Cornus sanguinea* L.) and the abandoned orchard of cherry plum (*Prunus cerasifera* Ehrh.) and apple (*Malus domestica* Borkh.) (Fig. 1). Among the herbaceous species, *Aegopodium podagraria* L. (Apiaceae) predominated on the sampling site, in places in combination with

several Poaceae species. The soil of the site is alluvial heavy sandy loam containing no stones or gravel particles and almost no remains of decaying wood fragments. Soil litter is not well developed.

The quantitative soil samples from the site were collected monthly during the periods of April–November 2012 and May–October 2013. The size of an individual sample was 0.25 m² (50 x 50 cm) at 50 cm depth. The soil from each sample was excavated, processed twice manually and the number of adults, larvae and pupae of the species was counted. Detected individuals were fixed in 70% alcohol during collecting or transferred live in perforated Eppendorf tubes with a little amount of the soil from the sample site for laboratory rearing. Each month a single sample from the sampling site was processed. Vertical distribution of *Ch. robustus* has been monitored from July to October 2013. In this period individuals were counted separately for 0–20 and 20–50 cm soil layers of each sample. Additional material of *Ch. robustus* from the sampling site, beside the quantitative samples, was collected in 2011–2014. The data obtained in these collections are included in the list of distribution and also used at the discussion of the species biology.



Fig. 1. Sampling site at the Zemen Gorge, SW Bulgaria.

Rearing experiments with larvae and eggs of *Chaetonyx* were conducted at room temperature (20–25°C). For pupation, third instar larvae were placed separately in 50 cm³ tubes with soil from the sampling site. Eggs hatched in Petri dishes or in perforated Eppendorf tubes with soil from the site. The head capsules of the larvae were measured on the preserved in 70% alcohol material under a stereomicroscope Olympus SZ61 using an ocular-micrometer. The photographs (Figs. 2 and 4) were taken with a digital color camera Olympus Color View I and a stereomicroscope Olympus SZ61.

Identification of the adults and larvae was confirmed with Baraud (1992) and Barbero & Palestini (1993), respectively. Conspecificity of the eggs, larvae and pupae with *Ch. robustus* was also confirmed by the laboratory rearing. The material used in this study is deposited in the collections of the Department of Zoology and Anthropology, Faculty of

Biology, Sofia University "St. Kliment Ohridski" and Zoological Institute RAS, Saint-Petersburg (ZIN).

Results

Distribution of *Ch. robustus* at the Zemen Gorge

Material examined (listed by localities): 1). Near town of Zemen, left bank of Struma Riv., 42°28.00'N, 22°43.22'E, 580 m a.s.l., poplar, alder vegetation, 11-IV-2009, 6 ♂♂, 1 ♀, beneath soil litter and in soil, leg. YP & DG; the same locality, 23-IX-2009, 2 ♀♀, in soil, leg. DG; the same locality, 14-V-2011, 1 ♂, in soil, leg. DG; the same locality, 03-X-2011, 1 ♂, in soil, leg. DG; 13-VII-2014, 1 ♂, 1 ♀, in soil, leg. DG; 2). Near town of Zemen, right bank of Struma Riv., 42°27.80'N, 22°42.85'E, 580 m a.s.l. (the sampling site), mixed riverside vegetation next to an orchard, 22-IV-2011, 3 ♂♂, 2 ♀♀, in soil, leg. DG; the same locality, 05-V-2011, 4 ♂♂, 6 ♀♀, in soil, leg. DG; the same locality, 03-X-2011, 7 ♂♂, 11 ♀♀, in soil, leg. YP & DG; the same locality, 27-V-2013, 2 ♂♂, 2 ♀♀, in soil, leg. DG; the same locality, 15-VI-2013, 2 ♂♂, 8 ♀♀, in soil, leg. DG; the same locality, 24-25-X-2013, 16 ♂♂, 15 ♀♀, in soil, leg. DG, Boyan Zlatkov & Ognyan Sivilov; the same locality, 13-VII-2014, 3 ♂♂, 3 ♀♀, in soil, leg. DG; the same locality, 03-VIII-2014, 9 ♂♂, 9 ♀♀, in soil, leg. DG; the same locality, 18-IX-2014, 4 ♂♂, 9 ♀♀, in soil, leg. DG; 3). Saraya Place, right bank of Struma Riv., 42°27.57'N, 22°42.60'E, 570 m a.s.l., poplar, alder vegetation, 23-VII-2002, 1 ♂, in soil, leg. DG; the same locality, 30-IV-2011, 1 ♀, in soil, leg. DG & Boyan Vagalinski; 4). Near Aydutsite Mahala, right bank of Struma Riv., 42°27.38'N, 22°42.79'E, 570 m a.s.l., poplar, alder vegetation, 03-VI-2012, 1 ♂, 1 ♀, in soil, leg. DG & Georgi Hristov; 5). Bliznatsite Place, left bank of Struma Riv., next to karst spring, 42°26.82'N, 22°42.73'E, 570 m alt., poplar, alder vegetation, 02-VI-2012, 1 ♀, in soil, leg. DG & Georgi Hristov; 6). Near Razhdavitsa Vill., right bank of Struma Riv., 42°24.11'N, 22°41.68'E, 520 m a.s.l., alder vegetation, 04-V-2009, 1 ♂, 4 ♀♀, beneath soil litter and in soil, leg. YP & DG; 7). Near Razhdavitsa Vill., left bank of Struma Riv., 42°23.78'N, 22°42.16'E, 510 m a.s.l., poplar, alder vegetation, 04-V-2009, 4 ♂♂, 1 ♀, beneath soil litter and in soil, leg. YP & DG.

Ch. robustus was found in seven different localities along the Zemen Gorge. The total number of collected adult specimens is 143 (66 ♂♂ and 77 ♀♀), not including the data obtained within the monthly quantitative collections from the sampling site (the latter are presented in Table 1). In all cases the specimens were found in alluvial riverside soil (at a distance up to 15 m from the river), inside the soil or at the boundary between the soil and the soil litter. The tree vegetation in the habitats usually was represented by black poplar (*Populus nigra* L.) and black alder (*Alnus glutinosa* (L.)). The forests never have formed dense canopy and in the most cases the habitat was situated next to herbaceous communities.

Density of the population of *Ch. robustus* in soil

Fourteen quantitative soil samples from the sampling site were processed during the period of investigation (Table 1). A total of 412 adults, 66 pupae and 2320 larvae of *Ch. robustus* have been excavated from the samples. Both larvae and adults have been found during all months of the study. The observed density of the adults and larvae for all the months was on average 29.4 ± 6.44 (from 2 to 89) and 165.7 ± 41.30 (from 19 to 581) individuals per 0.25 m², respectively. The maximal recorded density of the pupae was 38 specimens per 0.25 m².

Table 1. Occurrence of *Chaetonyx robustus* in the soil samples (leg. & obs. D. Gradinarov). In the sample marked with asterix, not all the individuals were sexed.

Date	Larvae	Adults			Dark individuals (%)	Pupae
		Males	Females	Total		
30-IV-2012	70	10	5	15	6 (40)	0
11-V-2012	88	11	10	21	21 (100)	0
02-VI-2012	116	9	6	15	15 (100)	0
01-VII-2012	63	14	14	28	28 (100)	3
04-VIII-2012	101	16	14	30	8 (26.7)	1
02-IX-2012	80	21	16	37	17 (45.9)	0
06-X-2012	108	6	8	14	5 (35.7)	0
03-XI-2012	156	5	4	9	5 (55.6)	0
02-V-2013	19	3	3	6	2 (33.3)	0
05-VI-2013	39	0	2	2	2 (100)	0
06-VII-2013*	346	4	2	33	33 (100)	20
03-VIII-2013	286	25	29	54	38 (70.4)	38
01-IX-2013	267	40	49	89	15 (16.9)	4
24-25-X-2013	581	29	30	59	4 (6.8)	0
Total	2320	193	192	412	199 (48.3)	66

Adults and oviposition

From late April to early August, adults have been found mainly in the soil layer from 0 to 20 cm deep (Table 2). In this period, the findings of adults at greater depths were considerably rarer. Since the beginning of August to October–November the adults showed a more uniform distribution in the studied depth range, reaching up to 50 cm depth in the soil. Adults with varying intensity of coloration have been observed throughout the different months in the both years of the survey (Table 1). In April and May, dark-brown adults and lighter ones, reddish-brown in color, have been excavated. In June and July all the collected individuals were uniformly dark-brown. In August, except for dark-brown individuals, yellowish or orange adults were also observed (Fig. 2). Such “pale” individuals were found at different depths, and constituted the major part of the adults under 0–20 cm soil layer (Table 2). In the sample of August 2013, most of these adults were yellowish, with soft and easy to deform exoskeleton. In the corresponding sample of the previous year, such slightly sclerotized adults were rarer. From August to November 2012, the proportion of dark-brown beetles increased (Fig. 3A, B), and ranged from 26.6% to 55.6% of all collected beetles (Table 1). In contrast, during the same period of 2013 this proportion showed clear decrease, reaching 6.8% at the end of October. In additional samples in 2014, only dark beetles were presented in the collections from July and August, while in the collection from September, 12 of all 13 found individuals were clearly lighter in color. No mating individuals were observed during the study period. In July 2012 and August 2013, dead intact dark-brown adults were found in the soil of the sampling site.

Table 2. Vertical distribution of *Chaetonyx robustus* in the soil in summer and autumn of 2013.

Month of sampling	Soil layer (cm)	Larvae (%)	Adults (%)			Pupae (%)
			Total	Dark	Light	
July	0–20	273 (78.7)	29 (87.9)	29 (87.9)	0 (0)	15 (75.0)
	20–50	74 (21.3)	4 (12.1)	4 (12.1)	0 (0)	5 (25.0)
August	0–20	171 (69.8)	50 (92.6)	38 (100)	12 (75.0)	30 (78.9)
	20–50	74 (30.2)	4 (7.4)	0 (0)	4 (25.0)	8 (21.1)
September	0–20	233 (87.3)	69 (77.5)	15 (100)	54 (73.0)	3 (75.0)
	20–50	34 (12.7)	20 (22.5)	0 (0)	20 (27.0)	1 (25.0)
October	0–20	384 (66.1)	35 (57.4)	3 (75.0)	32 (58.2)	0 (0)
	20–50	197 (33.9)	26 (42.6)	1 (25.0)	23 (41.8)	0 (0)

Numerous eggs of *Ch. robustus* have been observed in the upper soil layer of the sampling site (0–20 cm deep) at the beginning of July 2013 and in early August of 2014. In August 2013, only one egg was found in the sample. The eggs were relatively large, with a length of 1.20 ± 0.05 mm (1.13–1.28 mm) and a width of 0.93 ± 0.02 mm (0.9–0.96 mm) ($n = 11$). Hatching of the eggs in the laboratory continued during a week after collection of the eggs. One egg hatched in July 2013 and three eggs in August 2014 (Fig. 4A). In some of the eggs, collected on 03–VIII–2014, the developing embryos were observed (Fig. 4B).

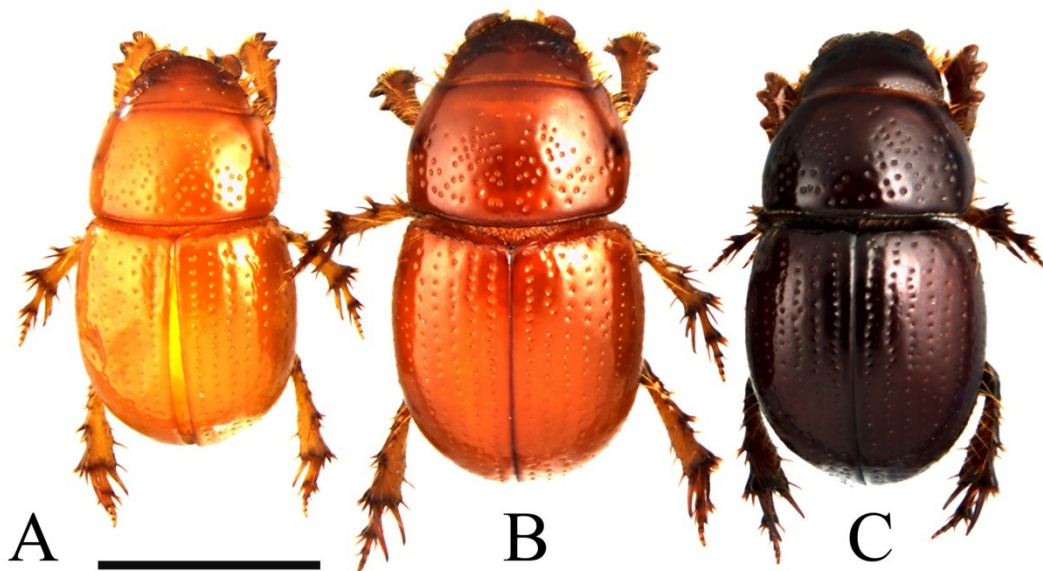
**Fig. 2.** Adults of *Chaetonyx robustus* with different degrees of sclerotization, collected on 04–VIII–2012 from the sampling site. A: Slightly sclerotized, just emerged from the pupae; B: Moderate sclerotization; C: Fully sclerotized, adult from previous generation. Scale bar: 2.5 mm.



Fig. 3. Adults of *Chaetonyx robustus*, excavated from the site near town of Zemen (in situ imaging). A: sampling on 04–VIII–2012; B: sampling on 02–IX–2012.

Larvae

Along with the eggs, small larvae, apparently first instar, were detected in samples from July and August of 2013. Some of these larvae in the samples taken during these two months had light abdomen with little or no food particles in the gut. The maximal width of the head capsule of these larvae was 0.65 ± 0.015 mm (0.62–0.68 mm, $n = 38$). Small larvae with width of the head capsule up to 0.70 mm have been found in samples of September, October and November, as well as in May 2013. Visibly larger larvae, probably of both second and third instars, have been found in the soil during all months of the study. The larvae preparing to pupate (prepupae) were found in the soil of the site in early June to early August of both 2012 and 2013. These larvae were significantly less mobile, with bright and partially transparent abdomen. The maximal width of the head capsule of the prepupae was 1.28 ± 0.07 mm (1.18–1.45 mm, $n = 16$). No prepupae were observed before June and after August. However, a significant amount of larger larvae were presented in the soil during September, October and November, along with the group of small larvae. Moulting of such larvae was repeatedly observed in August and September of both 2012 and 2013 in the soil samples during collection and during laboratory rearing.

The highest larval density was found in the soil layer from 0 to 20 cm, but significant number of larvae was detected also deeper up to the maximal excavated depth of 50 cm (Table 2). During July–October 2013, the percentage of the larvae found below 20 cm ranged from 7.3% to 33.9%. Some differences in vertical distribution, depending on the larval instar, were observed during the investigation. First instar larvae were observed only up to 20 cm depth, while the prepupae occurred in both the upper and the deep layers, up to 50 cm deep.

Pupae and pupation

Pupae of *Ch. robustus* (Fig. 4C) have been found in the soil of the sampling site in July and August 2012 and in July, August and September 2013 (Table 1). During additional sampling in July 2014, pupae have not been found. Most of the pupae have been found in the soil layer from 0 to 20 cm deep (Table 2), but about 25% have been found deeper, up to 50 cm deep. In July 2013, all pupae were white in color. In August of the same year, some pupae were darkened before adult emergence. All four pupae found in early September 2013 were darkened. Both white and darkened pupae were found in early August 2014. At the laboratory, ten larvae, collected in early May 2013 from the soil of the site, built cocoons from soil particles in the end of the same month. The cocoons were fixed to the bottom of the experimental tubes and in the middle of June four adults and four pupae were obtained.

The cocoons had smooth interior surface, thin walls and were very fragile. One prepupa in similar cocoon was observed in the soil of the site in July 2013.

At the beginning of July of the both years of the quantitative sampling, adults, eggs, larvae of the all three instars, and pupae were observed together in the soil of the sampling site (Fig. 4D).

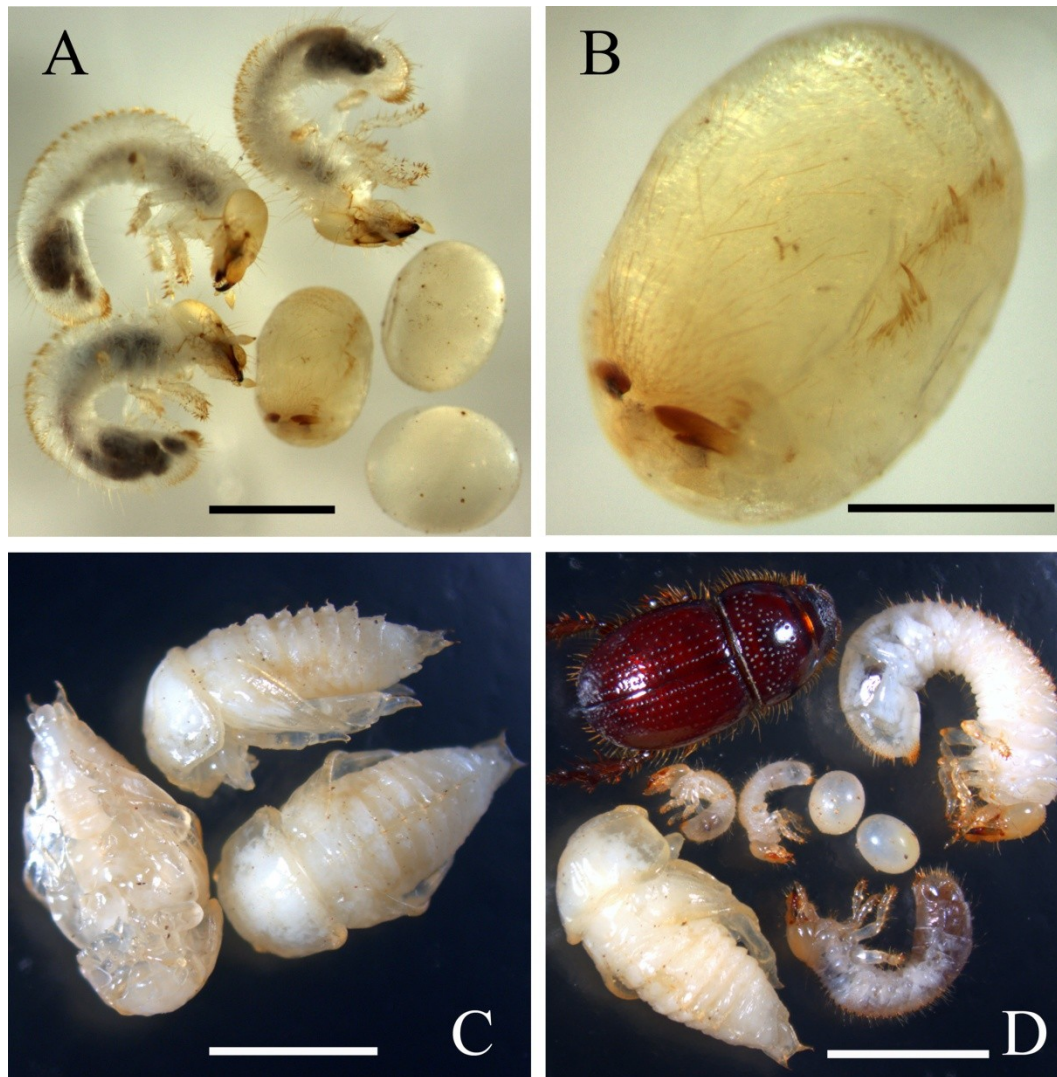


Fig. 4. Different stages of *Chaetonyx robustus*. A, B: Eggs and first instar larvae (laboratory rearing, eggs collected on 03-VIII-2014); C: Pupae, collected on 07-VII-2013 (in vivo imaging); D: Adult, pupa, larvae from the three instars and eggs (in vivo imaging, material was collected on 07-VII-2013). Scale bars: A: 1 mm; B: 0.5 mm; C, D: 2.5 mm.

Discussion

Distribution of *Ch. robustus* in Bulgaria

The areal of *Ch. robustus* includes mainly the Balkan and the Apennine peninsulas, as the species is recorded from Albania, Bulgaria, Greece, Italy, Macedonia, Romania, Serbia, Montenegro, Turkey and Hungary (López-Colón 2006). There are several records from Bulgaria, mainly from the mountain areas. For Stara Planina Mts., the species was reported from the regions of Murgash Peak (Joakimov 1904), Gorna Malina Vill. (Zacharieva-Stoilova 1974) and Vrachanska Planina Mts. (Petrova *et al.* in press), as well as without a specified locality by Nedelkov (1905). For Rila Mts., the species was listed by Mikšić (1957),

with denotation of the locality as „Rilo-Kalinin“ (possibly the area of Kalin Peak, NW Rila Mts.) and Král & Malý (1993) from the vicinity of Trestenik Hut. The latter authors also reported the species for Pirin Mts., Liljanovo Vill. (Král & Malý 1993). Additionally, Joakimov (1904) reported the species from Kurubaglar (currently known as the Lozenets district of Sofia city).

Our data shows that the species is common and locally abundant in riverside habitats in the region of Zemen Gorge. The most of the old records of the species from Bulgaria did not include quantitative data (Joakimov 1904, Nedelkov 1905, Mikšić 1957). The data of Král & Malý (1993) on *Ch. robustus* are based on a total of four specimens, found under stones (a routine method often used by entomologists, in contrast to soil excavations). According to Král & Malý (1993), the rarity of *Chaetonyx* species is likely due to their cryptic habitats. However, Zacharieva-Stoilova (1974) reported repeated findings of *Ch. robustus* (a total of 40 specimens) in the soil samples up to 30 cm deep. Our research confirms the assumption that *Ch. robustus* is geobiont species and can inhabit considerable soil depths (up to 50 cm at least). Probably the findings of the *Chaetonyx* specimens under stones are rather accidental, and have led to the false conclusion about their rarity. Soil excavations are obviously more suitable for detection of the *Chaetonyx*, which is confirmed also by the other studies (Zacharieva-Stoilova 1974, Petrova *et al.* in press).

Habitat preference of *Ch. robustus* in Bulgaria

Published data about habitat preferences as well as other observations on the ecology of *Ch. robustus* in Bulgaria are scarce. However, the species has been reported from different types of habitats, including typical mountain ones. Joakimov (1904) indicated collecting «under fallen damp foliage», probably in beech forest, in regard to the locality of Stara Planina Mts. Zacharieva-Stoilova (1974) and Král & Malý (1993) found the species in pastures (at about 500–700 m a.s.l. in Stara Planina Mts. and 1700 m a.s.l. in Rila Mts., respectively). Recently, the species was detected in the soil of xerotherm oak-hornbeam forest at 500 m a.s.l. in Vrachanska Planina Mts. (Petrova *et al.* in press). During the present research, *Ch. robustus* was commonly found in the sandy alluvial soils of sparse river valley forests, at 500–600 m a.s.l. The old records lack information about the soil type in the species habitats and about the proximity to rivers or other water bodies. The locality at Vrachanska Planina Mts., mentioned above, is situated relatively close to Iskar River (approximately 200 m from the shore), but 100 m higher due to very steep riverbank (Petrova *et al.* in press). The soil of this habitat is quite different from that at the localities in Zemen Gorge: it is of brown forest type, stony, close to rendzinas. It seems that the sandy soil and the close proximity to rivers or other water bodies are not essential for the presence of populations of the species. Taking into account the results obtained in the present research and the reports of the earlier authors, it can be assumed that in Bulgaria *Ch. robustus* has significant environmental plasticity, at least in regard to the altitude, soil type and the type of vegetation community.

Density and vertical distribution in the soil

The average density of the species in all samples during our study is 117.6 adults and 662.8 larvae per m². The differences in the number of larvae and adults between samples most probably results from the random selection of sampling sites rather than seasonal dynamics of the population. However, these data show that the population of *Ch. robustus* in the riverbank soils in the Zemen Gorge is impressively dense. Higher abundance of *Chaetonyx* larvae has been observed in the upper soil layer (up to 20 cm deep), although the larvae have been found also at greater depth (Table 2). In the upper soil layer, roots of *Aegopodium podagraria* and other herbaceous plants, which decaying parts could be used as a food source by the larvae, are developed. On the other hand, this layer is warmed more

intensely and earlier in the spring, which is favorable both for the larval growth and pupation. The spreading of the larvae on greater soil depths probably is an adaptation for more complete use of the food resources and for reducing the intraspecific competition. Such more even distribution of the larvae particularly leads to the decrease of the antagonistic relationships between them within population. If placed together in a little amount of soil, live larvae often injure each other, causing significant mortality. The pupation occurs both in the warmer upper soil layer and in greater depths. In July and August 2013, the percentage of the pupae found in the soil layer of 20–50 cm deep is comparable with that of the larvae. This suggests that the larvae do not migrate for pupation. Young adults remain close to the places of emergence for a certain time, which results in an increase of the proportion of adults found in greater soil depths in September–October. At the same time, oviposition and hatching takes place in the upper soil layer, which implies migration of the adults to the surface for reproduction and migration of a part of the young larvae to deeper layers during their subsequent development.

Development and life cycle of *Ch. robustus*

The research on the local population at the Zemen Gorge indicates that the whole development of *Ch. robustus* occurs in the same habitat. Due to its aptery, the species is colonial and can reach high local abundance in the soil of suitable habitats. The studies of biology and life cycle of the species, conducted on the sampling site, covered three consecutive years – quantitative surveys during 2012 and 2013 and additional collections in 2014. It should be noted that the latter two years – 2013 and especially 2014 were characterized by the uncommonly, for the region, cool and wet summers. This apparently affected the beginning and duration of the different stages of the life cycle of the species in these years.

Pupation, adult emergence, reproduction and lifespan

Both pupation and emergence of the adults of *Ch. robustus* are asynchronous within population and in the investigated habitat they occurred during all summer months. First pupae were observed in the quantitative samples in early July and it seems that the pupation under favorable conditions begins in late June. Finding of the pupae in early September 2013, as well as their delayed occurrence in 2014, were probably due to unfavorable temperatures during both years. At the laboratory, pupation was observed about one month earlier than in the field. It seems that at least a part of the third instar larvae are ready for pupation after overwintering and the process is initiated by the raise of the soil temperature. Lower temperatures at soil depth additionally delays the pupation and subsequent adult emergence when larval development takes place in deeper soil layers, increasing asynchronicity in the life cycle of the species.

Under favorable conditions, adult emergence probably begins in late July. In 2014, the emergence occurred later in accordance with the delayed pupation and the lower temperatures. Lighter colored beetles, that have been found at the beginning of August in both years, as well as in September 2014, belonged to the new adult generation. The slightly sclerotized yellowish adults in the sample from the early August 2013 obviously have had just emerged. In the following months, the slow progress of sclerotization of the exoskeleton of the young adults occurred, resulted in gradual darkening of the beetles. In some individuals, this process completed after wintering which explains the presence of lighter, reddish-brown beetles in the spring. In June and July, before and at the time of oviposition, only dark-brown adults have been found.

In the literature, there is no information on the lifespan of the adult orphnines. Data from this study indicates that adults of *Ch. robustus* from different generations coexist in the population at least for a few mounts. In early August to early September, they are

recognizable by the degree of sclerotization. With the developing of the sclerotization of the young adults in the autumn of 2012, the differences between young and older beetles decreased (Fig. 3). In 2013, because of the later adult emergence, all young beetles did not complete their sclerotization by the end of October and even the darkest of them were easily distinguished from those belonging to the older generation. The percentage of the completely sclerotized dark-brown adults in August to October in this case clearly indicate the proportion of the old individuals in the population (Table 1). This proportion showed a clear decline trend towards to the autumn and it seems that the new adult generation displaces the previous. Thus, in the sample from October 2013 only four of 59 excavated adults belonged to the older generation. Some of the old adults die after oviposition, as dead dark-brown beetles have been found in samples from July and August during our study. It is possible that some of the older adults can overwinter and even reproduce next summer. It is unlikely, however, that these adults normally represent significant part of the population. It also seems improbable that adults can survive more than two years. The common lifespan of adults in studied population appears to be about one year, as they overwinter and reproduce only once. Reproduction begins before emergence of the adults from the new generation, during the period of pupation of the previous generation, and the new adults do not reproduce in the same year. Oviposition takes place at the warmer upper layer of the soil, where the dark-brown adults are gathering before and during reproduction. In late July 2014, in the colony of *Ch. robustus* in Vrachanska Planina Mts. we have observed adults, eggs, larvae of different instars, and a single pupa (Petrova *et al.* in press). This suggests that both reproduction and pupation occur during the summer also in the other regions and habitat types in Bulgaria.

Larval development

Larvae of apparently different instars have been observed during all months of the study. This, along with the life span of the adults, suggests at least two generations overlap. It appears that both larvae and adults overwinter. It is however unclear how many times the specimens overwinter in their life cycle and whether the number of overwinterings is constant in different environmental conditions. It seems that all three larval instars are able to overwinter. Apparently most of the first instar larvae that hatch in the summer overwinter as second instar, while the rest overwinter as first. The overwintering first larvae likely molt in the spring which resulted in their absence in the soil in June. Prepupae have not been found in the soil after the beginning of August, and it seems that not all of the older larvae pupate in the same summer. Some of these larvae apparently overwinter again as second or third instar. In summary, the life cycle of *Ch. robustus* seems to be perennial and in studied habitat may include two overwinterings as larval stage and one as adult (Fig. 5).

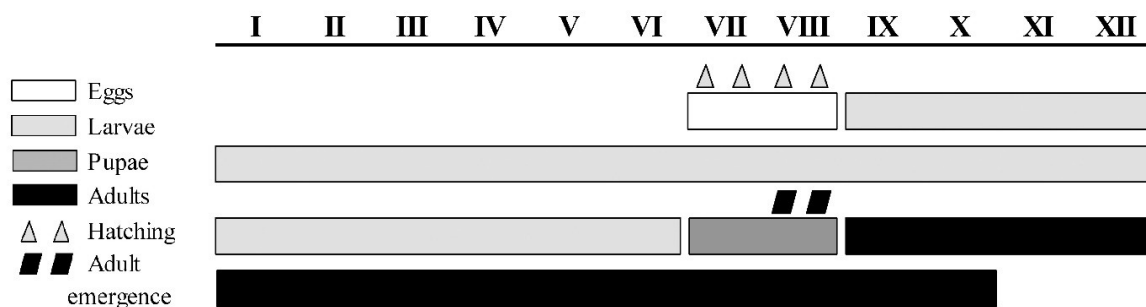


Fig. 5. Life cycle of *Chaetonyx robustus*. Diagram is constructed based on the investigation of the local population in riverside habitat at the Zemen Gorge, SW Bulgaria, in April 2012 to November 2014.

Biology of the Orphninae

Although little information is available on the biology of the other Orphninae genera, the habitat preferences and life cycle of *Chaetonyx* is probably unique among the orphnines and reflect its morphological and behavioral adaptations to subterranean life in temperate regions. *Chaetonyx* comprises the smallest orphnines possessing no head or pronotum armature – the character of allometric sexual dimorphism found in the majority of the Orphninae genera. All *Chaetonyx* are apterous, with fused elytra, and almost blind with the eyes reduced to only a few facettes. These are the adaptation to the subterranean life style and the species apparently do not leave soil at all stages of their life cycle. Due to the aptery and low dispersal potential, *Chaetonyx* live in large colonies. The ecological niche occupied by *Chaetonyx* apparently did not allow it for reasonable diversification in comparison to another geophilous genus, *Hybalus*.

The only other orphnine genus, which biology was studied in some detail, is *Hybalus*. This genus comprises over 30 species distributed mostly in the Atlas Mountains of North Africa (Baraud 1991). Similar to *Chaetonyx*, *Hybalus* are apterous and their elytra are fused, although the two genera are probably not closely related and the aptery was gained by them independently (Frolov, unpublished data). *Hybalus* are geophilous but not so much adapted to subterranean life as *Chaetonyx*. They are larger (body length 6.0–11.5 mm) and with small but well developed eyes. Although rarely encountered on soil surface, the beetles may come out on rainy days (Keith 2005). According to Gourvès (1988), who studied biology of *H. rotrovi* Petrovitz, in the eastern Middle Atlas, the adults were found in rainy season, mainly from November to March in soil 3 to 5 cm deep. Mating took place in soil and the 2-instar larvae diapaused during the dry season. Larvae fed on grass roots and resumed feeding activity with the beginning of the rainy season in October to quickly finish development, pupate, and give new generation of adults in November. Palmã (1938) described the similar, annual life cycle of *H. cornifrons* (Brullé) in Sicily and noted reasonable damage to wheat crops caused by the beetles in some years (probably this record refers to *H. benoitii* Tournier, since *H. cornifrons* doesn't occur in Sicily according to Baraud, 1991).

Biology of the tropical Orphninae, especially their life cycles and nesting behavior, is virtually unknown. The data from the collection specimens labels suggest that imago of most species are litter dwellers inhabiting forest and savanna biotopes. In Afrotropical Region and, especially, in Madagascar, adult orphnines were collected in pitfall traps mostly baited with fish (Frolov & Montreuil 2006, 2009). The larvae of *Triodontus nitidulus* (Guérin-Méneville, 1844) were found in upland rice plantations in Central Madagascar (Randriamanantsoa *et al.* 2010). Although it is unclear if the larvae of this species cause any damage to the crops, the adaptation to the development on plantations might explain the relative abundance of *T. nitidulus* throughout the island. The immature stages of only one Neotropical species, *Aegidium cribratum* Bates, were described (Morón 1991). The larvae and pupa of this species were found in association with adults in rotten logs, which suggest mycetophagy rather than detritophagy at least at the larval stages. An unexpected association of the Orphninae with epiphytes was recently discovered in the Andean cloud forest in Ecuador: the adults of an undescribed taxon were collected from the dead plant materials accumulated between epiphytes and tree bark up to 16 m high (Frolov & Vaz-de-Mello 2015). Available data, although scanty, suggest that the tropical Orphninae occupy a number of rather distinct ecological niches but apparently lack colonial geobiont specialists similar to *Chaetonyx*.

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The first report of Japanese oak silkmoth *Antheraea yamamai* (Guérin-Méneville, 1861) (Lepidoptera: Saturniidae) in Montenegro

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Abstract. Japanese oak silkmoth *Antheraea yamamai* (Guérin-Méneville, 1861) is reported here for first time in the scientific literature from Montenegro (Republic of Crna Gora). In two localities, not far away each other, moths were collected at light. We report this species from Serbia as well. All collected specimens are males.

Keywords: Saturniidae, *Antheraea yamamai*, Montenegro, Serbia, introduced species

Introduction

Antheraea yamamai (Guérin-Méneville, 1861) is a native of Japan, but it can be found also in South and North Korea, China and Taiwan (Gongyin & Cui, 1996; Goel & Krishna Rao, 2004). It is introduced to Ceylon (Rougeot & Viette, 1978) and in Northern India for silk production (Macek *et al.* 2007). *A. yamamai* is very large and distinctive species with a wingspan of 110-140 mm. In Europe it can be confused only with its closely related species Chinese Oak Silkmoth *Antheraea pernyi* (Guérin-Méneville, 1855). Differences between these two species are figured in many articles. *A. yamamai* differs at glance from the other representatives of family Saturniidae by its wing shape and colour, also the flight period overlaps with only one member of the family which looks like quite different. It has one generation per year. Adults are on the wings from late July to October (Kranjčev, 2005; Kühnert, 1971). Larvae feed mainly with *Quercus* spp. but also accept *Ulmus* spp., *Fagus sylvatica* L., *Castanea sativa* Mill., *Carpinus* spp., *Rosa* spp., *Cydonia* spp., *Sorbus aria* (L.) Crantz, *Pyracantha coccinea* M. Roem., *Mespilus germanica* L., *Crataegus* spp., *Photinia glabra* (Thunb.) Franch. & Sav., *Chaenomeles japonioca* (Thunb.) Lindl. ex Spach and *Acer campestre* L. (Kranjčev, 2005; Živojinović & Vasić, 1963; Wallace, 1867). It seems they are polyphagous on different deciduous trees and bushes. Moths are active during the night and are attracted to artificial light.

This species was introduced from Japan to Europe for silk production. In Southern Europe it has stable population (Macek *et al.* 2007). According to Wallace (1867) in Europe it was first introduced in France in 1861 but rearing experiments could not provide propagation of the species. In 1863 it was introduced again in Netherlands and sent to some other European countries (Wallace, 1867). It was successfully reared by Mach family in Veliki Slatnik near Novo Mesto in Slovenia from where it escaped in 1867 and started to spread (Hafner, 1910; Mušić, 1955). So far it is reported from Austria (Walzl, 1985), Bosnia & Herzegovina (Hanjalić & Lelo, 2014), Croatia (Kovačević & Franjević-Oštrc, 1978), Germany (Schmidt & Weigert, 2006), Hungary (Kovács, 1957; Macek *et al.* 2007), Italy (Cesale, 1973), Romania (Pittaway, 2000-2015), Serbia (Živojinović & Vasić, 1963), Slovenia

(Michieli, 1963). According to Macek *et al.* (2007) in Czech Republic it is not registered, but it may occur there, which was confirmed in Pittaway (2000-2015). Damages are reported only from Serbia in *Quercus* forests in Srem area in 1960-1962 (Živojinović & Vasić, 1963) and having this in mind designation of *A. yamamai* as an invasive species in Serbia (Zatezalo, 2014) seems unjustified since there are no additional data to support this. Damages were not noticed in any other European country.

Hanjalić & Lelo (2014) and Krpac *et al.* (2015) mentioned this species to be present in Montenegro but without supporting literature. Glavendekić *et al.* (2005) reported *A. yamamai* for Serbia and Montenegro. As these two countries were one country at that time and this is just a paper from proceedings it is not clear whether it occurs in Serbia or Montenegro or both present-day countries. By contacting the authors we got information that data on *A. yamamai* refer to literature records from Srem in Serbia (Glavendekić, pers. comm.).

Material and Methods

We collected 8 nights in Montenegro and Western Serbia using 3 portable light traps and lamps plugged in generator. Light traps were pot with funnel with one 8Wactinic/or 368 nm and one 8W “blacklight” tubes powered by 12V batteries. 220V generator was used for powering Finish type “tent-like” trap with 160W MVL at the top and pot with funnel with 20W 368nm black light below. Another pot with funnel with 20W 368nm lamp was used in distance of 60-70m from the “tent-like” trap. Distance between the traps was of several hundred meters. All traps ran all over the nights.

Results

Montenegro

Pivska planina Mt., Polje Crkvičko village, Rudine, 43°19'48" N, 18°53'41" E, altitude 1117 m a.s.l., 07.08.2015, 1 male in the pot of the “tent-like” trap (Fig. 1)

Pivska planina Mt., Boričje village, 43°08'14" N; 18°54'18" E, 1182 m a. s. l., 09.08.2015, 2 males at the “tent-like” trap and 1male on the ground near the trap with 20W 368nm lamp

Serbia

Javor Mt., Vasilin vrh peak, 43°26'28" N; 20°03'06" E, 1489 m a.s.l., 12.08.2015, 1 male at portable light trap



Fig. 1. *Antheraea yamamai* (Guérin-Méneville, 1861) male, Montenegro, Pivska planina Mt., Polje Crkvičko village, Rudine, 07.08.2015, in coll. NMNHS. Photo: Stoyan Beshkov.

Discussion

These are the first records of *A. yamamai* from Montenegro in scientific literature since it has been only photographed from Mt. Durmitor (Verspui, 2013). *A. yamamai* was known in Serbia only from Srem area in lowland forests (Živojinović & Vasić, 1963) and on Mt. Fruška Gora (Stojanović, 2012). In Bosnia & Herzegovina it was observed for the first time in 1996 but since 2004 reports are regular (Mihajlović & Stanivuković, 2009; Neimarlija & Merdan, 2012; Hanjalić & Lelo, 2014). In Croatia it is distributed throughout the country (Mladinov, 1976; Kovačević & Franjević-Oštrc, 1978; Kranjčev, 2005; Vignjević *et al.* 2010; Vojvodić, 2011). Considering its wide distribution in neighboring countries of Bosnia & Herzegovina and Croatia together with lack of continuous research on moths in Serbia and Montenegro, we assume that *A. yamamai* is already widely distributed in northern Montenegro and western Serbia. Its distribution in Serbia can be seen in an Internet database (Hric, 2014). The further spread on the Balkans should be expected in near future.

All collecting localities are more or less open area near forest with *Quercus* spp., *Fagus sylvatica*, *Acer* spp. and *Corylus* spp. The low density and the presence of male specimens only supports the opinion that perhaps collected specimens are dispersed individuals and this is not migration or colonization of new territories.

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Published data and new records to the fauna of Eupelmidae (Insecta: Hymenoptera) in Bulgaria

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Abstract. This study presents information about published data on eupelmid fauna of Bulgaria and new records of 16 species distributed in the country. Some of them are reared from Cynipidae (Hymenoptera) galls on *Quercus*, *Rosa*, *Hypocoum*, as well as from Cecidomyiidae (Diptera) developing in stems of *Eryngium campestre* L. Other species are reared from pods of *Astragalus glycyphyllos* L. and seeds of *Dianthus giganteus* d'Urv. New host associations are established. As a result of the study 4 species and 1 genus are new to the fauna of Bulgaria.

Key words: Eupelmidae, Bulgaria, new records, new host associations.

Introduction

Eupelmidae Walker, 1833 is a worldwide distributed family with the highest species diversity in tropical regions. Currently, there are over than 970 species in 49 genera recognized as valid and about 120 species are reported for European continent (Noyes 2015). The majority of eupelmid wasps in larval stages are primary idiobiont ectoparasitoids or predators of beetle larvae, but many other insect hosts are attacked from Diptera, Lepidoptera and Hymenoptera orders and spiders that mostly are concealed or protected in plant tissue. Polyphagy and hyperparasitism are typical for many eupelmid species (Gibson 1995).

The Eupelmid fauna in Bulgaria has not been a subject of special investigations. Only 21 species have been published in entomological papers till present study.

The first record of Eupelmidae from Bulgaria is given by (Chorbadzhev 1928). Later, many authors recorded additional species to Bulgarian fauna (Bouček 1965, 1967; Bouček & Andriescu 1967; Popov 1968; Angelov 1970; Pelov 1975; Kalina 1981, 1988). More intensive taxonomical and faunistical studies were carried out on eupelmid species that are associated with Lepidoptera pests on coniferous and some deciduous trees (Keremidchiev & Ganchev 1971, 1973; Tsankov & Mirchev 1983; Tsankov *et al.* 1984, 1996a, 1996b, 1997, 1998a, 1998b; Mirchev *et al.* 1998a, 1998b, 2003, 2010, 2011, 2012, 2014; Mirchev & Tsankov 2001; Tomov & Hristov 2007).

The great variety of habitats in Bulgaria and the geographical position of the country between Europe and Asia, give bases to presume existing of rich entomofauna there, including many endemic and relict forms. The present study is a first step of future investigations on eupelmids that are distributed in the region.

The aim of this paper is to summarize all accessible published data on Eupelmidae family from Bulgaria, as well as to present new faunistic records.

Material and Methods

The accessible published data on Eupelmidae family in Bulgaria was summarized. The original material was collected between 2003 and 2015 in different regions of Bulgaria mainly by sweeping in grass localities. Some specimens were reared at laboratory conditions from galls, pods, seeds and stems of different plants. The galls were put in the dark plastic bottles and boxes with an aluminum foil and misdirected test tubes. Emerged insects were fixed in 96° ethyl alcohol. An additional material was collected by Malaise trap. Complete data with names of the localities, GPS – coordinates and altitude are given in Table 1.

The species were determined after previously dehydration with isopropanol and placement on card plates. Determination was made after: Nikolskaya (1952); Kalina (1981, 1984, 1988); Askew & Nieves-Aldrey (2000); Gibson (2010, 2011); Al khatib *et al.* (2014).

The list of the established species comprises the following data: the valid taxa name, locality names, date of collecting, number and sex of specimens, host (in case of rearing), name of the collector. The species new to the Bulgarian fauna are marked with an asterisk (*) in the faunistic list; new genus to the Bulgarian fauna is marked with double asterisk (**). The material is preserved as card-mounted specimens in the authors' collection (University of Plovdiv).

Table 1. Collecting localities of Eupelmidae species in Bulgaria.

Locality	GPS - coordinates	Altitude
Vitosha Mts., Vladaya Village	N 42°37'26.4" E 023°10'50.9"	853 m.
Vitosha Mts., Marchaevo Village	N 42°37'12.4" E 023°11'03.9"	918 m.
Rhodope Mts., Madzharovo	N 41°39'04.3" E 025°53'01.3"	245 m.
Rhodope Mts., Dolni Glavanak Village, Kromleh loc.	N 41°40'51.4" E 025°49'28.7"	295 m.
Rhodope Mts., Hrabrino Village	N 42°02'10.0" E 024°39'33.9"	529 m.
Osogovo Mts., Vratsa Village, Dervena loc.	N 42°15'35.4" E 022°33'39.9"	959 m.
Osogovo Mts., Granitsa Village	N 42°15'30.0" E 022°44'26.2"	559 m.
Osogovo Mts., Eremiya Village	N 42°12'53.8" E 022°50'11.3"	600 m.
Ihtimanska Sredna Gora Mts., Verinsko Village	N 42°29'09.6" E 023°45'12.0"	743 m.
Thracian Lowland, Plovdiv, Dzhenem Tepe loc.	N 42°08'11.2" E 024°43'55.6"	307 m.
Stara Planina Mts., Vlasatili Village	N 42°49'19.6" E 025°35'30.8"	855 m.
Stara Planina Mts., above Vlasatili Village	N 42°48'48.7" E 025°35'32.6"	758 m.
West Stara Planina Mts., Vrachanski Balkan Nature Park	N 43°11'16.8" E 023°30'18.2"	709 m.
Stara Planina Mts., Plachkovtsi	N 42°48'04.2" E 025°28'09.7"	594 m.
Stara Planina Mts., above Gorni Stoevtsi Village, Rata loc.	N 42°47'41.1" E 025°29'03.8"	817 m.

Mezdra, Darmantsi Village	N 43°07'45.3" E 023°43'23.0"	276 m.
Veliko Tarnovo, Arbanasi Village	N 43°05'50.1" E 025°40'38.3"	417 m.
Black Sea Coast, Tsarevo	N 42°10'44.1" E 027°50'33.2"	10 m.
Black Sea Coast, Sinemorets, Silistar Protected Area	N 42°03'09.6" E 027°59'20.6"	22 m.
Strandzha Mts., The Mouth of Veleka River Protected Area	N 42°03'21.4" E 027°58'07.4"	41 m.
Belasitsa Mts., Kongura Hut	N 41°20'54.7" E 023°11'18.9"	1273 m.
Belasitsa, Ribnik Village	N 41°29'13.5" E 023°15'44.5"	96 m.
Ropotamo River	N 42°18'26.3" E 027°43'27.5"	35 m.

Results and Discussion

All accessible information concerning Eupelmidae species in Bulgaria till present study is presented in Table 2. Twenty-one species belonging to 5 genera were recorded in entomological papers. The species are arranged in alphabetic order.

Table 2. List of species of Eupelmidae family reported for Bulgaria.

Valid name	Literary source
1. <i>Anastatus bifasciatus</i> (Geoffroy, 1785)	Zanati 1978; Tsankov 1981, 1990; Tsankov & Mirchev 1983; Zaharieva 1983; Tsankov <i>et al.</i> 1984, 1996a, 1996b, 1997, 1998a, 1998b; Vidal 1993; Georgiev 1996; Zaharieva <i>et al.</i> 1997; Mirchev <i>et al.</i> 1998a, 1998b, 2003, 2010, 2011, 2012, 2014; Mirchev & Tsankov 2001
2. <i>Anastatus catalonicus</i> Bolivar y Pieltain, 1935	Pelov 1975
3. <i>Anastatus japonicus</i> Ashmead, 1904	Chorbadzhiev 1928; Thompson 1955; Stefanov & Keremidchiev 1961; Keremidchiev & Ganchev 1971, 1973; Herting 1976; Ganchev <i>et al.</i> 1980; Tsankov <i>et al.</i> 1984
4. <i>Calosota metallica</i> (Gahan, 1922)	Gibson 2010
5. <i>Calymmochilus dispar</i> Boucek & Andriescu, 1967	Bouček & Andriescu 1967; Bouček 1977; Trjapitzin 1978
6. <i>Eupelmus annulatus</i> Nees, 1834	Tsankov <i>et al.</i> 1991; Melika <i>et al.</i> 2002; Georgiev 2004; Stojanova 2006

Valid name	Literary source
7. <i>Eupelmus aseculatus</i> (Kalina, 1981)	Kalina 1981
8. <i>Eupelmus atropurpureus</i> Dalman, 1820	Angelov 1970
9. <i>Eupelmus australiensis</i> (Girault, 1913)	Bouček 1965
10. <i>Eupelmus azureus</i> Ratzeburg, 1844	Askew <i>et al.</i> 2013
11. <i>Eupelmus bulgaricus</i> Kalina, 1988	Kalina 1988
12. <i>Eupelmus cerris</i> Förster, 1860	Stojanova 2006
13. <i>Eupelmus confusus</i> Al khatib, 2014	Al khatib <i>et al.</i> 2014
14. <i>Eupelmus falcatus</i> (Nikolskaya, 1952)	Bouček 1966, Kalina 1981
15. <i>Eupelmus impennis</i> (Nikolskaya, 1952)	Kalina 1981
16. <i>Eupelmus maculatus</i> (Ferrière, 1954)	Fusu 2009
17. <i>Eupelmus microzonus</i> Förster, 1860	Popov 1968; Nieves-Aldrey & Melika 2005; Askew <i>et al.</i> 2006
18. <i>Eupelmus muellneri</i> Ruschka, 1921	Pelov 1975
19. <i>Eupelmus urozonus</i> Dalman, 1820	Popov 1968; Pelov 1975, 1999; Tsankov & Markova 1992; Vidal 1993; Georgiev & Pelov 1995, 1996; Tsankov <i>et al.</i> 1995; Markova 1997; Mirchev <i>et al.</i> 2001; Georgiev 2004; Georgiev <i>et al.</i> 2004; Stojanović & Marković 2004; Stojanova 2006; Pelov <i>et al.</i> 2007; Tomov & Hristov 2007; Daneshvar <i>et al.</i> 2009; Todorov <i>et al.</i> 2012; Askew <i>et al.</i> 2013

Valid name	Literary source
20. <i>Eupelmus vesicularis</i> (Retzius, 1783)	Angelov 1970; Pelov 1975; Tsankov <i>et al.</i> 1996a, 1996b; Mirchev <i>et al.</i> 1998b; Mirchev & Tsankov 2001; Stojanova 2006; Askew <i>et al.</i> 2006; Pelov <i>et al.</i> 2007; Todorov <i>et al.</i> 2012
21. <i>Eusandalum walkeri</i> (Curtis, 1836)	Bouček 1967

Faunistic list

Anastatus Motschulsky, 1859

**Anastatus giraudi* (Ruschka, 1921)

Material: Vitosha Mts.: Vladaya Village, 14.VIII.2014, 1 ♀ (M. Antov).

Anastatus japonicus Ashmead, 1904

Material: Ropotamo River, 15.VIII.2012, 16 ♀ (Malaise trap) (St. Petrov).

Calosota Curtis, 1836

Calosota metallica (Gahan, 1922)

Material: Vitosha Mts.: Vladaya Village, 14.VIII.2014, 14 ♀ (M. Antov); Stara Planina Mts.: Vlasatili Village, 21.VII.2014, 1 ♀ (M. Antov).

Eupelmus Dalman, 1820

Eupelmus annulatus Nees, 1834

Material: Rhodope Mts.: Madzharovo, 03.VI.2015, 1 ♀ (M. Antov) (emerged from *Andricus quercustozae* (Bosc, 1792) (Hymenoptera: Cynipidae) galls on *Quercus* sp.).

Eupelmus aseculatus (Kalina, 1981)

Material: Thracian Lowland: Plovdiv, Dzhendem Tepe loc., 16 – 18.V.2015, 7 ♀ (A. Stojanova) (emerged from *Aylax hypecoi* (Trotter, 1913) (Hymenoptera: Cynipidae) galls on *Hypecoum imberbe* Sibth. & Sm.).

Eupelmus atropurpureus Dalman, 1820

Material: Vitosha Mts.: Marchaevu Village, 14.VIII.2014, 1 ♀ (M. Antov); Ihtimanska Sredna Gora Mts.: Verinsko Village, 13.VIII.2014, 1 ♀ (M. Antov); Black Sea Coast: Tsarevo, 11.VI.2014, 1 ♀ (M. Antov).

Eupelmus australiensis (Girault, 1913)

Material: Ihtimanska Sredna Gora Mts.: Verinsko Village, 13.VIII.2014, 2 ♀ (M. Antov); West Stara Planina Mts.: Vrachanski Balkan Nature Park, 20.VIII.2014, 20 ♀ (M. Antov); Mezdra: Darmantsi Village, 21.VIII.2014, 4 ♀ (M. Antov); Stara Planina Mts.: Plachkovtsi, 22.VII.2014, 1 ♀ (M. Antov); Belasitsa Mts.: Ribnik Village, 07.VIII.2014, 2 ♀ (M. Antov); Rhodope Mts.: Hrabrino Village, 24.VI.2014, 1 ♀ (M. Antov).

Eupelmus azureus Ratzeburg, 1844

Material: Rhodope Mts.: Dolni Glavanak Village, Kromleh loc., 30.V.2014, 2 ♀ (P. Boyadzhiev) (emerged from *Biorhiza pallida* (Olivier, 1791) (Hymenoptera: Cynipidae) galls on *Quercus* sp.); Black Sea Coast: Sinemorets Village, Silistar Protected Area, 16.X.2014, 2 ♀ (M. Antov) (emerged from *Andricus lucidus* (Hartig, 1843) (Hymenoptera: Cynipidae) galls on *Quercus* sp.); Silistar Protected Area, 14.X.2014, 1 ♀ (M. Antov) (emerged from *Andricus quercustozae* (Hymenoptera: Cynipidae) galls on *Quercus* sp.); Strandzha Mts.: The Mouth of

Veleka River Protected Area, 16.X.2014, 4 ♀, 2 ♂ (M. Antov) (emerged from *Andricus caputmedusae* (Hartig, 1843) (Hymenoptera: Cynipidae) galls on *Quercus* sp.).

****Eupelmus cicadae* Giraud, 1872**

Material: Rhodope Mts.: Hrabrino Village, 24.VI.2014, 1 ♀ (M. Antov).

***Eupelmus falcatus* (Nikolskaya, 1952)**

Material: Black Sea Coast: Tsarevo, 12.VI.2014, 1 ♀ (M. Antov); Mezdra: Darmantsi Village, 21.VIII.2014, 1 ♀ (M. Antov).

***Eupelmus impennis* (Nikolskaya, 1952)**

Material: Black Sea Coast: Sinemorets Village, Silistar Protected Area, 06.VIII.2003, 50 ♀, 29 ♂ (A. Stojanova) (emerged from *Lasioptera eryngii* (Vallot, 1829) (Diptera: Cecidomyiidae) galls in stems of *Eryngium campestre* L.); 16.X.2014, 4 ♀, 4 ♂ (M. Antov) (emerged from *Lasioptera eryngii* (Diptera: Cecidomyiidae) galls in stems of *Eryngium campestre*).

****Eupelmus linearis* Förster, 1860**

Material: Osogovo Mts.: Vrattsa Village, Dervena loc., 08.VII.2014, 1 ♀ (M. Antov); Granitsa Village, 09.VII.2014, 1 ♀ (M. Antov).

***Eupelmus microzonus* Förster, 1860**

Material: Thracian Lowland: Plovdiv, Dzhendem Tepe loc., 16 – 18.V.2015, 4 ♀, 2 ♂ (A. Stojanova) (emerged from *Aylax hypocoi* (Hymenoptera: Cynipidae) galls on *Hypocoum imberbe*); Veliko Tarnovo: Arbanasi Village, 01.VIII.2015, 9 ♀ (M. Antov) (emerged from seeds of *Dianthus giganteus* d'Urv).

***Eupelmus urozonus* Dalman, 1820**

Material: Osogovo Mts.: Granitsa Village, 17.XI.2014, 18 ♀, 2 ♂; 13.IV.2015, 9 ♀, 1 ♂ (M. Antov) (emerged from *Diplolepis rosae* (Linnaeus, 1758) (Hymenoptera: Cynipidae) galls on *Rosa* sp.); Vitosha Mts.: Marchaevo Village, 07.XI.2014, 2 ♀; 01.V.2015, 1 ♀ (emerged from *Diplolepis rosae* (Hymenoptera: Cynipidae) galls on *Rosa* sp.); Vladaya Village, 01.V.2015, 1 ♀ (emerged from *Diplolepis rosae* (Hymenoptera: Cynipidae) galls on *Rosa* sp.).

***Eupelmus vesicularis* (Retzius, 1783)**

Material: Belasitsa Mts.: Kongura Hut, 06.VIII.2014, 1 ♀ (M. Antov); Ihtimanska Sredna Gora Mts.: Verinsko Village, 13.VIII.2014, 83 ♀ (M. Antov); Vitosha Mts.: Vladaya Village, 14.VIII.2014, 5 ♀ (M. Antov); Marchaevo Village, 14.VIII.2014, 2 ♀ (M. Antov); West Stara Planina Mts.: Vrachanski Balkan Nature Park, 20.VIII.2014, 1 ♀ (M. Antov); Stara Planina Mts.: above Vlasatili Village, 21.VII.2014, 1 ♀ (M. Antov); Stara Planina Mts.: above Gorni Stoevtsi Village, Rata loc., 22.VII.2014, 1 ♀ (M. Antov); Mezdra: Darmantsi Village, 21.VIII.2014, 1 ♀ (M. Antov); Osogovo Mts.: Vrattsa Village, Dervena loc., 08.VII.2014, 5 ♀ (M. Antov); Granitsa Village, 13.04.2015, 1 ♀ (M. Antov) (emerged from *Diplolepis rosae* (Hymenoptera: Cynipidae) galls on *Rosa* sp.); Eremitiya Village, 04.IX.2015, 7 ♀ (M. Antov) (emerged from pods of *Astragalus glycyphyllos* L.); Veliko Tarnovo: Arbanasi Village, 01.VIII.2015, 2 ♀ (M. Antov) (emerged from *Lasioptera eryngii* (Diptera: Cecidomyiidae) galls in stems of *Eryngium campestre*); Black Sea Coast: Tsarevo, 11.VI.2014, 2 ♀ (M. Antov); 15.VI.2014, 3 ♀ (M. Antov).

*****Metapelma* Westwood, 1835**

****Metapelma nobile* (Förster, 1860)**

Material: Ropotamo River, 15.VIII.2012, 2 ♀ (Malaise trap) (St. Petrov).

Sixteen eupelmid species belonging to 4 genera are herein presented. Of these, 4 species (*Anastatus giraudi*, *Eupelmus cicadae*, *E. linearis* and *Metapelma nobile*) and one genus – *Metapelma*, are new to the Bulgarian fauna. For 4 species data for new associations are pointed out for the first time.

With respect to the recently known data, the information above extends the range of some eupelmids. Newly established for Bulgarian fauna species are distributed mainly in Central and South Europe and for all of them these records are the most Southeastern localities of the areal (Noyes 2015).

There is no information in the literature about host-parasitoid relationships for *Eupelmus aseculatus* and the rearing of the species from *Aylax hypecoi* (Hymenoptera: Cynipidae) galls on *Hypecoum imberbe* is new data.

Kalina (1981) bred *Eupelmus impennis* from *Boucheella artemisiae* (Bouché, 1834) galls (Diptera, Cecidomyiidae); in our study we reared over than 70 specimens from *Lasioptera eryngii* galls on *Eryngium campestre* and this result extends the information about host association of the species.

Eupelmus vesicularis and *E. microzonus* are polyphagous species with wide range of hosts from Coleoptera, Hymenoptera, Diptera and Lepidoptera orders (Noyes 2015), but plant associations with *Astragalus glycyphyllos* and *Eryngium campestre* for the first species and with *Dianthus giganteus* for the second one are mentioned for the first time.

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Is the Kotschy's Gecko *Mediodactylus kotschyi* (Steindachner, 1870) (Reptilia: Gekkonidae) active during the winter?

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Abstract. The current communication reports few cases of unusual winter activity of the Kotschy's Gecko – *Mediodactylus kotschyi rumelicus* from the city of Plovdiv and *Mediodactylus kotschyi daniliewskii* from Hrishteni Village (Stara Zagora District) in Bulgaria.

Key words: *Mediodactylus kotschyi*, winter activity, Bulgaria.

The Kotschy's Gecko - *Mediodactylus kotschyi* (Steindachner, 1870) is one of the representatives from the Gekkonidae family, occurring in Bulgaria, which is considered to be a synanthrope (Beshkov & Nanev 2002). The species inhabits predominantly human settlements, as only few "wild" populations from Bulgaria are known (Beshkov & Nanev 2002, Stojanov et al. 2011).

The seasonal activity of this species, according to Stojanov et al. (2011) is from mid-March to the beginning of November, without giving specific dates. According to the unpublished MSs Thesis of Dobrev (1986) the earliest observations of active geckos from Bulgaria are from Elhovo Town on March 31st 1983 and the latest – Tsarevo Town on October 12th 1984. According to Undzhian (2000) the seasonal activity of the species is October (sometimes November) to March-April.

Data about unusual winter activity in lizards in Bulgaria is quite scarce in the herpetological literature. So far winter activity in the country has registered only for two species. Activity during the coldest months - December, January and February was recorded in *Podarcis muralis* by Buresh & Tsonkov (1933), Beshkov (1977), Undzhian (2000), Beshkov & Nanev (2002), Westerström (2005) and Tsankov et al. (2014). Winter activity in *Podarcis erhardii* was recorded by Buresh & Tsonkov (1933), Beshkov (1977) and Pulev & Sakelarieva (2011). Both lizard species are petrophylic (prefer rocky habitats). Their winter activity can be explained by the warmer microclimate in between the cracks of the rocks in sunny and warm weather.

We observed an active *M. k. rumelicus* from "Halm na Osvoboditelite" Hill (Bunarzdik Tepe) in Plovdiv (UTM LG16), Bulgaria. The observation was made on January 18th 2007 by I. Mollov and by far is the earliest observation of an active gecko in the country. The hill is located at the center of the city and is declared as "nature monument" by the Bulgarian legislation (Mollov 2005). The observed gecko was recorded in one of the support walls of the

alleys (which is a common microhabitat for this species on the hills of Plovdiv), on the west side on an unusually hot sunny January day.

Several active sub adult and adult specimens of *M. k. daniliewski* were observed on January 23rd 2015 at Hrishteni Village (Stara Zagora District – UTM LH90, 190 m a.s.l.), by D. Georgiev in an yard of a house, under some stone plates, also on unusually hot for the season day.

Also unusual late gecko activity (*M. k. rumelicus*) due to the unusual high temperatures was observed on November 9th 2015 and November 18th, 2015 in the building of the University of Food Technologies in Plovdiv (UTM LG16) by S. Basheva (Fig. 1).



Fig. 1. The observed specimens of *M. kotschy rumelicus* from the building of the University of Food Technologies in Plovdiv (Bulgaria). A – an adult specimen, observed on 09.11.2015; B - an adult specimen, observed on 18.11.2015. Photos: S. Basheva.

So, it appears that if the temperatures are high enough and they can maintain in certain levels especially in micro habitats, such as stone cracks, in between rocks, under tiles in buildings, etc., the geckos can be active as long as temperatures allow it. According to Shterback & Golubev (1986) the minimum temperature at which active gecko's are observed is 12 °C. Also, since this species is mainly a synanthrope (Mollov 2014) and inhabits mostly urban areas and human settlements, we should consider the “urban heat island theory” (Oke 1972, Camilloni & Barros 1997), since the temperatures in the center of the cities and urban areas is 1-2 degrees Celsius higher than the city surroundings. This theory would partially explain the observed by us unusual gecko activity.

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