



Data on the Trophic Spectrum of *Ablepharus kitaibelii* (Reptilia: Scincidae)

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Abstract: The snake-eyed skink (*Ablepharus kitaibelii*) is one of the smallest European lizards and is still insufficiently studied, especially in regard to its trophic preferences. We studied a total of 100 individual faecal samples of *A. kitaibelii* from two sites in Western Bulgaria and collected information for the potential trophic resource (using pit-fall traps). The results showed that Araneae and Auchenorrhyncha have the largest relative share in the trophic spectrum of the species, which, to some extent, confirms what was found in previous studies. The presence of five higher taxa of invertebrates (Gastropoda, Acari, Blattodea, Orthoptera and Raphidioptera) in the diet of *A. kitaibelii* was recorded for the first time. Several cases of keratophagy (a phenomenon that has not been reported for this species) were also recorded. Regarding the main physical characteristics of the prey (evasiveness and hardness), it was found that *A. kitaibelii* prefers sedentary and medium moving prey regardless of its hardness. We found no differences between sexes in food preferences.

Key words: Sauria, diet, prey electivity, keratophagy

Introduction

The skinks (Scincidae family) inhabit all continents except Antarctica, with 15 species found in Europe (Speybroeck et al. 2020). The Snake-eyed Skink *Ablepharus kitaibelii* Bibron et Bory de Saint-Vincent, 1833 is a small lizard species (up to 120 mm in total length according to Gruber 1981) which occurs in SE Europe (mainly the Balkans, but also in the Pannonian Plain) and western Asia Minor (Sillero et al. 2014, Speybroeck et al. 2016). It inhabits a variety of habitat types, but most often has been found in

glades, open oak forests, meadows, and brushwood (Herczeg et al. 2007, Speybroeck et al. 2016, and Vacheva et al. 2020). In Bulgaria, *A. kitaibelii* occurs sporadically throughout the country from the sea level up to cca. 1000, and in some areas up to 1200-1550 m a.s.l. (Stojanov et al. 2011).

Studies on the diet of *Ablepharus kitaibelii* are relatively few. Gruber (1981), based on a summary of the data available up to that time (five publications from the period 1912-1962), wrote that the food spectrum of the species includes small insects and their larvae, as well as small spiders and har-

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vestmen, land isopods and tiny earthworms. More detailed data for the food composition of *A. kitaibelii* have been obtained by Herczeg et al. (2007) based on an analysis of the stomach contents of 41 specimens from the area of Budapest, Hungary. Specifically for Bulgaria, the diet of the species has been studied on the basis of stomach content of 10 specimens (Angelov et al. 1966) and on the basis of faecal samples of 17 specimens (Natchev et al. 2015).

The aim of the present study was to investigate (by a non-invasive method) the trophic spectrum of *Ablepharus kitaibelii* in two areas in Bulgaria with a view of: (1) diet composition; (2) comparison of prey consumed and available food resources; (3) differences in diet between adult males and females.

Material and Methods

Study area and sampling

Field work was conducted in two sites, situated in southern and northwestern Bulgaria, respectively: the area of Gabrovitsa Village in the Sredna Gora Mts. (N42.260°, E23.921°, 430-570 m a.s.l.) and the eastern shore of the Ogosta Reservoir, Predbalcan Mts. (N43.374°, E23.209°, 180-240 m a.s.l.); detailed descriptions of the two sites are given by Vacheva et al. (2020). The area of Gabrovitsa was visited during spring and summer of 2017 and 2018 (a total of 25 visits), and that of Ogosta – irregularly from 2013 to 2016 (28 visits).

Skinks were captured by hand and kept in separate plastic boxes for up to 48 hours, after that they were released at the place of capture. The faecal pellets obtained during captivity were collected in separate Eppendorf tubes with 75% ethanol. In the Gabrovitsa area, 24 pit-fall traps (plastic containers, 9.5 cm wide and 12 cm deep, filled with propylene glycol) were used for collecting of invertebrates with a view to assessing the potential food resource. Traps were situated in different microhabitat types (four series, each of six traps, 10 meters apart) and were exposed for 23 and 17 days in spring (in 2017 and 2018 respectively), and for 16 and 23 days in summer (2017 and 2018).

Data processing and statistics

Captured skinks were conditionally considered adults or subadults on the basis of snout-vent length, respectively above or below 38 mm (see Vergilov et al. 2018a, b). Sexes (in adults only) were distinguished based on body proportions (see Ljubisavljevic et al. 2002 and Vergilov & Tzankov 2014), but in some individuals this was not sufficiently certain

(these skinks were not included in the comparison between sexes). Collected material (prey remains from the faecal pellets and invertebrates from the pit-fall traps) was examined under a stereomicroscope (magnification 10-40×) and after an identification to the lowest possible systematic level was grouped into “operational taxonomic units” (from here on as OTU/OTUs). In addition to taxonomic affiliations, the identified invertebrates were categorized in regard to their evasiveness [sedentary (E1), intermediate (E2), and evasive (E3)] and body’s hardness [soft (H1), intermediate (H2), and hard (H3)] according to Verwajen et al. (2002) and Vanhooydonck et al. (2007).

Correlation between the frequency and abundance of identified prey items was analyzed using Spearman’s rank correlation coefficient (Rho). The comparison of sexes in terms of diet diversity was carried out using Rényi’s index family (diversity profiles), which has been considered one of the most useful methods for ordering samples according to their diversity (Tóthmérész 1995). Regarding prey evasiveness and hardness, the comparisons between males and females were done by Chi-square (χ^2) test. Prey selection (for the population from Gabrovitsa only) was analyzed by comparing the relative abundance of OTUs from faecal samples with those from pit-fall traps using the electivity index of Vanderploeg & Scavia (1979). This index (E^*) is a modification of the Ivlev’s forage ratio, but has better theoretical justification (Lechowicz 1982). It takes values from -1 to +1 and can be interpreted as a measure of the deviation from random feeding ($E^* = 0$); here, the index range was divided into three parts and the OTUs were categorized as preferred ($E^* > 0.5$), neutral ($0.5 \geq E^* \geq -0.5$) or avoided ($E^* < -0.5$) [OTUs, represented by low abundance (below 5 individuals), were excluded, because according to Lechowicz (1982) the index is vulnerable to sampling errors for food types that are rare]. Statistical procedures were done via PAST 4.07 (Hammer et al. 2001), except for the electivity index, which was calculated in Microsoft Excel (2010) after input of the respective formulas.

Results

Faecal samples were collected from a total of 100 *Ablepharus kitaibelii* (38 males, 43 females, 12 subadults, and 7 adults which were not sexed). The remains of invertebrates in the faecal pellets were attributed to a total of 291 specimens belonging to 20 OTUs (respectively 195/15 from the Gabrovitsa and 96/15 from the Ogosta sample; Appendix 1).

The average number of individual remains found in the faecal pellets was 2.98 (3.03 for Gabrovitsa and 2.92 for Ogosta), and the maximum was 11 for Gabrovitsa and 6 for Ogosta.

In the sample from Gabrovitsa, two of the OTUs (Araneae and Auchenorrhyncha) were found most often (in above 30% of the faecal pellets) and the same OTUs also prevailed in number of individual remains (Fig. 1). In the sample from Ogosta, the only predominant OTU (both in frequency and abundance) was Araneae. Correlation between the abundance and frequency of occurrence of OTUs in the faecal pellets was strong positive with a high level of statistical significance in both samples (Ga-

brovitsa: $Rho = 0.981$, $p < 0.001$; Ogosta: $Rho = 0.986$, $p < 0.001$). The comparison of sexes showed a (little) higher diversity of OTUs in females in both samples (Fig. 2), but there were no statistically significant differences between sexes (Table 1).

Invertebrates collected by the pit-fall traps from the Gabrovitsa area were attributed to 25 OTUs, with Formicidae and Araneae being the most abundant (Fig. 1; Appendix 2). According to the electivity index values (Table 2) three of the OTUs were categorized as preferred prey of *A. kitaibelii* (Heteroptera, insect larvae and Auchenorrhyncha) and the rest as neutral. When dividing the sample by sex, the index values showed that two of the OTUs

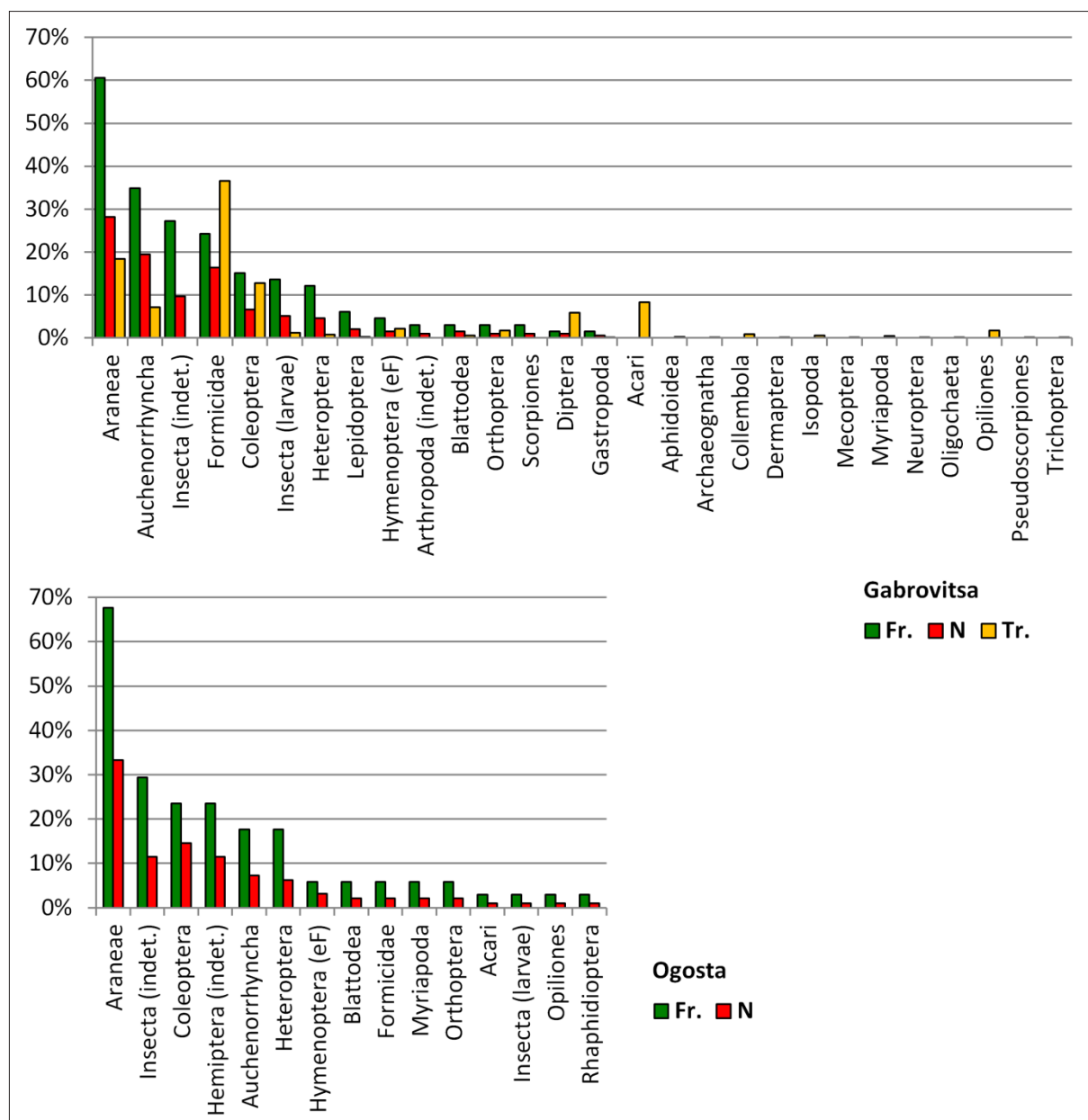


Fig. 1. Percentage share of the OTUs according to: number of faecal samples of *A. kitaibelii* in which the OTU was found (Fr.); number of specimens registered in the faecal samples (N); number of specimens, collected by pit-fall traps (Tr.).

Table 1. Simpson's index values regarding diversity of OTUs in the faecal pellets and statistical significance (Permutation p) of the differences between sexes.

	Males	Females	Perm. p
Gabrovitsa	0.7517	0.7766	0.4601
Ogosta	0.7755	0.7693	0.8941

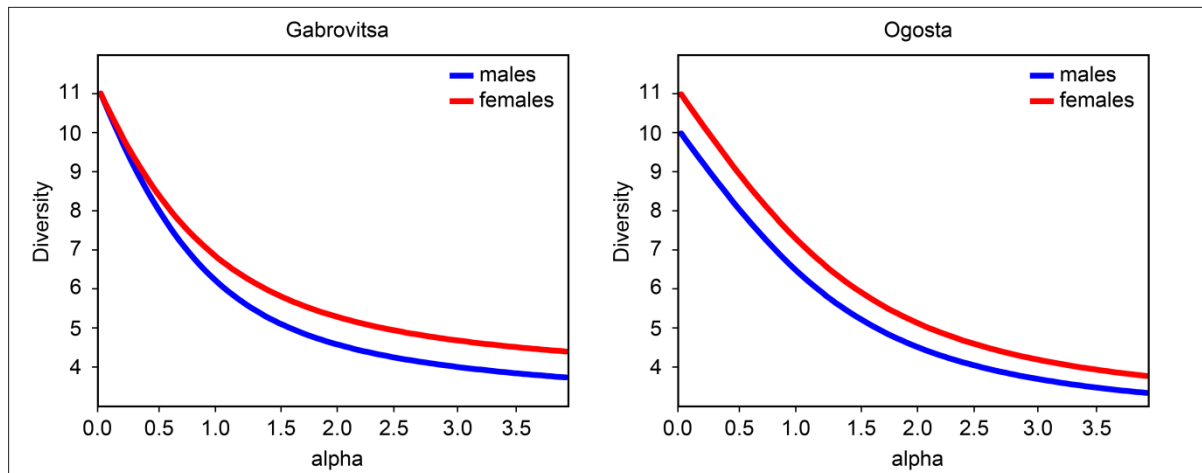
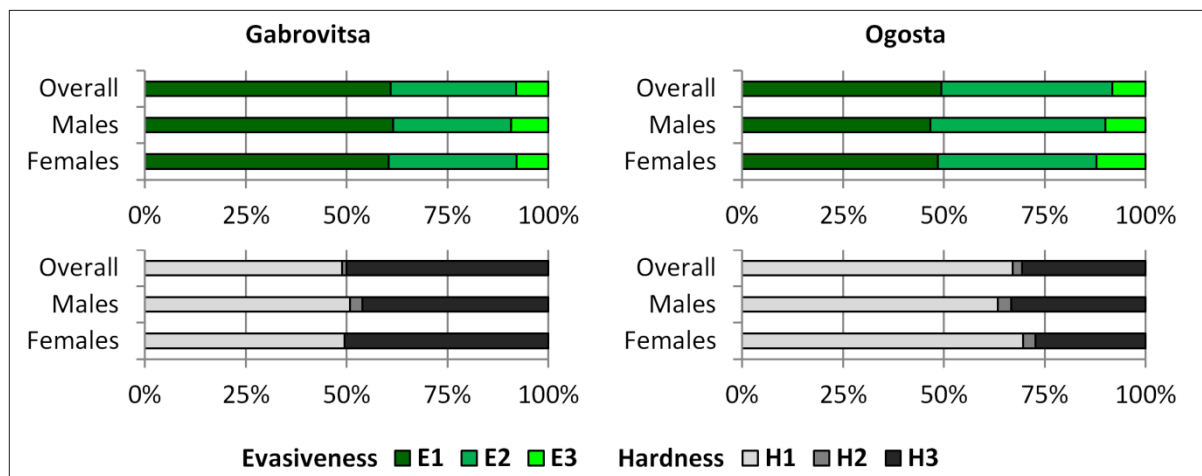
Table 2. Vanderploeg & Scavia index values for the sample from Gabrovitsa; symbols [$>$] and [=] denote preferred and neutral prey, respectively.

OTU	Overall	Males	Females
Araneae	0.43 [=]	0.62 [$>$]	0.34 [=]
Auchenorrhyncha	0.64 [$>$]	0.68 [$>$]	0.62 [$>$]
Coleoptera	-0.08 [=]	n/a	-0.19 [=]
Formicidae	-0.15 [=]	0.17 [=]	-0.26 [=]
Heteroptera	0.81 [$>$]	n/a	0.85 [$>$]
Insecta (larvae)	0.75 [$>$]	0.87 [$>$]	0.67 [$>$]

can be considered preferred by both sexes (insect larvae and Auchenorrhyncha), and two others – preferred only by males (Araneae) or only by females (Heteroptera).

Regarding prey evasiveness the sedentary invertebrates predominated in both samples, while in terms of hardness the prevalent category for the Ogosta sample was that of soft prey, but for the Gabrovitsa sample soft and hard prey had an equal share (Fig. 3). In both samples, there was no statistically significant difference between males and females in either evasiveness or hardness of the prey (for all combinations $\chi^2 < 3.28$, $df = 2$, $p > 0.05$).

Besides invertebrates, remains of lizard shed skin were also found in the faecal pellets of *A. kitaibelii* from Gabrovitsa, which is an evidence for the presence of keratophagy in this population. Such type of remains was detected in the faecal samples of two males and one female.

**Fig. 2.** Diversity profiles of the diet of *A. kitaibelii* based on the material from the faecal samples.**Fig. 3.** Percentage share of the categories of evasiveness and hardness according to the number of categorized prey items from the faecal samples of *A. kitaibelii* (E1 = sedentary, E2 = intermediate, E3 = evasive, H1 = soft, H2 = intermediate, and H3 = hard).

Discussion

The results of the present study indicate that the trophic spectrum of *Ablepharus kitaibelii* includes several higher taxa of invertebrates (Gastropoda, Acari, Blattodea, Orthoptera, and Raphidioptera) that do not appear to have been reported as components of the species' diet. On the other hand, components such as Araneae and Auchenorrhyncha, which according to our data are the most common and most abundant in the diet, appear to be so according to other similar studies (although based on significantly smaller samples): Angelov et al. (1966) and Herczeg et al. (2007) found the most frequent presence of Araneae and Homoptera (lower systematic category not specified, but in all probability it is Auchenorrhyncha), and Natchev et al. (2015) – of Hemiptera (lower systematic category also not specified) and Araneae. Regarding Auchenorrhyncha, it can be argued that in our case this OTU appears as positively selected prey (together with Heteroptera and Insect larvae), while Araneae (with higher occurrence and higher abundance than Auchenorrhyncha in the faecal pellets) together with Coleoptera and Formicidae (also with relatively high frequency and abundance) appear as accidental prey (Table 2).

In regard to the basic physical characteristics of the prey, our finding of predominance of slow- and medium-moving invertebrates in the *A. kitaibelii* diet is somewhat consistent with the statement of Herczeg et al. (2007), that it is a generalist predator of small, mainly flightless arthropods. This is easily explained given the species' presumed low ability to pursue fast prey due to the small relative length of the limbs, but it could also represent an adaptation to the environment given the semi-fossorial lifestyle of *A. kitaibelii*. As for the hardness of the prey items Angelov et al. (1966) did not find hard insects in the food, but our data show a different picture: preponderance of soft prey in one sample and almost equal shares of soft and hard prey in the other sample, while the intermediate category of hardness is very poorly represented in both samples. Natchev et al. (2015) have described the specific feeding kinematics that allows *A. kitaibelii* to feed on relatively large prey, despite its small body size and fragile skull. In view of the above, it can be assumed that feeding success depends much more on the speed of movement of the prey than on its hardness.

The ingestion of the shed skin (keratophagy), which we found in 4.5% of individual faecal samples from Gabrovitsa, is a phenomenon not previously reported for the genus *Ablepharus*. It could be interpreted as a mechanism to reclaim epidermal

proteins or vitamin D, although other hypotheses also exist (for a review see Mitchell et al. 2006).

In previous studies on the feeding of *A. kitaibelii*, the two sexes have not been considered separately. According to our results, there appears to be no difference between males and females either in terms of diet diversity or physical characteristics (evasiveness and hardness) of the prey items. Differences between the two sexes were also not found when examining the habitat preferences of *A. kitaibelii* in the same sites (Vacheva et al. 2020). Unlike a number of other European lizard species, in *A. kitaibelii* the sexual dimorphism in external morphology is weakly expressed: females are slightly larger than males, but males have relatively larger heads and longer limbs (Stojanov et al. 2011, Vergilov & Tzankov 2014, Vergilov et al. 2018b), which probably compensates for the difference in absolute sizes. In this sense, the established similarity between the sexes in terms of feeding can be considered a reflection of the similarity in external morphology.

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Appendix 1: Distribution of the material from the faecal samples of *Ablepharus kitaibelii* per OTU [E = evasiveness (E1, E2, and E3 refer to sedentary, intermediate, and evasive, respectively); H = hardness (H1, H2, and H3 refer to soft, intermediate, and hard, respectively)]; Fr = number of the faecal samples in which the OTU occurs; N = number of identified individual remnants. The total number of individuals (Overall column) is greater than the sum of males and females because it includes subadult individuals as well as adults whose sex we could not determine with sufficient certainty.

Gabrovitsa:

Phylum: Class	OTU	Overall (n = 66)		Males (n = 26)		Females (n = 30)	
		Fr.	N	Fr.	N	Fr.	N
Arthropoda	Arthropoda (indet.)	2	2	1	1	0	0
Arthropoda: Arachnida	Araneae [E1; H1]	40	55	19	23	20	30
Arthropoda: Arachnida	Scorpiones [E1; H1]	2	2	1	1	1	1
Arthropoda: Insecta	Auchenorrhyncha [E1; H3]	23	38	9	11	11	24
Arthropoda: Insecta	Blattodea [E3; H1]	2	3	0	0	2	3
Arthropoda: Insecta	Coleoptera [E2; H3]	10	13	2	3	7	7
Arthropoda: Insecta	Diptera [E3; H1]	1	2	1	2	0	0
Arthropoda: Insecta	Formicidae [E2; H3]	16	32	9	15	7	17
Arthropoda: Insecta	Heteroptera [E2; H1]	8	9	1	1	7	8
Arthropoda: Insecta	Hymenoptera (eF*) [E3; H3]	3	3	1	1	2	2
Arthropoda: Insecta	Insecta (indet.)	18	19	4	4	7	7
Arthropoda: Insecta	Insecta (larvae) [E1; H1]	9	10	5	5	4	5
Arthropoda: Insecta	Lepidoptera [E3; H1]	4	4	1	1	3	3
Arthropoda: Insecta	Orthoptera [E3; H2]	2	2	2	2	0	0
Mollusca: Gastropoda	Gastropoda [E1; H3]	1	1	0	0	1	1
	keratophagy	3		2		1	

* eF = except Formicidae

Ogosta:

Phylum: Class	OTU	Overall (n = 34)		Males (n = 12)		Females (n = 13)	
		Fr.	N	Fr.	N	Fr.	N
Arthropoda	Myriapoda [E2; H1]	2	2	1	1	0	0
Arthropoda: Arachnida	Acari [E1; H1]	1	1	0	0	0	0
Arthropoda: Arachnida	Araneae [E1; H1]	23	32	9	12	8	12
Arthropoda: Arachnida	Opiliones [E1; H1]	1	1	0	0	1	1
Arthropoda: Insecta	Auchenorrhyncha [E1; H3]	6	7	2	2	2	2
Arthropoda: Insecta	Blattodea [E3; H1]	2	2	1	1	1	1
Arthropoda: Insecta	Coleoptera [E2; H3]	8	14	3	5	2	5
Arthropoda: Insecta	Formicidae [E2; H3]	2	2	2	2	0	0
Arthropoda: Insecta	Hemiptera (others) [E2; H1]	8	11	3	4	4	5
Arthropoda: Insecta	Heteroptera [E2; H1]	6	6	1	1	2	2
Arthropoda: Insecta	Hymenoptera (eF*) [E3; H3]	2	3	1	1	1	2
Arthropoda: Insecta	Insecta (indet.)	10	11	3	3	3	3
Arthropoda: Insecta	Insecta (larvae) [E1; H1]	1	1	0	0	1	1
Arthropoda: Insecta	Orthoptera [E3; H2]	2	2	1	1	1	1
Arthropoda: Insecta	Rhaphidioptera [E2; H1]	1	1	0	0	1	1

* eF = except Formicidae

Appendix 2: Distribution of the material, collected by the pit-fall traps in Gabrovitsa, per OTU; N = number of specimens.

Phylum: Class	OTU	N	Phylum: Class	OTU	N
Annelida: Clitellata	Oligochaeta	10	Arthropoda: Insecta	Diptera	586
Arthropoda	Myriapoda	42	Arthropoda: Insecta	Formicidae	3661
Arthropoda: Arachnida	Acari	832	Arthropoda: Insecta	Heteroptera	81
Arthropoda: Arachnida	Araneae	1839	Arthropoda: Insecta	Hymenoptera (eF*)	217
Arthropoda: Arachnida	Opiliones	168	Arthropoda: Insecta	Insecta (larvae)	122
Arthropoda: Arachnida	Pseudoscorpiones	2	Arthropoda: Insecta	Lepidoptera	27
Arthropoda: Insecta	Aphidoidea	22	Arthropoda: Insecta	Mecoptera	1
Arthropoda: Insecta	Archaeognatha	9	Arthropoda: Insecta	Neuroptera	3
Arthropoda: Insecta	Auchenorrhyncha	714	Arthropoda: Insecta	Orthoptera	168
Arthropoda: Insecta	Blattodea	55	Arthropoda: Insecta	Trichoptera	2
Arthropoda: Insecta	Coleoptera	1281	Arthropoda: Malacostraca	Isopoda	57
Arthropoda: Insecta	Collembola	83	Mollusca: Gastropoda	Gastropoda	16
Arthropoda: Insecta	Dermaptera	4			

* eF = except Formicidae

