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Diversity and abundance of Collembola (Hexapoda) under native and invasive trees – a case study in Moldova

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Abstract. The planting of alien plant species is an issue that is contributing to the disappearance of biodiversity in natural habitats, to the spread of invasive species and to increased pests. In this study, the species composition and abundance of springtails (Collembola) in the natural oak forest of the Vila Nisporeni Protected Area in the Republic of Moldova is compared to that in the *Robinia pseudoacacia* plantation nearby. A total of 68 species were recorded. The number of Collembola species with a preference to reside in the natural forest was 19 compared to the *Robinia pseudoacacia* plantation with 9 species.

Key words: Vila Nisporeni Protected Area, natural oak forest, *Robinia pseudoacacia* plantation.

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I. INTRODUCTION

The Republic of Moldova has heterogeneous natural conditions and a geographical position that contributes to the formation of diverse types of soil and supports a high diversity of flora and fauna.

In the Republic of Moldova, 51 forest reserves, 9 medicinal plant plantations and three mixed ones (e.g. forest and medicinal plants or a steppe and forest) with a total area of 8,009 ha are currently registered. These reserves represent natural areas, which are valuable from a scientific point of view as they are intended to preserve and to restore one or more components of nature, in order to maintain the correct ecological balance. At the same time, in the Republic of Moldova, according to the Institute of Forestry Research and Management, false acacia (*Robinia pseudoacacia* – hereinbelow referred to as 'acacia') plantations occupy 125,000 ha, which is about 33.1% of the total forested area in the country.

Despite the fact that the acacia is considered to be an invasive tree species in Europe, in the territory of the Republic of Moldova it is intensively planted all over the country. Over the years, there have been multiple plantings with acacia in degraded and erosion-affected areas, as the acacia is considered as the only species capable of restoring degraded land, including areas adjacent to rivers.

The second biggest river in the country is the Prut River. Its length in the territory of the Republic of Moldova is 695 km (with its catchment representing about 24% of the territory of country). One of the protected areas on the basin of the Prut River is Vila Nisporeni.

The present study of collembolan fauna is the first one to be carried out in the natural forest and acacia plantation situated in the basin of the Prut River (preliminary results were presented in WEINER & BUŞMACHIU 2018). All previous studies were carried out in the riverine ecosystems of the northern and southern parts of the Republic of Moldova on the banks of the Prut and Dniester Rivers (BUŞMACHIU 2006; BUŞMACHIU & WEINER 2013; BUŞMACHIU et al. 2017).

According to BUŞMACHIU (2021a), a total of 270 collembolan species have been identified in the Republic of Moldova, most of which have a preference for forest habitats. For example, in the Plaiul Fagului Reserve, the best studied reserve in the country, 149 species of Collembola were identified (BUŞMACHIU 2021b). Most of the identified species are associated with wood in various stages of decay.

An article dedicated to the invertebrates of other studied protected areas, such as the Prutul de Jos Biosphere Reserve, was published in 2023, which includes the first data on collembolan species identified in this flooded area covered mainly by lakes and meadows (BUŞMACHIU et al. 2023).

The aim of the present research was to identify the species diversity and distribution of Collembola in the natural deciduous forest on the basis of *Quercus* sp. and the *Robinia pseudoacacia* plantation from the Vila Nisporeni Landscape Reserve in a comparative aspect, including the population structure, as well as the presence of dominant and rare species, which revealed the influence of invasive acacia species on the populations of Collembola. The performed species inventory is very valuable for research on the biogeographic distribution and species preferences in these habitats.

II. MATERIAL AND METHODS

Study sites

The Vila Nisporeni Protected Area (47°04′N 28°12′E) was created in 1975 and is located in Nisporeni District, Republic of Moldova (Fig.1 A, B). The 3499 ha of Vila Nisporeni is covered by several types of natural mixed deciduous forest with a basis of *Quercus* species: *Quercus pubescens* and *Q. petraea* in combination with *Tilia tomentosa*, *Fraxinus excelsior*, etc. (Fig 2 A, B), including a total of 314 species of vascular plants, with 20 of them considered rare species.

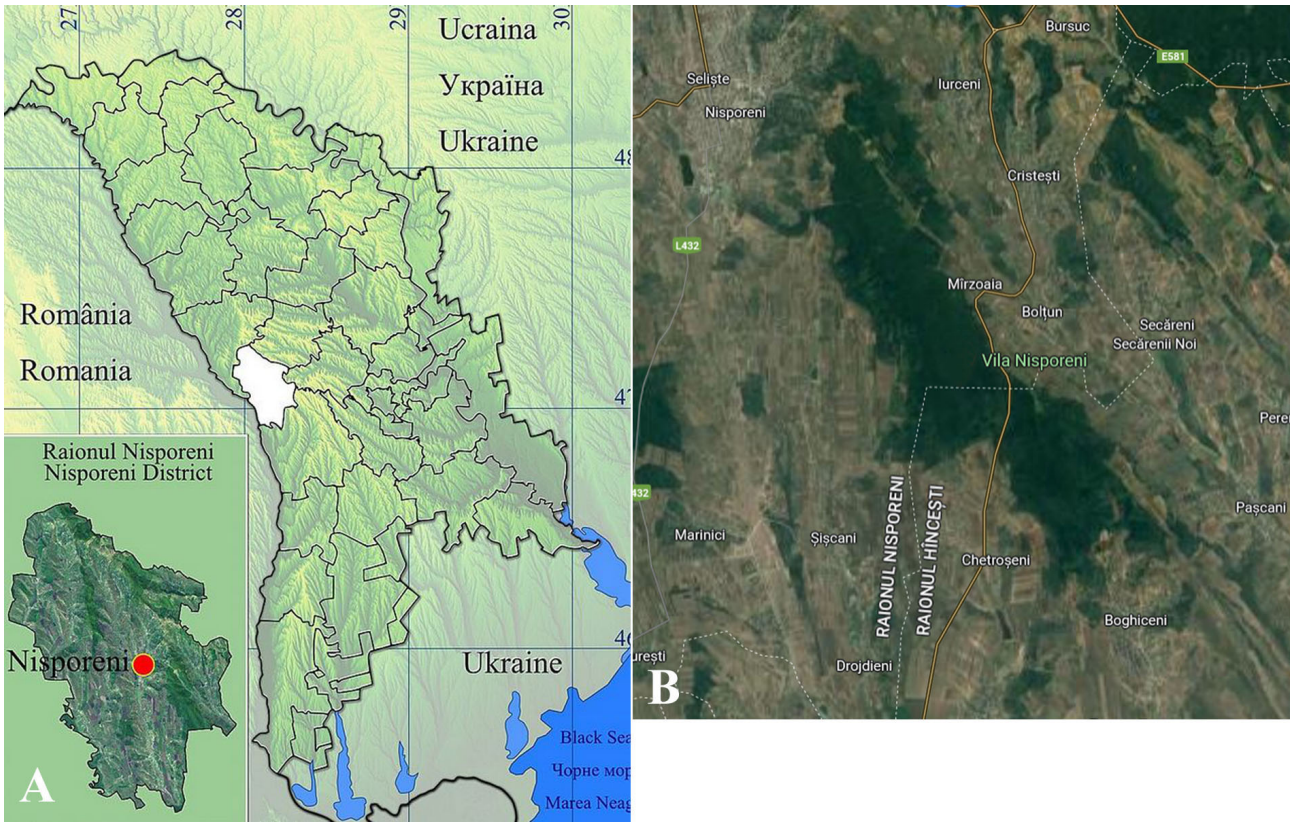

Fig. 1. Map of the Vila Nisporeni Landscape Reserve location: A – Nisporeni District (https://ro.wikipedia.org/wiki/Vila_Nisporeni); B – Vila Nisporeni Landscape Reserve (the forest on the map in dark green is a Landscape Reserve) (https://www.google.com/maps/place/Vila+Nisporeni)

Fig. 2. A – natural mixed deciduous forest; B – acacia plantation.

In the 1980s, a forest belt of *Robinia pseudoacacia* was planted along the edge of the natural oak forest, which has grown rapidly, advancing towards the natural forest and gradually replacing the native species of trees. Vila Nisporeni belongs to Category V (protected landscape), according to the International Union for Conservation of Nature (IUCN).

Sampling and extraction

The study was performed in two types of habitats: the natural oak forest (Fig. 2A); and an acacia plantation (Fig. 2B) covering the cut land of the natural forest.

Samples were collected from the soil, litter and rotten trunks of *Quercus* sp., covered by moss, in July and December 2015, July and October 2017, March 2019, March 2020, and March and August 2021. The litter and soil were sampled by a metallic square frame measuring 25 cm^2 to a 5 cm depth, with each sample including 4 subsamples. From both types of habitats, the same number of samples were taken.

The specimens were extracted using the modified flotation method of Buşmachiu et al. (2015) and were fixed in 96% ethyl alcohol. They were sorted, cleared in lactic acid and KOH, and mounted on permanent slides using Marc-Andre II solution. The specimens were identified using a phase contrast microscope LEICA 2500.

Identification and collection of Collembola

For the identification of Collembola, the determination keys published within the Synopses on Palaearctic Collembola (Bretfeld 1999; Potapov 2001; Thibaud et al. 2004; Dunger & Schlitt 2011; Jordana 2012) and Fjellberg (1998, 2007) were used, as well as the original descriptions. The geographic distribution and ecological data of the species were excerpted from the same literature. The Collembola life forms were analysed according to Gisin (1943) and Rusek (2007); the habitat preferences of the species are provided in the Appendix.

The collection of slides is stored in the Moldova State University, Institute of Zoology and Institute of Systematics and Evolution of Animals PAS.

Data analyses

A similarity percentage (SIMPER) test was employed to identify the collembolan species that contributed the most to significant dissimilarities between the two forest types. The analysis was performed in R version 4.0.3 using the vegan package version 2.5-7 (Oksanen et al. 2020). The VennDiagram package (Chen 2022) was used to draw the Venn diagram. Diversity indices were calculated in PAST 4.03 (Hammer et al. 2001) and included the following: dominance (D), Shanonn index, even-

ness, Margalef's index and the Berger–Parker index.

The dominance ranges from 0 to 1. Zero indicates that all taxa are equally present, while 1 means that a single taxon dominates the community. The Shannon index (entropy) is a diversity index taking into account the number of individuals as well as the number of taxa. It varies from 0 for communities with only a single taxon, to high values for communities with many taxa, each with few individuals. Evenness describes the commonness and ranges from 0 to 1. When species are present in similar proportions, the evenness increases and dominance decreases. Margalef's index is an index describing the richness (number of species) related to the number of individuals in a community. The Berger-Parker index is another way of describing dominance, which relates the number of individuals in the dominant taxon compared to the total number of individuals (Reference manual, PAST 4.03, Hammer et al. 2001).

III. RESULTS

Collembola diversity

As a result of our investigation, 68 species belonging to 39 genera and 15 families were revealed in both the studied habitats of the Vila Nisporeni Landscape Reserve (Protected Area). The species number identified in the natural forest was 59, but in the acacia plantation it was 49. There were 40 species common to both types of the habitats studied.

The most species rich was the family Isotomidae represented by 17 species, followed by the families Entomobryidae with 16 species, Tullbergiidae and Hypogastruridae with 7 species each, and Neanuridae with 6 species; another 10 families included a small number of species. One genus – *Jesenikia* – and 5 species, namely *Jesenikia filiformis* Rusek, 1997, *Folsomia fimetaria* (Linnaeus, 1758), *Folsomia dovrensis* Fjellberg, 1976, *Orchesella pannonica* Stach, 1960 and *Vertagopus haagvari* Fjellberg, 1996 are new for the fauna of the Republic of Moldova and marked with an asterisk (*) in the Appendix.

According to the previous study by Buşmachiu et al. (2015), collecting faunistic material in the warm winter of Moldova, before the first frost, allows researchers to highlight a wide range of collembolan species, especially Isotomidae. In our present study, we confirmed this finding, and for the most part species listed for the first time in the Republic of Moldova belonged to this family.

Among the studied entomobryid species, the genus *Pseudosinella* is represented by 5 species, four of them with 5+5 eyes found in the same samples. Three species, namely *Pseudosinella moldavica*, *P. pygmaea* and *P. variabilis*, were described from the Republic of Moldova. At the present time, *Pseudosinella moldavica* is known to occur in Romania and Ukraine, while the other two species have been described from natural deciduous European and Mediterranean forest types situated in the central and southern part of the country.

In the family Hypogastruridae three species of the genus *Xenylla* namely, *Xenylla mediterranea*, *Xenylla corticalis* and *Xenylla uniseta*, of the existing eight species (Buşmachiu & Weiner 2008; Buşmachiu & Weiner 2017) and only one species of *Ceratophysella* and *Hypogastrura* genera were found. Before now, *Xenylla uniseta* was cited once from a forest of the calcareous canyon of the Dniester River (Buşmachiu et al. 2015).

Among the family Neanuridae, the subfamily Neanurinae was represented by two genera with one species each. The *Deutonura* species was found in this habitat, while in other natural silvicolous ecosystems, especially in the basin of the Dniester River, the species of this subfamily are very abundant and a wide range of species from the genera *Deutonura*, *Neanura*, *Endonura* and *Lathriopyga* are present (Buşmachiu et al. 2015). The new locality in the Vila Nisporteni of *Thaumanura carolii* confirms the uniqueness of its occurrence. In the Republic of Moldova, this species has been found only in natural deciduous forests (mainly with oaks) such as the Codrii and Plaiul Fagului Reserves, Dobruşa Landscape Reserve and Orhei National Park (Donici locality).

In the smallest Collembola from the family Tullbergiidae, six identified species are widespread in the country. However, only *Jevania weinerae* was identified until now in two localities: Lalova and Vila Nisporeni (Buşmachiu 2021a, b).

Species from the Onychiuridae family are unusually poorly represented in the Prut River basin. *Protaphorura sakatoi* and *Protaphorura pannonica* inhabited the studied habitats. We recorded a similar result in our previous study carried out on the banks of the Prut River in the northern part of the Republic of Moldova (Buşmachiu et al. 2017). A few individuals of *Deuteraphorura silvaria* were present in three samples from the natural forest (Appendix).

Species from the Odontellidae family are very rare in the Republic of Moldova, being indicators of old natural oak forests, in which there is wood in an advanced stage of decomposition. In Vila Nisporeni, two species of this family were identified, with one of them *Superodontella montemaceli*, common in natural forests, and one specimen of a species possibly new to science.

In the case of the families Tomoceridae and Cyphoderidae, only one species of each with a few specimens were found. The Symphypleona families were also poorly represented: only 9 species from 8 genera and 6 families with a total number of 178 specimens were found. For the most part, these are widespread in the country. One species *Sminthurus wahlgreni* was registered in only three localities in the Republic of Moldova, including the Plaiul Fagului Reserve, Lalova and the Vila Nisporeni Landscape Reserve (Buşmachiu 2021a, b).

The numbers of collembolan species revealed in summer and winter were similar, i.e. 38 and 40, respectively. However, the genera of the Entomobryidae family (especially *Orchesella* and *Pseudosinella*) as well as Arrhopalitidae, were represented by a higher number of species and individuals in summer. In the winter, they were present only in rotten trunks, whilst most of the Isotomidae species have been found in this season.

Five species: *Mesaphorura critica*, *Protaphorura sakatoi*, *Parisotoma notabilis*, *Lepidocyrtus lignorum* and *Pseudosinella horaki* were present in all the samples from both habitats.

Several collembolan species: *Deuteraphorura silvaria*, *Thaumanura carolii*, *Superodontella montemaceli*, *Jevania weinerae* and *Tetracanthella pilosa*, showed a preference for the natural forest.

Comparison of collembolan species structure between the studied habitats

The SIMPER analysis indicated a medium degree of variability between the natural deciduous forest and the acacia plantation, with a 37.05% average dissimilarity. There were eight species with an average value of dissimilarity higher than 1%, out of which six species (*Protaphorura sakatoi*, *Isotomiella minor*, *Lepidocyrtus lignorum*, *Tetracanthella pilosa*, *Folsomia quadrioculata* and *Folsomia manolachei*) were characterised by contributions higher than 2%, explaining ca 60% of the variability (Table 1).

A change in the structure of the Collembola assemblages was observed under the acacia plantation, with a loss of diversity and increased dominance of a single species in comparison to the natural forest. The species which was the most abundant in the natural forest was *Protaphorura sakatoi* (336 individuals) and its number was even higher under the acacias (526 individuals).

Table 1

Results of the SIMPER analysis of the examined natural deciduous forest and acacia plantation, with the top eight species primarily responsible for the observed differences (average dissimilarity > 1%). The overall average dissimilarity between the two sites amounted to 35.05%

Species	Dissimilarity %	Natural forest, abundance	Acacia plantation, abundance	Cumulated contribution %
Protaphorura sakatoi	5.19	336	526	14
Isotomiella minor	4.72	236	63	26.75
Lepidocyrtus lignorum	3.74	191	54	36.85
Tetracanthella pilosa	3.19	117	0	45.47
Folsomia quadrioculata	2.81	146	43	53.06
Folsomia manolachei	2.78	112	10	60.57
Heteromurus major	1.45	3	56	64.48
Pseudosinella horaki	1.06	176	137	67.35

The other major species in the natural forest – *Isotomiella minor*, *Lepidocyrtus lignorum*, *Folsomia quadrioculata* and *Folsomia manolachei* – were less abundant under the acacias, where their numbers were lower by 3 to 4-times or even 11-times (*Folsomia manolachei*). In addition, one of the more abundant species in the natural forest, *Tetracanthella pilosa* (117 specimens), was completely absent under the acacias. In total, 19 species were missing under the acacias in comparison to the natural forest and nine new species appeared (Fig. 3), of which two species, *Micranurida pygmaea* and *Sminthurus wahlgreni*, appeared in considerable numbers (13 and 15 specimens, respectively). As a result, the Shannon diversity index was lower under the acacias (2.69) in comparison to the natural forest (2.91), and the dominance was 0.08 in the natural forest and 0.14 in the acacia plantation (Table 2).

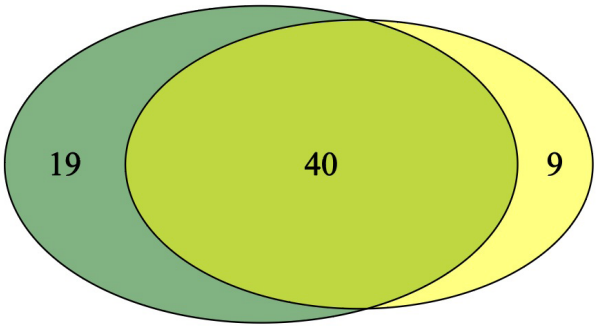

Fig. 3. Venn diagram showing the number of Collembola species in the natural forest (green oval) and in the acacia plantation (yellow oval).

Table 2

Diversity indices of Collembola in the samples from the examined natural forest and acacia plantation

Habitat	Natural forest	Acacia plantation
Number of species	59	49
Individuals	2044	1619
Dominance	0.08	0.14
Shannon	2.91	2.69
Evenness	0.31	0.30
Margalef	7.61	6.50
Berger-Parker	0.16	0.32

The majority of the species had a wide geographic occurrence; namely, a Palaearctic (13), Holarctic (14), European (14) and Cosmopolitan (11) distribution, with four species having a Mediterranean range. *Pseudosinella pygmaea* and *P. variabilis* were described from the Republic of Moldova (GAMA & BUŞMACHIU 2004). Comparing the forest and plantation, there were a similar numbers of species considering the biogeographic distribution, with some differences in the case of European species that were represented by 16 species in the natural forest and 10 in the *Robinia pseudoacacia* plantation. The abundance and number of collembolan species distributed in the Palaearctic range was two times higher in the natural forest (463 individuals), and in the Holarctic was 3-times higher (632) than in the acacia plantation (227 and 242, respectively).

According to the classification of life forms, 27 collembolan species were epiedaphic, 22 hemiedaphic and 20 euedaphic. Looking at the collembolan life forms in the natural forest and acacia plantation, the euedaphic species had a two-times higher abundance in the natural forest (487) than under the acacias (218 individuals), and one third higher in the hemiedaphic form, with 738 individuals in the natural forest vs 532 under the acacias. The richness of species divided into specific life forms did not differ between the studied oak forest and the acacia plantation.

IV. CONCLUSIONS

The present work focused on the diversity and habitat preferences of collembolan species from the Vila Nisporeni Landscape Reserve and the adjacent acacia plantation. A total of 68 species were recorded in both habitats, of which *Deuteraphorura silvaria*, *Thaumanura carolii*, *Superodontella montemaceli*, *Jevania weinerae* and *Tetracanthella pilosa* showed a preference for the natural forest. One genus – *Jesenikia* – and 5 species, namely *Jesenikia filiformis* RUSEK, 1997, *Folsomia fimetaria* (LINNAEUS, 1758), *Folsomia dovrensis* FJELLBERG, 1976, *Orchesella pannonica* STACH, 1960 and *Vertagopus haagvari* FJELLBERG, 1996 are new for the fauna of the Republic of Moldova; while a further three species, *Pseudosinella moldavica*, *P. pygmaea* and *P. variabilis* found in Vila Nisporeni were previously described from the Republic of Moldova.

Comparative analyses revealed 40 species to be common for both habitats, along with 19 species found only in the natural forest and 9 species found only in the acacia plantation. Notably, the acacia plantation was established on the edge of the natural forest, most likely replacing the areas of natural forest where it had been cut down. Therefore, it is intriguing that almost two thirds (40) of the 68 species identified in this work were common for both areas – the acacia plantation and the natural forest. It is worth noting that among the common species there were 23 hemiedaphic and euedaphic species, which are less likely to disperse than the epiedaphic species. In particular, the euedaphic species are known to remain in changed habitats much longer than epiedaphic ones. A similar phenomenon was previously observed in the Pieniny Mountains, Poland (Weiner 1981). The fauna of the 'Pieniny meadows' (*Anthylii-Trifolietum*), which resulted from cutting down (cutting each year to preserve this type of meadow) parts of the natural forest, showed many common characteristics with the fauna of the natural forest, despite having a lower level of diversity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

APPENDIX

List of Collembola from Vila Nisporeni with the number of individuals collected, biogeographic distribution (BD) and life forms (see pages 24-26).

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APPENDIX

List of Collembola from Vila Nisporeni with the number of individuals collected, biogeographic distribution (BD) and life forms. Abbreviations for the habitats: NF – natural forest, RP – acacia plantation; C – cosmopolitan, E – European, H – Holarctic, P – Palaearctic, M – Mediterranean, R – known from the type locality only; e – epiedaphic, h – hemiedaphic, eu – euedaphic; * – species new to Moldova, ** – species probably new for science

Taxa	Habitat		BD	Life forms
	NF	RP		
Hypogastruridae				
Ceratophysella engadinensis (GISIN, 1949)	1	2	C	e
Hypogastrura manubrialis (TULLBERG, 1869)	0	1	C	e
Xenylla mediterranea GAMA, 1964	2	0	M	h
Xenylla corticalis BÖRNER, 1901; *in*: BÖRNER 1901b	9	0	P	h
Xenylla uniseta GAMA, 1963	0	2	M	h
Willemia intermedia MILLS,1934	2	1	H	eu
Willemia scandinavica STACH, 1949	0	6	H	eu
Neanuridae				
Deutonura albella (STACH, 1920)	14	20	E	h
Thaumanura carolii (STACH, 1920)	9	0	E	h
Micranurida pygmaea BÖRNER, 1901; *in*: BÖRNER 1901b	0	13	C	h
Pseudachorutes dubius KRAUSBAUER, 1898; *in*: Krausbauer 1901b	7	0	P	e
Pseudachorutes parvulus BÖRNER, 1901; *in*: BÖRNER 1901b	25	3	P	e
Pseudachorutes sp.**	1	0		e
Odontellidae				
Superodontella montemaceli ARBEA & WEINER, 1992	8	0	E	h
Superdontella sp.**	1	0		h
Onychiuridae				
Deuteraphorura silvaria (GISIN, 1952)	7	0	R	eu
Protaphorura pannonica (HAYBACH, 1960)	2	2	E	eu
Protaphorura sakatoi (YOSII, 1966)	336	526	E	eu

APPENDIX – CONT.

Taxa	Habitat		BD	Life forms
	NF	RP		
Tullbergiidae				
Doutnacia xerophila Rusek, 1974	31	51	E	eu
Jevania weinerae Rusek, 1978	5	0	E	eu
Mesaphorura critica Ellis, 1976	101	78	P	eu
Mesaphorura hylophila Rusek, 1982	51	71	P	eu
Mesaphorura krausbaueri Börner, 1901; *in*: Börner 1901a	12	0	C	eu
Mesaphorura macrochaeta Rusek, 1976	4	0	C	eu
Mesaphorura sylvatica (Rusek, 1971)	6	0	P	eu
Isotomidae				
Desoria intermedia (Schött, 1902)	3	4	P	e
Desoria propinqua (Axelson, 1902)	14	5	H	e
Desoria violacea (Tullberg, 1877)	1	0	E	e
Folsomia dovrensis Fjellberg, 1976*	2	7	H	h
Folsomia fimetaria (Linnaeus, 1758)*	4	0	H	eu
Folsomia quadrioculata (Tullberg, 1871)	146	43	H	h
Folsomia manolachei Bagnall, 1939	112	10	P	h
Jesenikia filiformis Rusek, 1997*	2	1	P	eu
Isotoma caerulea Bourlet, 1839	3	2	H	e
Isotoma viridis Bourlet, 1839	14	39	H	e
Isotomiella minor (Schäffer, 1896)	236	63	H	eu
Parisotoma notabilis (Schäffer, 1896)	204	176	C	h
Proisotoma minima (Absolon, 1901)	0	1	H	h
Proisotomodes bipunctatus (Axelson, 1903)	12	16	H	h
Tetracanthella pilosa Schött, 1891	117	0	P	e
Vertagopus haagvari Fjellberg, 1996*	1	0	P	e
Entomobryidae				
Entomobrya marginata (Tullberg, 1871)	4	1	E	e
Entomobrya muscorum (Nicolet,1842)	1	2	H	e
Orchesella bulgarica Stach, 1960	5	1	E	e
Orchesella spectabilis Tullberg, 1871	7	0	E	e
Orchesella pannonica Stach, 1960*	1	0	E	e
Orchesella multifasciata Scherbakov, 1898	24	24	E	e

APPENDIX – CONT.

Taxa	Habitat		BD	Life forms
	NF	RP		
Lepidocyrtus lignorum (FABRICIUS, 1775)	191	54	H	e
Lepidocyrtus paradoxus UZEL, 1891	0	1	H	e
Lepidocyrtus violaceus (GEOFFROY in FOURCROY, 1785) LUBBOCK 1873	7	1	H	e
Pseudosinella albida (STACH, 1930)	31	18	M	e
Pseudosinella horaki RUSEK, 1985	176	137	E	h
Pseudosinella moldavica GAMA & BUŞMACHIU, 2002	3	5	E	h
Pseudosinella pygmaea GAMA & BUŞMACHIU, 2004	10	0	R	h
Pseudosinella variabilis GAMA & BUŞMACHIU, 2004	9	2	R	h
Heteromurus major (MONIEZ, 1890)	3	56	M	h
Heteromurus nitidus (TEMPLETON, 1836)	14	39	C	h
Tomoceridae				
Tomocerus vulgaris (TULLBERG, 1871)	1	2	C	h
Cyphoderidae				
Cyphoderus bidenticulatus (PARONA, 1888)	3	4	M	eu
Arrhopalitidae				
Pygmarrhopalites secundarius (GISIN, 1958)	0	1	P	eu
Pygmarrhopalites terricola (GISIN, 1958)	1	3	E	eu
Neelidae				
Neelus murinus FOLSOM, 1896	10	31	C	eu
Megalothorax minimus WILLEM, 1900	6	18	C	eu
Sminthurididae				
Sphaeridia pumilis (KRAUSBAUER, 1898) ; *in*: Krausbauer 1901a	3	16	C	h
Katiannidae				
Sminthurinus aureus (LUBBOCK, 1862)	11	33	P	e
Gisianus flammeolus (GISIN, 1957)	18	11	P	e
Dicyrtomidae				
Dicyrtoma fusca (LUBBOCK, 1873)	0	1	H	e
Sminthuridae				
Sminthurus wahlgreni juv. STACH, 1920	0	15	P	e
Total abundance	2044	1610		