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# Mesovoid shallow substratum as a biodiversity hotspot for conservation priorities: analysis of oribatid mite (Acari: Oribatida) fauna

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**ABSTRACT** — The mesovoid shallow substratum (MSS) is a unique habitat that shelters and serves as a microrefuge for epigeal, endogean and hypogean invertebrate species. Understanding the MSS community's spatio-temporal structure and species diversity patterns in relation to environmental parameters plays a crucial role in conservation. In this study we investigated: i) the diversity and community structure of oribatid mites from the edaphic habitat, superficial MSS (*i.e.*, the upper layer of MSS) and deep MSS (*i.e.*, lower layer of MSS) in an alpine region of Southern Carpathians, Romania, and ii) the relationships between the oribatid mite communities and the environmental variables, such as temperature and humidity. The composition and the structure of oribatid communities differed along the three habitats indicating possible habitat specialization. The structure of the oribatid community was influenced by temperature and humidity. The rich and mixed oribatid communities in the MSS and the oribatid communities' response to surface and subsurface environmental variables suggest that the MSS may offer a micro-refuge for edaphic species when the conditions in the surface habitats become too harsh. Thus, we suggest that MSS should be prioritized for conservation because it may be a key component in maintaining biodiversity.

**KEYWORDS** — community structure; conservation; diversity; edaphic habitat; MSS; oribatid mites

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## INTRODUCTION

Exploring how community composition and diversity change across spatial and temporal scales is important for biodiversity conservation as it explains the sources of diversity and the processes that create or maintain diversity (Veech *et al.* 2002; Gering *et al.* 2003; Noda 2004). Work in this field of research using oribatid mite species has mostly focused on species-area relationships or arboreal communities (Maraun and Scheu 2000; Kaneko *et al.* 2005; Fischer *et al.* 2010). Subsoil compartments,

e.g. Mesovoid Shallow Substratum (MSS) communities are less known. Described in 1980 as "Milieu Souterrain Superficiel", MSS is an intermediate habitat between the base of the soil and the bedrock. It is composed by a network of small cracks and voids and is commonly situated in the lower levels of scree slopes (Juberthie *et al.* 1980; Juberthie 1983).

The MSS's particular environmental conditions, *i.e.*, absence of light and photoperiod, low temperature fluctuations (Culver and Pipan 2014), very high humidity (Giachino and Vailati 2010) and bi-

directional flux of organic material (Culver and Pipan 2014) make it a unique habitat that shelters and serves as a microrefuge for epigeal, endo-gean and hypogean invertebrate species (Nitzu *et al.* 2014). Therefore, understanding the MSS community structure and diversity as related to spatio-temporal variation of environmental factors is important.

Recent studies revealed that the MSS is inhabited predominantly by oribatid mites (Skubała *et al.* 2013). Oribatid mites are distributed worldwide, including the alpine regions, and are one of the richest and most abundant of the Acari taxa in soils with high content of decaying organic matter (Krantz and Walter 2009). Oribatid abundance is influenced by environmental variables such as temperature and humidity (Culver and Pipan 2014, Mumladze *et al.* 2015, Pipan *et al.* 2011). There is limited information on oribatid mites from MSS, and the patterns and drivers of their diversity and community structure are poorly understood (Jiménez-Valverde *et al.* 2015). Although the interest in evaluating the importance of the MSS as a hot spot for invertebrate diversity increased in the last years (Nae and Ilie 2004; Nitzu *et al.* 2006; Nitzu *et al.* 2010; Nitzu *et al.* 2014; Pipan *et al.* 2011), there is little information on oribatid community structure and spatio-temporal dynamics in these environments (Skubała *et al.* 2013).

In this study we aim to investigate: i) the diversity and community structure of oribatid mites from edaphic environment, superficial MSS (*i.e.*, the upper layer of MSS) and deep MSS (*i.e.*, lower layer of MSS) in a sub-alpine region in the Piatra Craiului National Park, Southern Carpathians, Romania; ii) the relationships between the environmental variables, such as temperature and humidity, and diversity and community structure of oribatid mites. We discuss the results in the context of increased interest in studying MSS habitats from an ecological perspective, and we provide here a first comprehensive study on the oribatid mite communities in MSS.

## MATERIALS AND METHODS

The study area is located in the Piatra Craiului National Park, Southern Carpathians, one of the most

important karst areas in Romania (Nitzu *et al.* 2014). Piatra Craiului Massif is a 20 km<sup>2</sup> limestone ridge with more than 500 caves and diverse types of talus and scree slopes, both covered and open (see Culver and Pipan 2014).

Three sampling sites were selected: (1) Cerdacul Stanciului – a mobile limestone scree situated near Stanciului Cave; (2) Marele Grohotiș – the largest mobile nude limestone scree accumulation from Piatra Craiului Massif; and (3) Valea Seacă – a stabilized type of MSS, covered by forest (spruce and beech).

Cerdacul Stanciului and Marele Grohotiș are sub-alpine habitats, classified as "calcareous and calcashist screes of the montane to alpine levels – *Thlaspietea rotundifolii*" (Doniță *et al.* 2005) and are listed in the 8210 habitat types following Natura 2000 habitats classification. Valea Seacă is a R6111 type of habitat – Carpathian South-East communities of fixed screes with *Geranium macrorrhizum*, *Sedum fabaria* and *Geranium lucidum* (Doniță *et al.* 2005).

All Oribatida material used in this study was collected as a part of a broader study concerning the diversity of epigeal invertebrates, commonly beetles and wandering spiders (Nitzu *et al.* 2014). Pitfall traps were used to collect invertebrates from edaphic habitat (EDAF), and drillings for MSS (López and Oromi 2010). Pitfall traps might not be the most effective sampling technique for oribatid mites, as the traps sample surface-active invertebrates, estimating the abundance of each species as a function of its activity during the sampling period and population density in the habitat (Brown and Matthews 2016). Thus this study provides data on oribatid species activity-density, *i.e.*, the abundance of a species in pitfall traps is an unknown function of that species' surface activity and density in the surrounding habitat, but for simplicity we refer to oribatid mite "abundance" throughout the paper.

The MSS was sampled at two depths: 0.5 m – the superficial MSS (SMSS) and 0.75 m – the deep MSS (DMSS). The MSS at each sampling site was sampled at two altitudes: Cerdacul Stanciului at 1637 m and 1672 m, Marele Grohotiș at 1579 m and 1580 m and Valea Seacă at 1087 m and 1200 m, respectively

(Table 1). However, the drilling at 1200 m was lost (represented by missing values (-) in Table 1).

To collect mites with drillings, inside of each drilling we placed a trap half filled with 70% ethanol. The upper part of the drilling was covered with a plastic lid to prevent the debris and rocks from falling inside. Each trap was emptied

once a month from April to November (8 months), in 2008 and 2009. This made a total of 80 MSS samples: Cerdacul Stanciului 8 months x 2 years x 2 depths; Marele Grohotiș 8 months x 2 years x 2 depths; Valea Seacă 8 months x 1 year x 2 depths.

To collect mites from EDAF, we selected four plots, one in Cerdacul Stanciului, two at Marele Gro-

TABLE 1: Sampling sites and sampling periods for the three habitats (EDAF – edaphic; SMSS – superficial MSS; DMSS – deep MSS), Piatra Craiului National Park, Romania. X denotes that the sampling was done

Sampling sites /Habitat type	EDAF		SMSS			DMSS			
	Month	Year		Month	Year		Month	Year	
		2008	2009		2008	2009		2008	2009
Cerdacul Stanciului									
	Mar	-	-	Mar	-	-	Mar	-	-
	Apr	-	-	Apr	X	X	Apr	X	X
	May	X	-	May	X	X	May	X	X
	Jun	X	-	Jun	X	X	Jun	X	X
	Jul	-	-	Jul	X	X	Jul	X	X
	Aug	-	-	Aug	X	X	Aug	X	X
	Sept	-	-	Sept	X	X	Sept	X	X
	Oct	-	-	Oct	X	X	Oct	X	X
	Nov	-	-	Nov	X	X	Nov	X	X
Marele Grohotiș									
	Mar	-	-	Mar	-	-	Mar	-	-
	Apr	-	-	Apr	X	X	Apr	X	X
	May	X	-	May	X	X	May	X	X
	Jun	X	-	Jun	X	X	Jun	X	X
	Jul	-	-	Jul	X	X	Jul	X	X
	Aug	-	-	Aug	X	X	Aug	X	X
	Sep	-	-	Sep	X	X	Sep	X	X
	Oct	-	-	Oct	X	X	Oct	X	X
	Nov	-	-	Nov	X	X	Nov	X	X
Valea Seacă									
	Mar	X	-	Mar	-	-	Mar	-	-
	Apr		-	Apr	X	X	Apr	-	-
	May	X	-	May	X	X	May	-	-
	Jun		-	Jun	X	X	Jun	-	-
	Jul	X	-	Jul	X	X	Jul	-	-
	Aug		-	Aug	X	X	Aug	-	-
	Sep		-	Sep	X	X	Sep	-	-
	Oct		-	Oct	X	X	Oct	-	-
	Nov		-	Nov	X	X	Nov	-	-

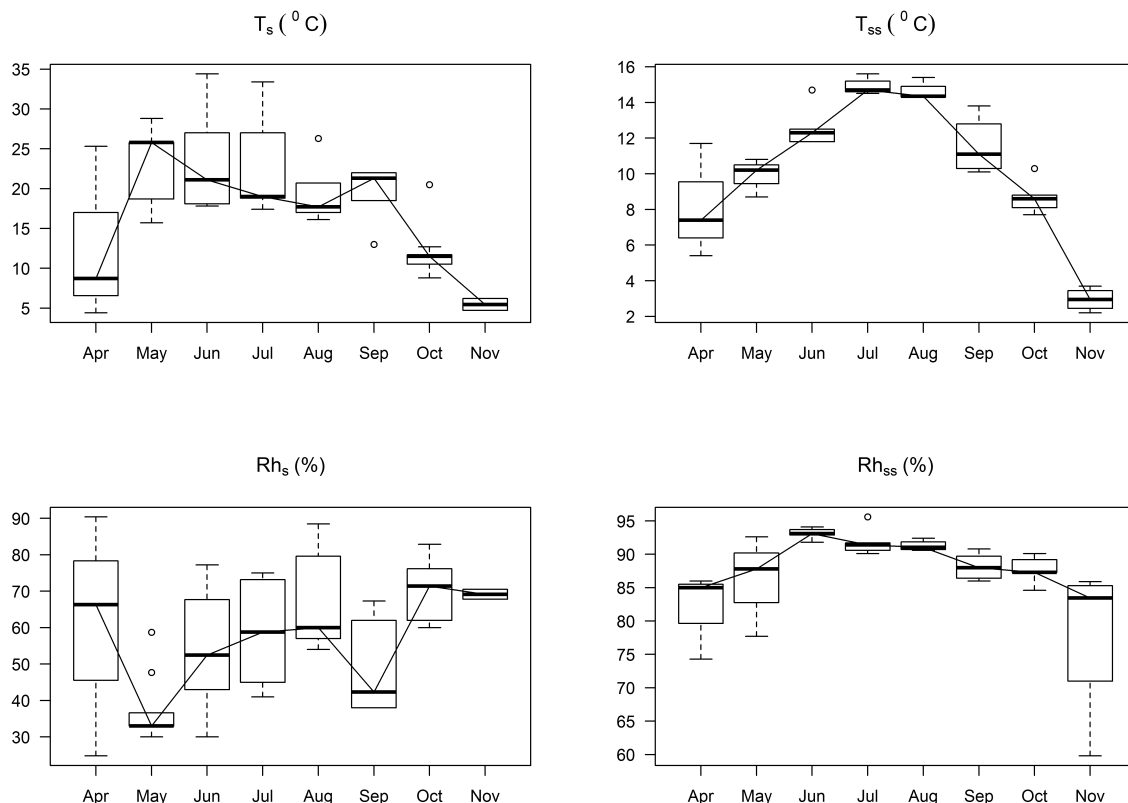


FIGURE 1: Monthly variation of soil ( $T_s$ ) and subsoil ( $T_{ss}$ ) temperature and soil ( $R_h$ ) and subsoil ( $R_{h_{ss}}$ ) relative humidity. The box-plots show the median, the upper and lower quartiles, the maximum and the minimum values and the outliers.

hotiș and one plot in Valea Seacă. In each plot five pitfall traps, with 70% ethanol were set at a depth of 9 cm, covering a perimeter of 25 m<sup>2</sup>. Each of the five traps per plot was considered as one independent sample. The traps were emptied at 5-day intervals, and the material collected in each trap pooled for each month. The pitfall traps were installed during May and June in Cerdacul Stanciului and Marele Grohotiș. In Valea Seacă the pitfall traps were installed during March, May and July. This made a total of 35 EDAF samples: Cerdacul Stanciului 5 traps x 1 plot x 2 months; Marele Grohotiș 5 traps x 2 plots x 2 months; Valea Seacă 5 traps x 1 plot x 3 months (Table 1). One pitfall trap did not collect any oribatid mites and 22 pitfall traps collected at list one specimen.

The collected mites were sorted and identified to

genus and species level. We used the identification keys published by van der Hammen (1952), Bernini (1978), Pérez-Inigo (1993, 1997), Weigmann (2006). The systematic ranking of the species was done after Subías (2004, updated in 2017). After identification the material was preserved in 70% ethanol and stored in the collection of “Emil Racoviță” Institute of Speleology, Bucharest, Romania. Original taxonomic descriptions are not included in the References.

The temperature ( $T$ ) ( $^{\circ}\text{C}$ ) and relative humidity ( $R_h$ ) (%) were measured using a humidity LogR thermo-hygrometer “Digi-Sense” Cole – Palmer.  $T$  and  $R_h$  were measured at the ground level (hereafter soil temperature ( $T_s$ ) and soil relative humidity ( $R_h$ )) and at 0.5 m and 0.75 m depth (hereafter subsoil temperature ( $T_{ss}$ ) and subsoil relative humidity

TABLE 2: Species, code (the abbreviation used in CCA graphs), abundance (N), dominances (DO) and constancies (C) of the oribatid mites collected from the three habitats (EDAF – edaphic; SMSS – superficial MSS; DMSS – deep MSS) in 2008-2009, Piatra Craiului National Park, Romania.

Species name	Code	EDAF			SMSS			DMSS		
		N	DO	C	N	DO	C	N	DO	C
<i>Atropacarus stricalus</i>	Atrostri	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Phthiracarus</i> sp.	Phthsp	75	18.20	69.57	37	4.58	37.50	23	6.59	37.50
<i>Phthiracarus laevigatus</i>	Phthlaev	3	0.73	8.70	0	0.00	0.00	0	0.00	0.00
<i>Phthiracarus longulus</i>	Phthlong	2	0.49	4.35	0	0.00	0.00	0	0.00	0.00
<i>Phthiracarus</i> ( <i>Archiphthiracarus</i> ) sp.	Archsp	1	0.24	4.35	5	0.62	4.17	1	0.29	3.13
<i>Steganacarus</i> sp.	Stegsp	5	1.21	17.39	1	0.12	2.08	1	0.29	3.13
<i>Camisia biverrucata</i>	Camibive	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Camisia horrida</i>	Camihorr	0	0.00	0.00	0	0.00	0.00	1	0.29	3.13
<i>Camisia</i> ( <i>Ensicamisia</i> ) <i>lapponica</i>	Camilapp	0	0.00	0.00	1	0.12	2.08	1	0.29	3.13
<i>Heminothrus</i> ( <i>Platynothis</i> ) <i>peltifer</i>	Hemipelt	1	0.24	4.35	2	0.25	2.08	0	0.00	0.00
<i>Hermannia convexa</i>	Hermconv	6	1.46	8.70	0	0.00	0.00	0	0.00	0.00
<i>Hermannia gibba</i>	Hermgibb	10	2.43	30.43	5	0.62	10.42	5	1.43	15.63
<i>Hermannia</i> ( <i>Heterohermannia</i> ) <i>nodosa</i>	Hermnode	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Belba</i> sp.	Belbsp	0	0.00	0.00	1	0.12	2.08	3	0.86	6.25
<i>Cepheus dentatus</i>	Cephdent	3	0.73	13.04	2	0.25	4.17	0	0.00	0.00
<i>Conoppia microptera</i>	Conomicr	0	0.00	0.00	5	0.62	4.17	1	0.29	3.13
<i>Conoppia palmicincta</i>	Conopalm	3	0.73	13.04	4	0.50	8.33	4	1.15	6.25
<i>Ommatocephus ocellatus</i>	Ommaocel	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Tritegeus bisulcatus</i>	Tritbisu	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Ceratoppia</i> sp.	Cerasp	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Ceratoppia bipilis</i>	Cerabipi	32	7.77	52.17	193	23.92	47.92	108	30.95	59.38
<i>Ceratoppia quadridentata</i>	Ceraquad	3	0.73	13.04	4	0.50	6.25	1	0.29	3.13
<i>Adoristes ovatus</i>	Adorovat	8	1.94	21.74	1	0.12	2.08	4	1.15	6.25
<i>Liacarus coracinus</i>	Liaccora	10	2.43	26.09	5	0.62	10.42	3	0.86	9.38
<i>Liacarus</i> ( <i>Dorycanosus</i> ) <i>acutus</i>	Doryacut	3	0.73	4.35	5	0.62	6.25	0	0.00	0.00
<i>Xenillus tegeocranus</i>	Xenitege	0	0.00	0.00	1	0.12	2.08	11	3.15	18.75
<i>Caleremaeus monilipes</i>	Calemone	0	0.00	0.00	0	0.00	0.00	1	0.29	3.13
<i>Eremaeus hepaticus</i>	Eramhepa	2	0.49	4.35	3	0.37	6.25	0	0.00	0.00
<i>Eremaeus oblongus silvestris</i>	Euersilv	1	0.24	4.35	1	0.12	2.08	0	0.00	0.00
<i>Pantelozetes</i> sp.	Pantlozet	3	0.73	4.35	0	0.00	0.00	0	0.00	0.00
<i>Pantelozetes paolii</i>	Pantpaol	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Multioppia</i> ( <i>Multilanceoppia</i> ) <i>carpatica</i>	Multcarp	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Rhinoppia</i> sp.	Rhinsp	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Quadroppia</i> ( <i>Coronoquadroppia</i> ) <i>monstruosa</i>	Quadmons	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Carabodes</i> sp.	Carasp	2	0.49	4.35	0	0.00	0.00	0	0.00	0.00
<i>Carabodes areolatus</i>	Carareo	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Carabodes femoralis</i>	Carafemo	3	0.73	13.04	0	0.00	0.00	0	0.00	0.00
<i>Carabodes labyrinthicus</i>	Caralaby	15	3.64	30.43	1	0.12	2.08	4	1.15	9.38
<i>Carabodes marginatus</i>	Caramarg	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Carabodes reticulatus</i>	Carareti	3	0.73	13.04	0	0.00	0.00	1	0.29	3.13
<i>Carabodes rigosior</i>	Cararugo	3	0.73	4.35	0	0.00	0.00	0	0.00	0.00
<i>Tectocephus velatus velatus</i>	Tectvela	7	1.70	17.39	0	0.00	0.00	0	0.00	0.00
<i>Cymbaeremaeus cymba</i>	Cymbeyb	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Scutovertex minutus</i>	Scutminu	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Eupelops acromius</i>	Eupearco	5	1.21	8.70	2	0.25	4.17	1	0.29	3.13
<i>Eupelops claviger</i>	Eupeclav	0	0.00	0.00	1	0.12	2.08	1	0.29	3.13
<i>Eupelops plicatus</i>	Eupeplic	6	1.46	8.70	6	0.74	8.33	2	0.57	3.13
<i>Eupelops subuliger</i>	Eupesubu	4	0.97	13.04	0	0.00	0.00	2	0.57	6.25
<i>Eupelops torulosus</i>	Eupetoru	3	0.73	13.04	4	0.50	6.25	2	0.57	3.13

TABLE 2: Continued.

Species name	Code	EDAF			SMSS			DMSS		
		N	DO	C	N	DO	C	N	DO	C
<i>Achipteria coleoptrata</i>	Achicole	10	2.43	26.09	0	0.00	0.00	0	0.00	0.00
<i>Achipteria elegans</i>	Achieleg	2	0.49	4.35	0	0.00	0.00	0	0.00	0.00
<i>Achipteria nitens</i>	Achinite	4	0.97	13.04	0	0.00	0.00	2	0.57	3.13
<i>Achipteria ellnicki</i>	Achisell	5	1.21	17.39	0	0.00	0.00	0	0.00	0.00
<i>Campachipteria fanzagoi</i> = <i>Parachipteria willmanni</i>	Parawill	0	0.00	0.00	6	0.74	10.42	5	1.43	9.38
<i>Parachipteria punctata</i>	Parapunc	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Oribatella</i> sp.	Oribsp.1	2	0.49	8.70	4	0.50	8.33	7	2.01	12.50
<i>Oribatella calcarata</i>	Oribcalc	11	2.67	26.09	10	1.24	16.67	8	2.29	18.75
<i>Oribatella foliata</i> = <i>O. brevipila</i>	Oribbrev	2	0.49	8.70	116	14.34	27.08	22	6.30	28.13
<i>Oribatella longispina</i>	Oriblong	29	7.04	30.43	236	29.24	52.08	43	12.32	43.75
<i>Oribatella ornata</i>	Oriborna	0	0.00	0.00	0	0.00	0.00	5	1.43	15.63
<i>Oribatella quadricornuta</i>	Oribquad	3	0.73	13.04	24	2.97	8.33	13	3.72	18.75
<i>Euzetes globulus</i>	Euzeglob	0	0.00	0.00	14	1.73	4.17	3	0.86	3.13
<i>Fuscozetes setosus</i>	Fuscseto	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Lepidozetes singularis</i>	Lepising	1	0.24	4.35	0	0.00	0.00	1	0.29	3.13
<i>Melanozetes meridianus</i>	Melameri	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Sphaerozetes piriformis</i>	Sphapiri	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Trichoribates (Latilamellobates) incisellus</i>	Tricinci	2	0.49	8.70	0	0.00	0.00	0	0.00	0.00
<i>Chamobates</i> sp.	Chamsp	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Chamobates alpinus</i>	Chamalpi	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Chamobates birulai</i>	Chambiru	1	0.24	4.35	4	0.50	4.17	0	0.00	0.00
<i>Chamobates cuspidatus</i>	Chamcusp	62	15.05	47.83	47	5.82	33.33	30	8.60	25.00
<i>Chamobates pusillus</i>	Champusi	2	0.49	4.35	0	0.00	0.00	0	0.00	0.00
<i>Chamobates (Xiphobates) voigsi</i>	Chamvoig	3	0.73	4.35	0	0.00	0.00	0	0.00	0.00
<i>Minuthozetes pseudofusiger</i>	Minupseu	17	4.13	13.04	1	0.12	2.08	0	0.00	0.00
<i>Mycobates</i> sp.	Mycosp	5	1.21	17.39	17	2.11	16.67	8	2.29	12.50
<i>Mycobates carli</i>	Mycocarl	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Mycobates parmellae</i>	Mycoparm	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Mycobates (Calypozetes) alpinus</i>	Mycocalpi	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Punctoribates punctum</i>	Puncpunc	1	0.24	4.35	1	0.12	2.08	1	0.29	3.13
<i>Oribatula</i> sp.	Oribsp	1	0.24	4.35	0	0.00	0.00	1	0.29	3.13
<i>Oribatula interrupta</i>	Oribinte	2	0.49	4.35	0	0.00	0.00	2	0.57	6.25
<i>Oribatula interrupta sitnikorae</i>	Oribsitn	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Oribatula pannonica</i>	Oribpann	0	0.00	0.00	2	0.25	2.08	2	0.57	6.25
<i>Oribatula tibialis</i>	Oribtibi	9	2.18	17.39	5	0.62	10.42	3	0.86	9.38
<i>Phauloppia</i> sp.	Phausp	0	0.00	0.00	3	0.37	4.17	0	0.00	0.00
<i>Scheloribates (Hemileius) initialis</i>	Scheinit	1	0.24	4.35	1	0.12	2.08	4	1.15	9.38
<i>Liebstadia</i> sp.	Liebsp	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Liebstadia humerata</i>	Liebhume	1	0.24	4.35	0	0.00	0.00	0	0.00	0.00
<i>Scheloribates (S.) cf. laevigatus</i>	Schelaev	1	0.24	4.35	1	0.12	2.08	6	1.72	12.50
<i>Neoribates aurantiacus</i>	Neoraura	0	0.00	0.00	2	0.25	4.17	2	0.57	6.25
<i>Acrogalumna longipluma</i>	Acrolong	1	0.24	4.35	1	0.12	2.08	0	0.00	0.00
<i>Galumna flagellata</i>	Galulflag	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Peragalumna nervosa</i>	Pergnerv	0	0.00	0.00	1	0.12	2.08	0	0.00	0.00
<i>Pilagalumna tenuiclava</i>	Pilotenu	0	0.00	0.00	7	0.87	6.25	0	0.00	0.00

(Rhss)). Figure 1 summarizes these measurements.

## DATA ANALYSIS

### Diversity and community structure

The structure of mite communities in the three habitat types (EDAF, SMSS and DMSS) was accessed based on the dominance and constancy of the species and the number of the unique species. The dominance of an individual species was calculated as  $DO = N_i / N * 100 (\%)$ , where  $N$  = the total number of individuals in each habitat,  $N_i$  = the total number of individuals of the  $i^{\text{th}}$  species. The species with  $DO \geq 5$  were considered dominant and species with  $DO \geq 10$  – eudominant. Constancy is the percentage of the samples in which the species occurred:  $C = L_s / L * 100 (\%)$ , where  $L$  = the total number of samples in each habitat,  $L_s$  = the number of samples in which the species was found. Species with  $C \geq 50$  were considered constant, and species with  $C > 75$  – euconstant. The unique species are species that occur at only one site.

Because the number of samples in each habitat type was not equal and the total abundance ( $N$ ) per habitat type was different (EDAF:  $N = 412$ ; SMSS:  $N = 807$ ; DMSS:  $N = 349$ ) the data was normalized before analysis. To normalize the data, (i) for abundance analysis, we used average abundances of species, i.e., number of individuals per sample / number of samples for each habitat; and (ii) for diversity analysis, we rarefied the abundance matrix, using 300 individuals per sample. The following diversity indices were chosen to investigate the community composition and how it differs among habitat types: species richness ( $S$ ), Shannon-Wiener index ( $H'$ ), Simpson's index ( $D$ ) and Pielou's ( $J'$ ) index (Magurán 2004).

General Linear Mixed Models (GLMM) were applied to test whether the main community features (abundance and species richness) were related to the habitat type (EDAF, SMSS and DMSS) and to the environmental variables ( $T_s$ ,  $R_h$ s,  $T_{ss}$  and  $R_{hss}$ ). The habitat type,  $T_s$ ,  $R_h$ s,  $T_{ss}$  and  $R_{hss}$  were introduced in the analysis as fixed factors, the sampling site (CS, MG and VS) was considered a random factor and month was included as a repeated

measure. We built a set of four candidate models and assess the relative performance of these models using a selection technique based on Akaike's information criterion corrected for sample size (AICc: Burnham and Anderson 2002; Johnson and Omland 2004). Models were ranked, and the one with the lowest AICc was used as the reference for calculating the AIC difference ( $\Delta_i$ ) and the likelihood of a model given the data and model weights ( $w_i$ ). Models within two AIC units of the AICmin were considered competitive and more plausible than others (Burnham and Anderson 2002).

The mite species assemblage relationships were modelled by applying a series of Constrained Correspondence Analyses (CCA) and the so-called "partial CCA". They can be used to model the multivariate response of a species assemblage to a matrix of explanatory variables (Borcard *et al.* 1992; Legendre and Legendre 1998). The Correspondence Analysis approach is appropriate because it preserves the chi-square distance of the sample and thus correctly handles species frequency.

In particular, the following two models were considered on abundance data pooled over each month:

CCA I:  $A \sim f[\text{habitat}]$

CCA II:  $A \sim f[\text{environmental variables}]$

Where  $A$  is the species abundance matrix, the function  $f$  is the linear combination of independent variables and the operator  $[\ ]$  describes the operation for partitioning out the component of variation described by the linear function within the square brackets. The independent variables included in the CCA analysis were the habitats type (EDAF, SMSS and DMSS) and the environmental variables ( $T_s$ ,  $R_h$ s,  $T_{ss}$  and  $R_{hss}$ ). The abundance matrix was  $\ln(x + 1)$  transformed to maintain normal distribution and to avoid the "arch effect" in CCA (Ter Braak 1986). The permutation procedure (based on 9999 cycles) was used to test the significance of explanatory variables in CCA for all eigenvalues (Oksanen *et al.* 2006).

All analyses were performed using R version 3.2.1 (R Development Core Team 2016). The abundance matrix rarefaction and the CCA were performed using vegan package (Oksanen *et al.* 2006).

The diversity analysis was done using BiodiversityR package (Kindt 2014) and the GLMM using the lme4 package (Bates and Maechler 2010).

## RESULTS

### Diversity and community structure

A total of 1568 oribatid mites belonging to 94 species, 57 genera and 28 families was collected (Table 2). The number of mites collected per sample ranged from 1 to 100, and the species richness from 1 to 21. Overall, in the three habitats, five species were eudominant, five species were dominant, three species were constant and none was euconstant (Table 2). Three species were eudominant and constant in one of the three habitats: *Phthiracarus* sp. in EDAF, *Oribatella longispina* (Berlese, 1914) in SMSS and *Ceratoppia bipilis* (Hermann, 1804) in DMSS. The latter species was also dominant and constant in EDAF. The most abundant species, *Ceratoppia bipilis* and *Oribatella longispina*, were present in all three habitats but had the highest abundance in SMSS. Four species were new records for Romania: *Achipteria elegans* (Schweizer, 1956), *Eupelops plicatus* (C.L. Koch, 1835), *Ommatocephus ocellatus* (Michael, 1882) and *Oribatella longispina* Berlese, 1915 (see Nae and Ivan 2015).

Twenty-four species were common to all three habitats. On the other hand, 47 species were unique in one of the three habitats (EDAF: 27 species; SMSS: 17 species; DMSS: 3 species). The species richness was higher in edaphic habitat (EDAF) than in MSS; the diversity indices (Shannon-Wiener, Pielou's and Simpson's) were lower in the superficial MSS than in the deep MSS or in EDAF (Table 3).

TABLE 3: Observed species abundance (N), observed number of species (S), Shannon-Wiener index ( $H'$ ), Pielou's index ( $J'$ ), Simpson's index (D) for oribatid mites in three different habitats (EDAF – edaphic; SMSS – superficial MSS; DMSS – deep MSS), 2008-2009, Piatra Craiului National Park, Romania.

	N	S	$H'$	$J'$	D
Habitat type					
EDAF	412	65	2.893	0.695	0.879
SMSS	807	58	2.265	0.557	0.802
DMSS	349	42	2.652	0.709	0.869

The model selection using AIC indicated that for both abundance and species richness, only the models including the Tss and Rhss were supported (Table 4).

The permutation tests for all two CCA models applied to oribatid mite abundance matrix indicated that explanatory variables accounted for a significant portion of species distribution variation (999 permutations,  $P < 0.05$ ). The first two canonical axes clarify the main community patterns (Figs 2–3: species assemblages show a gradient in species composition that is collinear with the spatial variation (Figure 2) and environmental factors (Figure 3).

The CCA of the association between oribatid mite species abundance and the habitat shows that species in the upper right quadrat – *Oribatella longispina*, *Chamobates birulai* (Kulczynski, 1902), and *Pilogalumna tenuiclava* (Berlese, 1908) – were associated with the SMSS (Figure 2). Four species were associated with the EDAF habitat: *Achipteria coleoptrata* (Linnaeus, 1758), *Ceratoppia quadridentata* (Haller, 1882), *Cepheus dentatus* (Michael, 1888) and *Phthiracarus* sp. (Figure 2). *Oribatella* sp., *Hemileius initialis* (Berlese, 1908) and *Schelorbates laevigatus* (Koch, 1835) were associated with the DMSS (Figure 2).

The temperature and relative humidity in the MSS present decreased variation in amplitude in comparison with the external temperature and relative humidity that show high fluctuations (Figs 1, 3). The CCA results for association of the oribatid mite species abundance and environmental variables showed that Ts and Rhs are the strongest determinants of Oribatida community composition

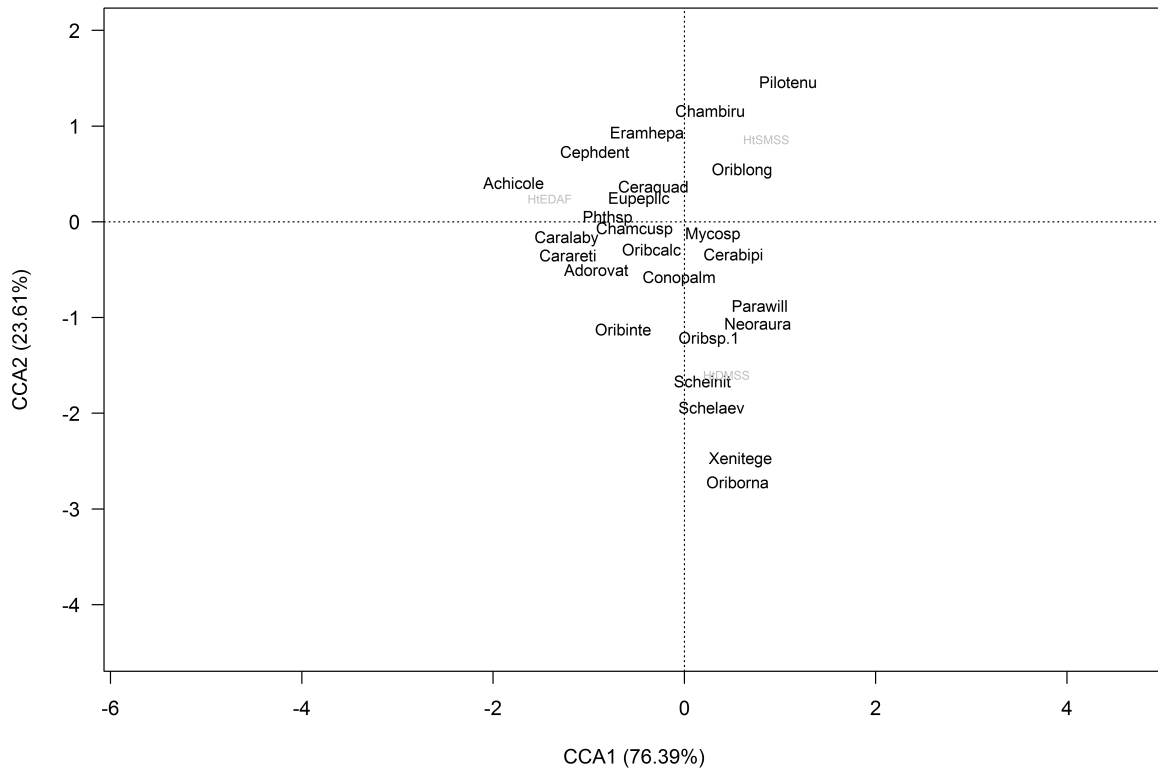


FIGURE 2: Biplots of the CCA model of the mite species abundance matrix in relation to habitat type; EDAF – edaphic, DMSS – deep MSS, SMSS – superficial MSS. Species names were abbreviated using the first four letters of the genus and species name, respectively. Abbreviations are shown in Table 2.

(Figures 1, 3). *Cepheus dentatus* and *Camisia biverrucata* (Koch, 1839) were the species correlated with Ts, while the presence of *Oribatula tibialis* (Nicolet, 1855), *Neoribates aurantiacus* (Oudemans, 1914) and *H. initialis* was influenced by RhS (Figure 3).

## DISCUSSION

This study highlights the ecological importance of MSS habitats for oribatid mite communities. We found rich and diverse oribatid mite communities in MSS habitats and we report rare (*A. elegans*) and new records (*O. ocellatus*) in edaphic habitat for the study area. We also report two species

new for the Romanian fauna from MSS (*E. plicatus* and *O. longispina*). *A. elegans* was first described by Schweizer (1956) from individuals collected in Switzerland (from spruce and larch forests with high humidity, acidic soils, lichen and moss beds and rocks) and was not reported again with new observations until now. *Ommatocephus ocellatus* is frequently found on tree bark and lichens (Weigmann 2006) and is a Palearctic species (Subías 2004). *Eupelops plicatus*, which has Holarctic distribution (Subías 2004) and prefers forest soils or tree bark (Weigmann 2006), was collected from all three environments. *Oribatella longispina* was the second

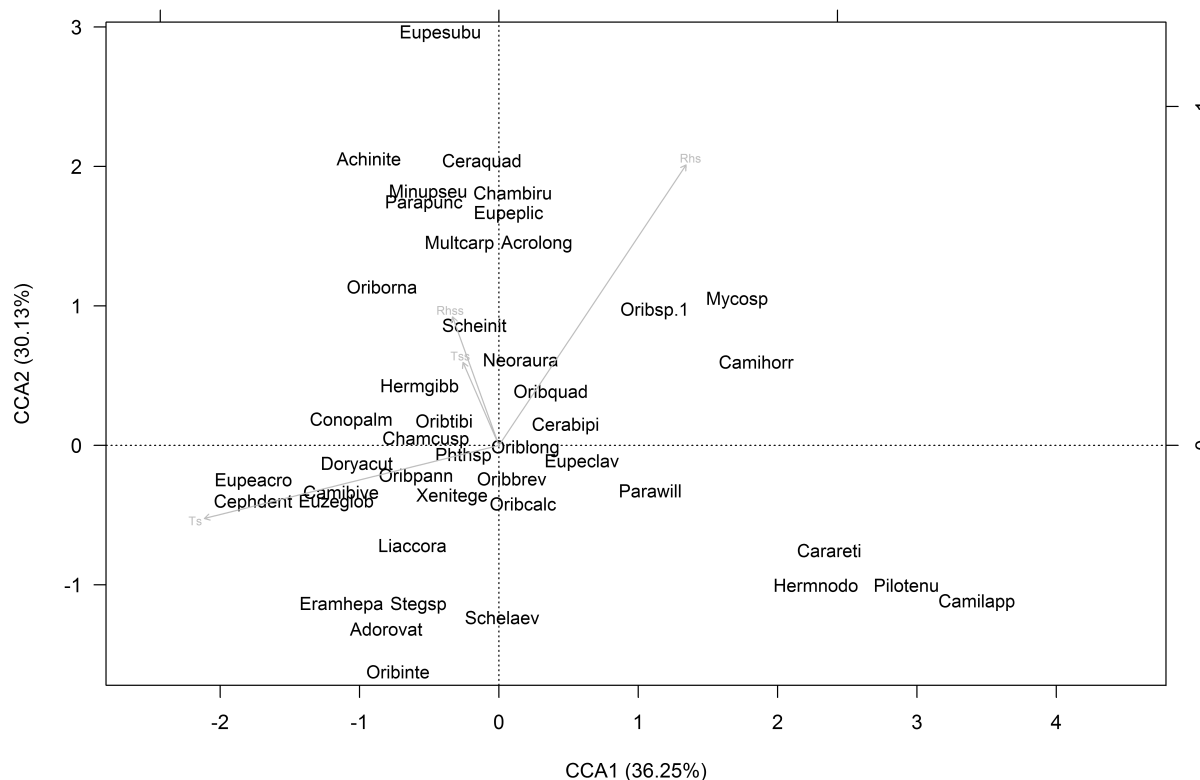


FIGURE 3: Biplots of the CCA model of the mite species abundance matrix in relation to environmental variables: Ts – soil temperature, Rhs – soil relative humidity, Thss – subsoil temperature, Rhss – subsoil relative humidity. Species names were abbreviated as explained in Figure 1. The arrows indicate environmental gradients; the length of arrows shows their correlation with the ordination axis. Longer arrows indicate greater importance of the factor for the species variation. Species near to or beyond the tip of arrows are strongly correlated and influenced by the factor. Those at opposite end are less strongly affected.

eudominant species in our sites with the highest abundance in MSS.

The composition of oribatid mite communities differed among the three habitats. This most likely indicates habitat specialization and explains the observed high levels of species diversity. However, the choice of the pitfall traps as sampling technique of the edaphic habitat may have influenced the type of mites that were sampled (Moreira *et al.* 2008) and thus the results must be carefully considered when compared to other findings.

The MSS showed the strongest evidence of habitat association (29 species were present only in the MSS), indicating dependence of oribatid mite communities on the particular environmental character-

istics of the MSS. A number of authors have brought arguments in favour of the MSS as a habitat for endemic and rare species – species and genera new for science (Honciuc and Stănescu 2003; Arillo *et al.* 1994), and the importance of MSS as a habitat “in its own right, one with a set of unique species” (Pipan *et al.* 2011; Culver and Pipan 2014). However, two of the most abundant species in our study, *C. bipilis* and *O. longispina*, were found both in EDAF and MSS, although neither of these species was previously reported from the MSS. The first species is widely distributed in Holarctic (Subías 2004) and the second is well represented in East Europe (Subías 2004). Bernini (1978) reported *O. longispina* as a mountain species.

The observed species richness of the oribatid mites decreased from edaphic habitat through SMSS, to the DMSS (but see the Material section). The diversity indexes, however, are similar for the DMSS and edaphic habitats, all higher than for the MSS. This suggests that the MSS, especially deep MSS, plays an important role as a refuge for edaphic species, which is in line with other studies (Pipan *et al.* 2011; Nitzu *et al.* 2014) and supports the hypothesis that the MSS is a gateway in the colonization of the subterranean realm (Pipan *et al.* 2011).

Furthermore, environmental variables, i.e., subsoil temperature and relative humidity, were important determinants of the species richness of oribatid mites. Temperature and humidity are key factors in determining the presence of invertebrate species in edaphic and MSS habitats (Růžička *et al.* 1995; Růžička and Zacharda 2010). In summer, when the outside temperature increases and soil relative humidity decreases, the species enter the MSS and screes, using it as a refuge (Nitzu *et al.* 2014).

Environmental variables were also the major parameter influencing the structure of the oribatid mite community. Soil temperature was the most important factor determining differences in community structure followed by soil relative humidity.

The CCA results also indicated that several oribatid mite species were affected by soil temperature and relative humidity. This is in line with previous studies which indicated that the temperature and humidity shape the species community structure in these scree habitats (Blair *et al.* 2000, Blesić and Mitrovski 2003, Nitzu *et al.* 2014).

Temperature can directly and indirectly influence oribatid mite communities through its effect on moisture conditions and subsoil temperature, and it may be a key factor determining the presence of certain species (Jiménez-Valverde *et al.* 2015). Thus, as pointed out by other studies, the two variables are interconnected and the higher the temperature, the greater the choice of organisms for higher humidity (Madge 1964). The low amplitude of temperature variation in MSS compared with high temperature variation at soil level (Figure 1) makes the MSS a micro-refuge for epigeal species when conditions in the surface habitats become too harsh (e.g., too hot) (Nitzu *et al.* 2014).

## CONCLUSION

The main findings of our study are: (i) the edaphic and MSS habitats have rich oribatid communities

TABLE 4: Akaike statistics for model including the species abundance and the species richness. LL – log likelihood; K – number of parameters; AICc – Akaike’s information criterion corrected for sample size;  $\Delta$ AICc – differences between the best model (smallest AICc) and each model;  $w_i$  – Akaike weights; Ts – soil temperature; Rhss – soil relative humidity; Tss – subsoil temperature; Rhss – subsoil relative humidity.

Model	Covariates	LL	K	AICc	$\Delta$ AICc	$w_i$
Abundance						
3	Tss + Rhss	-23.10	40	126.19	0.00	1
2	Tss + Rhss + Ts + Rhss	-29.90	42	143.80	17.60	0.00
4	Ts + Rhss	-56.56	40	193.12	66.93	0.00
1	Habitat type	-71.22	49	240.43	114.24	0.00
Species richness						
3	Tss + Rhss	-70.87	40	221.74	0.00	0.99
2	Tss + Rhss + Ts + Rhss	-73.59	42	231.19	9.45	0.01
4	Ts + Rhss	-164.79	40	409.57	187.83	0.00
1	Habitat type	-219.41	49	536.83	315.09	0.00

that are different among the habitats; (ii) the diversity and community structure of oribatid mites are sensitive to fluctuations of environmental factors, such as temperature and relative humidity. Our results highlight the role of MSS as a reservoir of edaphic oribatid mite species. Although we could not distinguish facultative or obligate subterranean species, the fact that some species were found exclusively in MSS indicates that the MSS may offer habitat to possible troglaxene or trogliphilic oribatid species (Mammola *et al.* 2016). Further studies are needed to confirm the role played by that the MSS in sheltering cave-dwelling mite species. However, we suggest that the MSS should be prioritized for conservation since it may be a key component in maintaining biodiversity.

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