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Protura (Arthropoda: Hexapoda) in Slovenian caves

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Abstract: Protura is a small class of Hexapoda, generally poorly known, and rather scarce data on its occurrence in caves are scattered throughout literature on fauna in caves and in some papers on Protura. Although the cave-dwelling fauna is relatively well studied in Slovenia, published records on Protura are rare. In this paper, data on the occurrence and abundance of Protura in Slovenian caves are considered. Various statistical analyses were performed to compare caves with Protura to those without in 60 intensively monitored cavities to detect any differences in the selected environmental conditions. No significant difference was obtained. Samples collected from 15 caves yielded 286 specimens identified to genus or species level. Ten species were identified: *Acerentulus confinis* (Berlese, 1908), *A. rafalskii* Szeptycki, 1979*, *Acerentomon affine* Bagnall, 1912*, *A. balcanicum* Ionesco, 1933*, *A. italicum* Nosek, 1969, *A. maius* Berlese, 1908*, *A. meridionale* Nosek, 1960, *Acerella muscorum* (Ionesco, 1930)*, *Eosentomon armatum* Stach, 1926* and *E. transitorium* Berlese, 1908*. The seven species marked with an asterisk are new records for Slovenia. Two specimens belonging to an unidentified species of the genus *Ionescuellum* Tuxen, 1960 were also found. As expected, Protura were most abundant at the cave entrance close to the surface, and none of the species were found exclusively in cavities. This suggests that proturans were introduced passively into the cavities via organic matter and surface soils.

Keywords: adaptations, cave fauna, microarthropods, microhabitats, subterranean ecosystem

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INTRODUCTION

Protura is a small, poorly known Class of Hexapoda. In particular, knowledge of their geographical distribution is strongly biased, partly due to the “proturologists” distribution effect (Galli & Rellini, 2020). This means that the observed distribution of proturans largely reflects the presence of specialists in different geographical areas, rather than a real biogeographical pattern. The same bias has been observed in other soil- and cave-dwelling organisms, such as paligrades (Mammola et al., in press) and diplurans (Sendra et al., 2020). Previously, only nine species of Protura had been identified from a few localities in Slovenia (Nosek, 1973; Szeptycki, 2007; Galli et al., 2016): *Acerentulus condei* Nosek, 1983, *A. confinis* (Berlese, 1908), *Acerentomon doderi* Silvestri, 1907, *A. kustorae* Nosek, 1983, *A. italicum* Nosek, 1969, *A. meridionale* Nosek, 1960, *A. microrhinus* Berlese, 1909, *Acerella tiarnea* (Berlese, 1908) (doubtful record) and *Eosentomon delicatum*

Gisin, 1945. Among these, two species were described from Slovenian type material: *A. kustorae* and *A. meridionale*. To date, the former is known only from the type locality (Szeptycki, 2007).

Protura ecology is also poorly known; however, these euedaphic microarthropods mainly, if not exclusively, feed on fungal hyphae (see Galli et al., 2019a, b). Some records of these organisms from caves are known from general faunistic papers (Vandel, 1964; Neuherz, 1974, 1975; Nosek, 1975; Novak, 2005; Szeptycki, 2007; Galli et al., 2019b; Shrubovych & Georgiev, 2020), but no specialized subterranean, troglolytic species has been described (Pass & Szucsich, 2011). However, Nosek (1977a,b) stated that Protura “were introduced also into caves” and that the only adaptation recorded to the “troglolytic mode of life” was an “enlargement of body size”. However, this notation is missing any further clarifications and has not been supported by further bibliographic reports. Generally, Protura are morphologically uniform, as observed between populations of the same species

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living in different environments and climates, and after long times of separate evolution (Nosek, 1975; Tuxen, 1978). Further, none of the papers published to date has dealt specifically with Protura in caves.

In general, due to difficulties in exploring and studying subterranean environments, knowledge about the subterranean fauna is largely incomplete (Mammola, 2018). A significant proportion of subterranean species are still undescribed (the Linnean shortfall), and the distribution of already described species (the Wallacean shortfall), their abundances and population dynamics (the Prestonian shortfall) are mostly unknown (Cardoso et al., 2011; Culver et al., 2012; Ficetola et al., 2018). However, the subterranean fauna in Slovenian caves is relatively well known and has been recognized globally as unique and a biodiversity hotspot for richness in troglobiont species along the “mid-latitude biodiversity ridge in terrestrial cave fauna” (Bole et al., 1993; Culver et al., 2006; Culver & Pipan, 2019). Yet, some taxa remain significantly understudied, and some other taxa, such as Protura, have never been studied in association with cave ecosystems.

In the present paper we provide the first comprehensive faunistic and ecological data on Protura in caves. We give a general overview of proturan distribution in intensively explored caves in Slovenia, and, for some caves, provide a more in-depth taxonomic analysis. Furthermore, we compare the environmental conditions in caves with and without proturans, and investigate the influence of the main cover types and the slope azimuth where the caves are located on the occurrence of Protura in caves. In addition, we present data on ecological conditions in cave sections where Protura have been found, and a general pattern of their spatial distribution in caves.

METHODS

Field methods

Since 1977, 64 cavities (caves and artificial tunnels) in northern and central Slovenia have been the object of ecological studies in January, April, July, and October, following a standardized sampling scheme (Novak, 2005). Most of these cavities are figured in Novak (2005), two further in Novak et al. (2004b) and Kozel et al. (2019). General data on caves were obtained from the Cave Cadastre (Slovenian Speleological Society), while for short caves not registered in the Cadastre, and for artificial cavities, our own data were provided. For simplicity, both caves and artificial cavities will be referred to as caves in the following text. Caves were divided into 785 sampling sections in total, with an average length of ~4 m each, i.e. an average of 12 sampling sections each. The distances from the entrance (polygonal distance) and from the surface (vertical distance) to the center of the sampling sections were calculated from the cave ground plan and the terrain profile above the cave, rounded up to 1 m accuracy each. Once per season, we took records of air temperature and relative air humidity in every sampling section using a psychrometer (initially an August psychrometer, later a handheld aspiration

psychrometer Ahorn FN A846, Germany). A mercury thermometer, and a probe thermometer (Checktemp 1, Hanna, Germany), were used to measure the ground temperature at a depth of 2 cm. The substrate moisture content was determined after drying ~100 g samples at 105°C until reaching the stable weight (Sheppard & Addison, 2008). Environmental factors that change only negligibly over the year in caves were measured only once (Kozel et al., 2019). Among these, the pH of the ground substrate was measured with a pH meter (91–02 Orion, USA Orion Ionalyser 407 A; Hanna Instruments HI221 Calibration Check, Microprocessor pH Meter), and the carbonate content in the substrate by means of Scheibler's calcimetry. Organic material content was calculated after the ignition of dry substrate samples (at 650°C) (Halikia et al., 2001) in a muffle furnace. Airflow was measured with a hand-held anemometer (Munro IM159) or – with all values lower than 0.3 ms⁻¹ not measurable with the anemometer – derived from the velocity of fog or smoke of a burned match or magnesium belt (Novak et al., 2004a). The main cover type (pine, deciduous, mixed wood, scrubland, meadow, bare rock) in the landscape around the cave was determined on the first visit. The slope azimuth in front the cave was determined on 1:25.000 maps.

Protura sampling, preparation and identification

A two-day exposure to baited pitfall traps (decomposing beef and apple juice, with a lacing of cherry and maraschino essence, and a few drops of detergent to reduce the surface tension), and Berlese-Tullgren funnel extraction of ~100 g samples from a ~0.25 m² area around the pitfall traps were applied in the study. In total, 3140 trap samples and 3092 Berlese-Tullgren samples were collected. For details see Novak (2005) and Kozel et al. (2019). Some other collecting methods, such as large Berlese-Tullgren funnel extraction, were only qualitatively analyzed as they were unsuitable for quantitative analysis. However, proturans extracted by means of such methods were studied and analyzed taxonomically (e.g., those from caves IDs 3 and 28). Voucher specimens of Protura were deposited in the zoological collection of DISTAV - University of Genova (Protura series: 479, 481–487, 724–731, 773–776). Protura collected in 15 of the caves listed in Table 1 (IDs: 3, 28, 31, 32, 35, 36, 37, 38, 40, 42, 45, 48, 49, 52, and 59) were prepared for species identification. For this purpose, they were incubated at 40–50°C for 24 hours in lactic acid to make them clear, then mounted on slides in Marc André II medium. Specimens were observed and identified to species and life-stage levels with the aid of an interference contrast microscope (Leica DM LB2), a Leica DFC 295 camera, and Leica Application Suite Vers. 3.8.

Statistical analyses

To compare environmental conditions between caves with and without proturans, 60 caves were evaluated, consisting of subequal numbers of both cave groups; 31 with and 29 without proturans (Table 1, Fig. 1) regardless of their natural (n = 56) or artificial origin

($n = 4$), since the relative faunas are quite comparable (Pretner, 1979). A Mantel test was applied to check for the spatial autocorrelation among caves with and without Protura using an *ade4* package (Dray et al., 2020). Due to the small number of Protura available

and bearing in mind that their ecology is poorly known but probably similar in various species (Galli et al., 2019 a,b) the analyses were carried out on the entire sample (for support in this regard, see Qiao et al., 2017; Smith et al., 2019).

Table 1. Cavities included in the analysis of the ecospace properties with Protura; 31 caves with and 29 caves without Protura. Cad. Nr, Cadastre number of the cavity; Presence (1)/absence (0) of Protura; N, E, coordinates; Elev., elevation of the (main) entrance [m]; Length res., length of the studied part of the cave [m]; Slope dir., direction of the slope on which the cave opens; n.r. not yet registered; * artificial cavity.

ID	Cavity	Protura	Cad. Nr.	N	E	Elev.	Length res.	Slope dir.
1	Jama pri taboru	0	367	46.28	14.24	463	42	100
2	Babja luknja	0	35	46.13	14.39	330	85	50
3	Jama 2 na Jurcetovih Percah	0	2327	46.10	14.42	408	42	140
4	Zijalka nasproti Ribče peči	0	4605	46.42	14.62	820	50	30
5	Korančevka	0	2503	46.48	14.78	1010	54	250
6	Lovrišnikova jama	0	758	46.15	14.80	480	12	190
7	Jama pri Votli peči pri Ravnah	0	3263	46.55	14.97	388	22	0
8	Skobirjeva votlica	0	3956	46.47	14.98	940	25	80
9	Mesarska lopa	0	563	46.38	14.90	1279	40	180
10	Brdajsova jama	0	3497	46.12	14.90	651	28	120
11	Lokoviška jama	0	3959	46.37	15.02	360	45	300
12	Jama v Burgi	0	1091	46.08	15.02	600	15	150
13	Zapečke peči	0	3208	46.55	15.22	610	20	180
14	Kapelarjevo brezno	0	4706	46.11	15.21	440	25	130
15	Fantovska luknja 2	0	3967	46.22	15.31	420	56	0
16	Umetni rov nad Šturmovo grabo*	0	U3	46.60	15.46	624	58	100
17	Jama v kamnolomu pri Suhem	0	4632	46.14	15.39	500	25	200
18	Glija jama	0	84	46.11	15.44	515	68	0
19	Jama 2 v Repoluskovih pečinah	0	4371	46.68	15.62	492	20	200
20	Gruska jama	0	1374	46.09	15.57	310	22	200
21	Jama pod južnim vrhom Tisnika	0	521	46.41	15.17	728	25	80
22	Železna jama	0	2678	46.14	14.64	344	86	200
23	Jernejeva jama	0	929	45.72	14.16	610	63	60
24	Mala jama v Kostanjeviški jami	0	518	45.84	15.43	170	70	150
25	Jama Kreščak	0	5849	45.85	15.64	298	170	250
26	Karbelova jama	0	10411	46.63	15.01	660	96	80
27	Krivčeva jama	0	9238	46.24	15.76	540	73	160
28	Knapovca	0	n.r.	46.63	15.55	380	23	70
29	Spodnja Stopenca	0	469	45.97	15.51	279	55	270
30	Štinetova jama	1	240	46.19	14.30	395	52	0
31	Godova jama	1	3462	46.08	14.25	528	25	220
32	Lisičja luknja	1	401	46.14	14.51	535	20	180
33	Rački pekel	1	465	46.39	14.72	590	38	200
34	Zijalka v Dovji Griči	1	376	46.30	14.67	1526	26	130
35	Boštonova jama	1	357	46.14	14.68	325	30	270
36	Ihanšica	1	46	46.12	14.65	416	56	200
37	Zamernikova jama	1	B2	46.40	14.78	857	8	180
38	Podkrajnikova zijalka	1	2697	46.27	14.78	820	28	280
39	Krapljetova jama	1	484	46.28	14.96	854	65	40
40	Jama v Lipovici	1	1182	46.16	14.89	510	58	220
41	Umetni rov v Dravogradu*	1	U1	46.58	15.02	349	25	90
42	Objekt pri Žnodru	1	B1	46.51	15.05	511	7	350
43	Rdečka jama	1	3488	46.45	15.01	858	67	10
44	Tajnshekova jama 2	1	2535	46.31	15.08	325	15	0
45	Umetni rov v Bistriškem grabnu*	1	U2	46.64	15.13	437	15	210
46	Jama Školjka	1	3311	46.41	15.17	552	44	100
47	Zgornja Steska jama	1	169	46.31	15.16	374	102	180
48	Špegličeva jama	1	3512	46.30	15.19	400	46	50

49	Ocvirkova jama v Štadlerjevem gozdu	1	5348	46.21	15.21	333	40	330
50	Jama pod Herkovimi pečmi	1	1849	46.63	15.26	538	75	90
51	Jaklova luknja	1	4636	46.48	15.28	1050	7	150
52	Lindeška jama	1	2304	46.35	15.32	525	12	220
53	Luknja pri Naceku na Planici	1	2407	46.47	15.56	735	10	180
54	Jama v kamnolomu nad Studenicami	1	252	46.30	15.62	400	32	310
55	Rov	1	1375	46.16	15.59	315	102	80
56	Jama pri Pruhi	1	4380	46.52	15.72	295	107	290
57	Pavlijeva luknja	1	3142	46.62	15.23	595	122	50
58	Ovčje peklo nad Radljami	1	3192	46.62	15.24	520	44	140
59	Umetni rov v Osku*	1	U5	46.58	15.92	262	85	0
60	Zguba jama	1	6290	45.80	14.21	561	122	270

Fig. 1. Map of studied cavities with (blue dots) and without (red dots) Protura in Slovenia. The numbers correspond to the IDs in Table 1.

The dimensionality due to seven ecological variables (Table 2) was reduced using Principal Component Analysis (PCA); all variables were transformed by z-standardization before incorporation. Using the ordination method, the multidimensionality of the ecological variables was presented in a two-dimensional ecospace, defined by the first and second ecological principal components (EPC1 and EPC2). Differences in the ecospace between sampling sections, with Protura and without Protura, were checked by subjecting EPC1 and EPC2 to a t-test. Ecospaces for the sampling sections, with and without Protura, were graphically presented with a biplot on EPC1 and EPC2 and polygons of the ecospace were presented using the Convex Hull method. The PCA was conducted in R environment in package *factoextra* (Kassambara & Mundt, 2020). The graphic projection of ecospace on the first two EPCs and the application of Convex Hulls were performed using software PAST version 4.02 (Hammer et al., 2001). For this analysis, we used data from the first two sampling sections, i.e., ~5 m inside the cave, as the majority of specimens were recorded there.

Preliminarily, we tested the presence-absence of Protura with respect to the environmental factors using Binomial Generalized Linear Model (GLM);

none of the factors had a statistically significant effect on their presence. Therefore, we checked whether the presence of Protura in the caves coincided with the main cover types in the landscape surrounding the cave. For this purpose, differences between the frequencies of habitat types with respect to the presence/absence of Protura were tested using the Chi square test. In addition, the Watson-Williams test for equal means and the Mardia-Watson-Wheeler test for equal distributions were used to check whether the presence of Protura in the cavities could be explained by orientation of the slopes on which the caves open. All three tests were performed in PAST version 4.02 (Hammer et al., 2001).

In order to investigate the general pattern of spatial distribution of Protura, the influence of the distance from the entrance, and of the distance from the surface on their abundance was examined using a GLM. Abundance was modeled using a negative binomial distribution family, the distribution-type recommended for overdispersed count data (Zuur et al., 2009), using MASS package (Ripley et al., 2020). In the models, only caves with Protura were included by pooling the data for each sampling section up to the deepest part of the caves (i.e., 24 m from the entrance and 20 m from the surface), where Protura

were recorded. Regression analyses were conducted in R environment (R CoreTeam, 2020) following the general protocol by Zuur & Ieno (2016).

Protura were putatively not attracted by the baits in pitfall traps, and displaced for short distances (speculatively ~ 0.5 m only – see also Balkenhol, 1996) during trapping in the caves. Consequently, the trapping results refer to ~ 1 m², and densities per m² were estimated using the following calculation:

$$\text{Density} = N_{\text{trap}} + 4 N_{\text{funnel}} \quad (1)$$

where N_{trap} and N_{funnel} are the number of individuals in a trap and Berlese-Tullgren sample, respectively.

The age-ratios (juveniles/adults) of identified specimens in different seasons were compared applying the Chi-square test to the numbers of juveniles (from larva I to pre-imago instar) and adults recorded each season. The statistical significance of the differences from the expected value (1) of the species sex ratio (males/females) was determined using the Chi-square test. Software PAST version 4.02 (Hammer et al., 2001) was used in both cases.

Table 2. Factor loadings from the first two ecological principal components (EPC), and eigenvalues and percentage of explained variation. Loadings > 0.60 are in bold. The acronyms of ecological variables are explained in Table 3.

Ecological variables	EPC1	EPC2
Tair	0.69	0.66
Tgr	0.63	0.65
RH	-0.53	-0.25
MC	-0.63	0.45
pHgr	0.40	-0.43
CC	0.42	-0.61
OM	-0.63	0.45
Eigenvalue	2.30	1.90
Variance %	32.90	27.10

RESULTS

Proturans were collected in 31 cavities and records of 135 individuals were eligible for statistical analyses in relation with environmental data. The descriptive statistics of the environmental conditions in sampling sections with Protura is presented in Table 3. The Mantel test revealed no spatial autocorrelation among caves with and without Protura ($r = -0.001$, $p = 0.397$).

Most of the seven ecological variables were correlated with each other. To avoid redundancy, these variables were reduced using PCA. The factor loadings and the amount of the explained variance for the first two ecological principal components (EPCs) are shown in Table 2. Increasing EPC1 is associated with an increase in air temperature and ground substrate temperature ($r > 0.5$), and with a decrease in air humidity, substrate moisture content, and organic material content ($r < -0.5$) (Table 2). Increasing EPC2 is well associated with an increase in the air temperature and ground substrate temperature ($r > 0.5$), and with the decrease in carbonate content ($r < -0.5$) (Table 2, Fig. 2).

The polygons of the ecospace based on samples with and without Protura largely overlapped, with the centroids of both groups close to each other (Fig. 2). Protura occupied most of the ecospace of the entrance areas of the 60 caves under study. They were absent only in very dry/wet sections, and very cold sections with substrates very poor or rich in organic matter. The t-test between EPCs, with and without Protura, returned a non-significant difference between the ecospace (EPC1: $t = 0.07$, $df = 106$, $p = 0.946$; EPC2: $t = 0.83$, $df = 106$, $p = 0.407$).

A Chi-square test showed no significant relation between the frequencies of Protura presence or absence and the main cover type in the landscape with the caves ($\chi^2 = 3.80$, $df = 5$, $p = 0.578$). Most caves with Protura were on the southern slopes (circular mean = 186.3°; 95% confidence 96.3°–276.3°). No significant differences were found when examining the variability in slope azimuths between the caves with and without Protura (Watson-Williams equal means U test = 1.16, $df = 57$, $p = 0.286$; a Mardia-Watson-Wheeler W test = 4.30, $df = 56$, $p = 0.117$).

Abundance of Protura decreased significantly with increasing distance from the entrance (*Estimated $\beta \pm SE$* : -0.1151 ± 0.0345 , $p = 0.001$) and from the soil surface (*Estimated $\beta \pm SE$* : -0.0855 ± 0.0286 , $p = 0.003$). The effect of both variables on the abundance of Protura is illustrated in Figure 3.

Estimated densities of Protura were very low in all the caves under study, with maximum values of 68 and 32 specimens/m² at the entrance of Ovčje peklo and Ihanšica, respectively.

Protura from 15 caves, totally 286 individuals, were examined in more detail. Identified Protura

Table 3. Descriptive statistics of the sampling sections with Protura. N, number of sampling sections; Mean, arithmetic mean; StD, standard deviation; min, minimum; max, maximum. For description of variables, see the first paragraph in the Field methods.

Variables	Acronyms	N; Mean $\pm$ StD (min–max)
Number of Protura specimens	Abundance	48; 2.5 $\pm$ 0.4 (1–17)
Distance from the entrance	DistE [m]	48; 5.4 $\pm$ 7.4 (0–24)
Distance from the surface	DistS [m]	48; 6.4 $\pm$ 4.8 (0–20)
Air temperature	Tair [°C]	43; 8.8 $\pm$ 5.1 (1.0–22.0)
Ground temperature	Tgr [°C]	48; 7.5 $\pm$ 3.7 (0.6–16.5)
Relative air humidity	RH [%]	43; 89.3 $\pm$ 11.8 (64–100)
Substrate moisture content	MC [%]	45; 21.2 $\pm$ 14.7 (0–53)
Airflow	Airflow [log cm·s ⁻¹]	48; 1.5 $\pm$ 0.7 (0–3)
Organic material content	OM [%]	34; 14.3 $\pm$ 17.9 (1.5–85.9)
pH of the ground substrate	pHgr [pH-unit]	34; 8.0 $\pm$ 0.7 (4.4–8.7)
Carbonate content	CC [%]	34; 24.9 $\pm$ 24.5 (0.0–87.0)

Fig. 2. Bivariate plot of the cave-ecospace polygons onto the first two ecological principal components (EPCs) derived from ordination of seven environmental variables. The percentage of variance explained by individual EPCs is in parentheses. The character vector diagram illustrates the relative contribution of the original variables (see Table 3 for acronyms) to each EPCs. The polygon with the Protura ecospace is dark grey, with black dots and outlined by solid line, and that without Protura is light grey, with open dots and outlined by the dotted line. Centroid identifiers: P – Protura present; A – Protura absent.

Fig. 3. Predicted relationships between the abundance of Protura and (A) distance from the entrance, and (B) distance from the surface obtained from the generalized mixed models. Black lines are predicted values, while grey areas are 95% confidence intervals.

belonged to five genera and ten species (Table 4). Seven of them were new to Slovenia: *Acerentulus rafalskii* Szeptycki, 1979, *Acerentomon affine* Bagnall, 1912, *A. balcanicum* Ionesco, 1933, *A. maius* Berlese, 1908, *Acerella muscorum* (Ionesco, 1930), *Eosentomon armatum* Stach, 1926 and *E. transitorium* Berlese, 1908. Further, an unidentified species of the genus *Ionescuellum* Tuxen, 1960 is also new to Slovenia. Among the identified Protura, 260 belong to the order Acerentomata, two belong to the family Hesperentomidae, genus *Ionescuellum*, and the others belong to the family Acerentomidae, genera *Acerentulus*, *Acerentomon* and *Acerella*. The remaining 26 specimens belong to the order Eosentomata, genus *Eosentomon*. These proturans did not show any obvious morphological differences from those of the same species collected elsewhere in Europe outside the caves.

The age ratios of juveniles to adults in spring and summer (the seasons accounting for the large majority of identified specimens), were 0.55 and 0.31 respectively, and differing at a significant level ($\chi^2 = 3.9454$, $df = 1$, $p < 0.05$). The sex ratio of the five dominant species is shown in Table 5. For three of the species (*A. confinis*, *A. maius*, and *A. meridionale*) the populations contained a greater number of females at a statistically significant level.

DISCUSSION

In this study, no differences were found in terms of the ecological characteristics between caves with and without Protura. The environmental analyses showed that Protura were mainly found close to the entrance of the cave. However, a small number of individuals were recorded deeper inside, probably having fallen or washed into the cave from surface soils through vertical fissures in the bedrock. The role

Table 4. Protura identified in 15 Slovenian caves. For cave ID, see Table 1. The following abbreviations were adopted: LI = Larva I, LII = Larva II, MJ = Maturus Junior, PI = Pre-Imago, F = Female, M = Male, Un = Unidentified, Aut = Autumn, Spr = Spring, Sum = Summer.

ID-Cave	3	28	31	32	32	35	36	37	38	40	42	45	48	49	52	59
Season	Spr	Spr	Spr	Spr	Sum	Spr	Spr	Spr	Spr	Sum	Spr	Spr	Spr	Sum	Spr	Aut
<i>Ionescuellum</i> sp.				1F				1 MJ								
<i>Acerentulus</i> sp.							1F			2M		1Un	2M 6F 2MJ 1LI			
<i>Acerentulus confinis</i>				1F	1M 2F			4M 9F 7PI 11MJ		5F		1F				
<i>Acerentulus rafalskii</i>														1M		1M
<i>Acerentomon</i> sp.	1LI 2Un		1LI	1LI	1Un		1MJ 1Un		2LII					1MJ		1LII 1LI
<i>Acerentomon doderoi</i> group									1LI							
<i>Acerentomon microrhinus</i> group					1M 2PI				1F 1LI		1M 2F					
<i>Acerentomon affine</i>						1M, 2F	18M 19F 2PI 7MJ 9LII 7LI 4Un									
<i>Acerentomon balcanicum</i>				2M 2F	15M 25F 7PI 2MJ 1LII 4Un											
<i>Acerentomon italicum</i>																1F
<i>Acerentomon maius</i>	15F 3MJ 1LI	1M														
<i>Acerentomon meridionale</i>					4M 12F 1PI 3MJ 4LII											
<i>Acerella muscorum</i>								2F							3F	1F
<i>Eosentomon</i> sp.								1M 3Un			1M 2F 1Un				1Un	
<i>Eosentomon delicatum</i> group								2M 5F								
<i>Eosentomon transitorium</i> group											1M 2F					
<i>Eosentomon armatum</i>			2M 1F 1MJ													
<i>Eosentomon transitorium</i>								1M							1M 1F	
Total	22	1	5	7	85	3	69	46	5	7	10	2	11	2	6	5

of the cave-entrance habitat, as an ecotone environment, has been discussed since the first empirical studies on cave biodiversity (e.g., Peck, 1976; Prous et al., 2004, 2015; White, 2012). In some cases, this ecotone habitat is also characterized by high endemism (e.g., Yao et al., 2016), but it was not the case with Protura in our study; specimens in the caves belonged to the same species elsewhere inhabiting soils of epigeal habitats. This result supports the previously available published data on Protura collected in caves (Vandel, 1964; Neuherz, 1974, 1975; Nosek, 1975; Novak,

2005; Szeptycki, 2007; Galli et al., 2019b; Shrubovych & Georgiev, 2020).

Density estimates recorded in the caves under study were lower than those of some hundreds/thousands individuals/m² previously known from literature for surface soil samples (Galli et al., 2019a). This is probably due to both a low amount of soil, and a limited volume of the substrate per unit area in such habitats.

The analyzed samples were too small to examine thoroughly the life cycles of single species. The overall

Table 5. Sex ratio calculated for five dominant species of Protura in Slovenian caves. M = number of males, F = number of females, sex ratio M/F, χ^2 and relative p-values for the sex ratio.

Specie	M	F	Sex ratio	χ^2 (df = 1)	p
<i>Acerentulus confinis</i>	5	18	0.28	7.35	<0.01
<i>Acerentomon affine</i>	19	21	0.90	0.05	0.823
<i>Acerentomon balcanicum</i>	17	27	0.63	1.15	0.283
<i>Acerentomon maius</i>	1	15	0.07	12.25	<0.01
<i>Acerentomon meridionale</i>	4	12	0.33	4.00	<0.05

analysis of the developmental instars of specimens evidenced the presence of juveniles in three seasons (spring, summer and autumn), indicating that in Slovenia Protura reproduce almost throughout the warm part of the year with a peak during spring. This result confirms the already investigated phenologies of some species (Galli et al., 2019a). For five dominant species it was possible to analyze the sex ratio. The results are similar to those obtained for the same species in Italy (Galli et al., 2019b): *A. confinis*, *A. maius*, and *A. meridionale* populations showed a bias towards females, while in *A. affine* and *A. balcanicum* populations showed a balanced sex ratio (for further information see also Galli et al., 2019b).

This research has increased the checklist of Protura species in Slovenia through the addition of: *Acerentulus rafalskii*, previously known only from Poland (Szeptycki, 2007); *Acerentomon affine*, widespread in Western Europe (Szeptycki, 2007); *A. balcanicum* in Southeast Europe and Ukraine (Szeptycki, 2007); *A. maius* in Central Europe and Italy (Szeptycki, 2007); *Acerella muscorum*, distributed in West and Central Europe and the Near East, and already recorded from all the countries surrounding Slovenia (Austria, Hungary, Italy, and Bosnia and Herzegovina) (Szeptycki, 2007); *Eosentomon armatum* and *E. transitorium*, both with a wide European distribution (Shrubovych & Bernard, 2018). However, Szeptycki (2007) considered the bibliographic record of *Acerella tiarnea* doubtful. The new record of *A. muscorum* mentioned above probably confirms the misidentification. Therefore, the current number of Protura species in Slovenia is 15.

Our research, the first one specifically concerning Protura in caves, confirms the absence of any morphological adaptations to such an environment in this taxon (Pass & Szucsich, 2011). Furthermore, in the collection of one of the authors (LG), some specimens of *Acerentomon doderoi*, *A. gallicum* Ionesco, 1933 and *A. maius* come from two caves in Liguria (NW-Italy), and three females of *Berberentulus travassosi* (Silvestri, 1938) come from a Minas-Gerais (Brazil) cave. Morphologically, all of them correspond to the species description based on specimens collected from surface soils (LG, unpublished data).

All the evidence shown above supports the hypothesis that Protura have entered caves passively within debris/litter by accident, mostly close to the entrance. In terms of speleobiological, ecological classification, they must be ranked among “accidentals” (sensu Barr, 1967). Eventually, in some other regions, Protura might migrate from soils or mesovoid shallow substratum (MSS) (Mammola et al., 2016) into adjacent cave portions and vice versa

over the year, but this does not seem to happen in Central Europe.

CONCLUSION

Protura is probably the least known group of hexapods, with scattered and fragmentary literature records from caves. This is the first contribution dealing exclusively with this topic, which despite the small number of specimens found provides important knowledge on proturans in caves because the research is based on over 6000 samples taken from caves in Slovenia.

Our research confirms previous observations that proturans occupy cave ecosystems through passive colonization after their accidental introduction from the surface soil of habitats near the entrance to, and above, caves. None of the identified species displayed morphological adaptations specific to a cave environment. And although the density of proturans was lower than that found in surface soils elsewhere, their presence recorded mainly near cave entrances was related with the ‘fallen-in’ organic matter onto these sites.

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data. PK took part in the field research, FJ and PK performed statistical analyses of the field data, and TN, FJ, and PK contributed to the corresponding texts.

REFERENCES

- Balkenhol, B., 1996. Activity range and dispersal of the Protura *Acerentomon nemorale* (Arthropoda: Insecta). *Pedobiologia*, 40, 212–216.
- Barr, T.C.Jr., 1967. Observations on the ecology of caves. *The American Naturalist*, 101, 475–491.
- Bole, J., Drovnik, B., Mršič, N., Sket, B., 1993. Endemic animals in hypogean habitats in Slovenia. *Naše jame*, 35, 43–55.
- Cardoso, P., Erwin, T.L., Borges, P.A.V., New, T.R., 2011. The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, 144(11), 2647–2655. <https://doi.org/10.1016/j.biocon.2011.07.024>
- Culver, D.C., Trontelj, P., Zgajmajster, M., Pipan, T., 2012. Paving the way for standardized and comparable subterranean biodiversity studies. *Subterranean Biology*, 10, 43–50. <https://doi.org/10.3897/subtbiol.10.4759>
- Culver, D.C., Deharveng, L., Bedos, A., Lewis, J.J., Madden, M., Reddel, J.L., Sket, B., Trontelj, P., White, D., 2006. The mid-latitude biodiversity ridge in terrestrial cave fauna. *Ecography*, 29, 120–128. <https://www.jstor.org/stable/3683502>
- Culver, D.C., Pipan, T., 2019. *The biology of caves and other subterranean habitats* (2nd Ed.). Oxford University Press, Oxford, 301 p.
- Dray, S., Dufour, A.-B., Thioulouse, J., 2020. Analysis of ecological data: Exploratory and euclidean methods in environmental sciences. R package Version 1.7-16. <https://CRAN.R-project.org/package=ade4>
- Ficetola, G.M., Canedoli, G., Stoch F., 2018. The Racovitza impediment and the hidden biodiversity of unexplored environments. *Conservation Biology*, 33, 214–216. <https://doi.org/10.1111/cobi.13179>
- Galli, L., Bartel, D., Capurro, M., Pass, G., Sarà, A., Shrubovych, J., Szucsich, N., 2016. Redescription and review of the most abundant conehead in Italy: *Acerentomon italicum* Nosek, 1969 (Protura: Acerentomidae). *Italian Journal of Zoology*, 83(1), 43–58. <https://doi.org/10.1080/11250003.2015.1114686>
- Galli, L., Capurro, M., Colasanto, E., Molyneux, T., Murray, A., Torti, C., Zinni, M., 2019a. A synopsis of the ecology of Protura (Arthropoda: Hexapoda). *Revue suisse de Zoologie*, 126(2), 155–164. <https://doi.org/10.5281/zenodo.3463443>
- Galli, L., Capurro, M., Molyneux, T., Torti, C., Zinni, M., 2019b. Ecology of Italian Protura. *Pedobiologia*, 73, 20–28. <https://doi.org/10.1016/j.pedobi.2019.01.004>
- Galli, L., Rellini, I., 2020. The geographic distribution of Protura (Arthropoda: Hexapoda): a review. *Biogeographia – The Journal of Integrative Biogeography*, 35, 51–69. <https://doi.org/10.21426/B635048595>
- Halikia, I., Zoumpoulakis, L., Christodoulou, E., Prattis, D., 2001. Kinetic study of the thermal decomposition of calcium carbonate by isothermal methods of analysis. *The European Journal of Mineral Processing and Environmental Protection* 1(2), 89–102. <https://doi.org/10.21426/B635048595>
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 1–9. <http://folk.uio.no/ohammer/past>
- Kassambara, A., Mundt, F., 2020. factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://CRAN.R-project.org/package=factoextra>
- Kozel, P., Pipan, T., Mammola, S., Culver, D.C., Novak, T., 2019. Distributional dynamics of a specialized subterranean community oppose the classical understanding of the preferred subterranean habitats. *Invertebrate Biology*, 138, e12254. <https://doi.org/10.1111/ivb.12254>
- Mammola, S., 2018. Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. *Ecography*, 41, 1–21. <https://doi.org/10.1111/ecog.03905>
- Mammola, S., Giachino, P.M., Piano, E., Jones, A., Barberis, M., Badino, G., Isaia, M., 2016. Ecology and sampling techniques of an understudied subterranean habitat: the Milieu Souterrain Superficiel (MSS). *Science Nature*, 103, 88. <https://doi.org/10.1007/s00114-016-1413-9>
- Mammola, S., Souza, M.F.V.R., Isaia, M., Lopes Ferreira, R., in press. Global distribution of microwhip scorpions (Arachnida: Palpigradi). *Journal of Biogeography*.
- Neuherz, H.V., 1974. Der erste Höhlenfund einer Protüre (Insecta, Apterygota) in Österreich. *Die Höhle*, 25, 25–30.
- Neuherz, H.V., 1975. Die Landfauna der Lurgrotte (Teil I). *Sitzungsberichte der österreichischen Akademie der Wissenschaften (Mathem.-naturw. Kl.)*, 183, 159–285.
- Nosek, J., 1973. *European Protura*. Genève, Muséum d'Histoire Naturelle, Geneva, 345 p.
- Nosek, J., 1975. Niches of Protura in biogeocoenoses. *Pedobiologia*, 15, 290–298.
- Nosek, J., 1977a. Adaptations in Protura. *Revue d'Écologie et de Biologie du Sol*, 14(1), 217–224.
- Nosek, J., 1977b. Proturan synusies and niche separation in the soil. *Ecological Bulletins*, 25, 138–142.
- Novak, T., 2005. Terrestrial fauna from cavities in northern and central Slovenia, and a review of systematically ecologically investigated cavities. *Acta Carsologica*, 34(1), 169–210. <https://doi.org/10.3986/ac.v34i1.285>
- Novak, T., Lipovšek Delakorda, S., Senčič, L., Pabst, M.A., Janžekovič, F., 2004a. Adaptations in phalangiid harvestmen *Gyas annulatus* and *G. titanus* to their preferred water current adjacent habitats. *Acta Oecologica*, 26, 45–53. <https://doi.org/10.1016/j.actao.2004.03.004>
- Novak, T., Sambol, J., Janžekovič, F., 2004b. Faunal dynamics in the Železna jama cave. *Acta Carsologica*, 33, 249–267. <http://carsologica.zrc-sazu.si/downloads/332/novak.pdf>
- Pass, G., Szucsich, N., 2011. 100 years of research on the Protura: many secrets still retained. *Soil Organisms*, 83(3), 309–334.
- Peck, S.B., 1976. The effect of cave entrances on the distribution of cave inhabiting terrestrial arthropods. *International Journal of Speleology*, 8, 309–321.
- Pretner, E., 1979. Podzemeljska koleopterološka fauna umetnih votlin. [Die unterirdische Käferfauna in künstlichen Hohlräumen.] *Acta Entomologica Jugoslavica*, 15(1–2), 89–102.
- Prous, X., Lopes Ferreira, R., Jacobi, C.M., 2015. The entrance as a complex ecotone in a Neotropical cave. *International Journal of Speleology*, 44(2), 177–189. <https://doi.org/10.5038/1827-806X.44.2.7>
- Prous, X., Lopes Ferreira, R., Parentoni Martins, R., 2004. Ecotone delimitation: epigean-hypogean transition in cave ecosystems. *Austral Ecology*, 29, 374–382. <https://doi.org/10.1111/j.1442-9993.2004.01373.x>

- Qiao, H., Peterson, A.T., Ji, L., Hu, J., 2017. Using data from related species to overcome spatial sampling bias and associated limitations in ecological niche modeling. *Methods in Ecology and Evolution*, 8(12), 1804–1812. <https://doi.org/10.1111/2041-210X.12832>
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Ripley, B., Venables, B., Bates, D. M., Hornik, K., Gebhardt, A., Firth, D., 2020. Support functions and datasets for Venables and Ripley's MASS. R package Version 7.3-53. <https://cran.r-project.org/package>
- Sendra, A., Palero, F., Jiménez-Valverde, A., Reboleira, A.S.P.S., 2020. Diplura in caves: diversity, ecology, evolution and biogeography. *Zoological Journal of the Linnean Society*, 20, 1–15. <https://doi.org/10.1093/zoolinnean/zlaa116>
- Sheppard, S.C., Addison, J.A., 2008. Soil Sample Handling and Storage. In: Carter, M.R., Gregorich, E.G. (Eds.), *Soil sampling and methods of analysis* (2nd Ed.), Canadian Society of Soil Science, Taylor & Francis, Boca Raton, p. 39–49.
- Shrubovych, J., Bernard, E., 2018. A key for the determination of European species of *Eosentomon* Berlese, 1909 (Protura, Eosentomata, Eosentomidae). *ZooKeys*, 742, 1–12. <https://doi.org/10.3897/zookeys.742.22664>
- Shrubovych, J., Georgiev, D., 2020. Protura records in Sarnena Gora Mountains. In Georgiev, D., Bechev, D., Yancheva, V. (Eds.), *Fauna of Sarnena Sredna Gora Mts*, Part 1. Zoonotes, Suppl. 9, 43–46.
- Smith, A.B., Godsoe, W., Rodríguez-Sánchez, F., Wang, H.H., Warren, D., 2019. Niche estimation above and below the species level. *Trends in Ecology & Evolution*, 34(3), 260–273. <https://doi.org/10.1016/j.tree.2018.10.012>
- Szeptycki, A., 2007. Catalogue of the world Protura. *Acta Zoologica Cracoviensia*, 50B(1), 1–210.
- Tuxen, S.L., 1978. Protura (Insecta) and Brazil during 400 million years of continental drift. *Studies on Neotropical Fauna and Environment*, 13(1), 23–50.
- Vandel, A., 1964. *Biospéologie. La biologie des animaux cavernicoles*. Guthier-Villars, Paris, 619 p.
- Yao, Z., Dong, T., Zheng, G., Fu, J., Li, S., 2016. High endemism at cave entrances: a case study of spiders of the genus *Uthina*. *Scientific Reports*, 6, 35757. <https://doi.org/10.1038/srep35757>
- White, W.B., 2012. Entrances. In: White, W.B., Culver, D.C. (Eds.), *Encyclopedia of caves* (2nd Ed.). Elsevier, Amsterdam, p. 280–284.
- Zuur, A.F., Ieno, E.N., Walkerm, N.J., Savaliev, A.A., Smith, G.M., 2009. *Mixed effect models and extensions in ecology with R*. Springer, New York, 574 p.
- Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type analyses. *Methods in Ecology and Evolution*, 7(6), 636–645. <https://doi.org/10.1111/2041-210X.12577>