COMPARATIVE STUDY ON THE PERFORMANCE OF SAMPLING METHODS FOR MYRIAPODA (DIPLOPODA, CHILOPODA, SYMPHYLA) AND ONISCIDEA FROM BUZĂU MOUNTAINS

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Abstract. In this paper we focus on some edaphic macroinvertebrates, Oniscidea, Diplopoda, Chilopoda and Symphyla, and their activity-density in relation with a short-term sampling protocol and a series of environmental factors. Our case study targeted the invertebrate community from Ivăneţu Massif near the Rupestral Assembly of Bozioru Mountains and tested the performance of direct sampling using tweezers, sifting leaf-litter with the Winkler sieve, extracting soil samples and using pitfall traps. In order to achieve a more realistic understanding of oniscidean and myriapod diversity patterns we recommend a combination of the abovementioned sampling methods.

Keywords: Oniscidea, Myriapoda, Sampling methods, Buzău Mountains, Romania.

1. INTRODUCTION

There are relatively few areas in Romania where the species richness and activity-density are thoroughly investigated as most of the territory is only fragmentarily and disproportionately known due to a rather random, haphazard way sampling of Oniscidea and Myriapoda as we pointed in previous studies (BABA ET AL., 2019; GIURGINCA, 2021; GIURGINCA, 2022).

Buzău Mountains are one of the understudied geographic areas from a faunal point of view, underscoring the need to establish a feasible sampling plan for short-term (but also long-term) studies that efficiently covers these groups of soil arthropods that includes booth predatory and detritivore species. As it often happens in many studies, it is more feasible to use sampling methods that cover as many arthropodal groups as possible and not use individual techniques for each specific group.

Previous studies on areas located near our study sites (targeting Myriapoda and Oniscidea) were carried out by NITZU ET AL. 2002 on the salt karst in the Meledic area, located at approximately 16 km to the northeast of the Ivăneţu

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Massif. Four chilopod species were thus identified: *Lithobius forficatus* (Linnaeus, 1758), *Lithobius parietum* Verhoeff, 1899, *Pachymerium antipai* Căpușe, 1968 and *Cryptops anomalans* Newport, 1944, one of Diplopoda – *Megaphyllum bosniense* (Verhoeff, 1897), respectively four of Oniscidea – *Hyloniscus riparius* (C.L. Koch, 1838), *Trachelipus nodulosus* (C.L. Koch, 1838), *Porcelio laevis* Latreille, 1804 and *Armadillidium vulgare* Latreille, 1804 (NITZU ET AL., 2002).

More information on the Chilopoda fauna of the Buzău Mountains predates 1967 and refers to 7 species, but the sampling was unsystematic leading to the capture of only some surface dwelling centipedes: *Lithobius crassipes* L. Koch, 1862 (Văzăunea Cave 1 from Peşterii Valley in Buzăului Mountains) (NEGREA, 1963; NEGREA, 1966); *Lithobius erythrocephalus* C.L. Koch, 1847 (beech forest in Răstoaca – Nehoiului Valley; beech forest from Casoca Forest – Buzău Valley; under the stones near the Siriu Lake); *Lithobius forficatus* (same sampling locations as *L. erythrocephalus*); *Lithobius schuleri* Verhoeff, 1925 (under the stones near the Siriu Lake); *Lithobius tenebrosus* Meinert, 1872 (beech forest in Răstoaca – Nehoiului Valley) Meinert, 1872, *Cryptops hortensis* (Donovan, 1810) (beech forest in Răstoaca – Nehoiului Valley) (MATIC & NEGREA, 1967) and *Cryptops parisi* Brolemann, 1920 (Văzăunea Cave 1 from Peşterii Valley in Buzăului Mountains) (NEGREA, 1963; NEGREA, 1966).

To highlight the importance of using a broad spectrum of sampling methods in accurately determining the specific richness and to evaluate the activity-density of abovementioned soil invertebrates in a particular area, it is necessary to refer to the adaptations of these species to the environment and in some cases to their vertical distribution. Geophilomorphs for example are mainly soil dwellers and prefer to feed on slower, but larger organisms such as lumbricids and Diptera larvae being linked with bacterial energy channel, on the other side species inhabiting mainly the surface of the soil, especially lithobiomorphs, feed on smaller and more active invertebrates such as springtails being linked with fungal energy channel (VOIGTLÄNDER, 2011; POTAPOV, 2023).

2. CONTEXT

There are only a few studies concerning the whole community on Oniscidea, Diplopoda and Symphyla. The first study is MATIC & CSENTERI, 1983 in Eastern Carpathians recording 17 species of millipedes. It was followed by GAVA, 2004 detailing 23 species of millipedes and 9 species of Symphyla from 3 forests (Făget, Zăvoi and Trivale) in the Pitești area. In 2014 NITZU ET AL. investigates the importance of scree habitats for one species of Oniscidea and 9 species of Diplopoda form Piatra Craiului Mountains. In comparison, the centipede communities have been the subject of relatively more numerous studies allowing us to build a context.

However, it is difficult to compare and establish accurately the composition of centipede community in various areas when different singular sampling methodologies were used. A significant bias is given also by the ability of the researcher to sample especially accounting the direct sampling and singling. Nonetheless we will try to comprise all the systematic studies made in Romania using at least one sampling method and capturing a minimum of 3 seasons in one year span on certain areas.

The first to address the centipede community as a whole in addition to faunistic inventories or taxonomic investigations was ZACHIU MATIC. He sampled a large amount of biologic material (1233 individuals) from steppic xerothermic habitats located in southern Transylvania using pitfall traps recording 10 species (6 lithobiomorphs, 2 scolopendromorphs and 2 geophilomorphs) (MATIC ET AL., 1979). Subsequently a forest with strong anthropic impact from northern Transylvania was investigated using only Barber traps in a two-year interval and 13 species were found (9 lithobiomorphs, 1 scolopendromorph and 3 geophilomorphs) (MATIC & HODOROGA, 1985). Subsequently, in the same area, MATIC & COLLABORATORS (1996) took in consideration multiple forest habitats using the same sampling technique finding of 11 species (6 lithobiomorphs, 1 scolopendromorph and 4 geophilomorphs). Călimani Mountains were also targeted by MATIC & CSENTERI, 1983 focusing on both millipedes and centipedes (14 lithobiomorphs, 2 scolopendromorphs and 6 geophilomorphs) and their altitudinal zonation, unfortunately the paper lacks relevant information about the sampling interval and methods used. Another study from MATIC & STUGREN, 1984, on a spruce-beech forest from Bihor Mountains accounted for only 7 species (3 lithobiomorphs, 1 scolopendromorph and 3 geophilomorphs) although the investigation included 3 seasons but used only direct sampling from soil and leaf litter.

Analyzing some historical data from ŞTEFAN NEGREA, a researcher that focused his activity mainly on subterranean habitats, we can find some interesting data, for example centipedes from South-Eastern Dobrogea in karstic areas were sampled mostly using pitfall traps (some of them being placed in drillings) uncovering 8 species of Chilopoda (5 lithobiomorphs, 2 scolopendromorphs and 1 scutigeromorph) (NEGREA, 2004).

An ample, long-term (3 years) ecological study on myriapods in three types of deciduous forests in the middle part of the Argeș Valley was conducted by GAVA, 2004. He analyzed no less than 23 species most of them geophilomorphs – 12 species, 8 lithobiomorphs and 3 scolopendromorphs, within approximately 3408 individuals. The author extracted fauna using the Tullgren funnel from leaf litter and soil.

Another comprehensive study on centipedes using multiple sampling techniques resulted in finding 19 valid species (13 lithobiomorphs, 1 scolopendromorph and 5 geophilomorphs) from Cloşani karstic area in a two-year interval (one of the Romanian biodiversity hotspots), this study included both forest and subterranean habitats (ILIE, 2003). The same author used multiple methods to investigate the Chilopoda fauna from Anina Mountains, 23 valid species (14 lithobiomorphs, 5 scolopendromorphs, 2 geophilomorphs and 1 scutigeromorph) being found in two years (ILIE, 2003).

We emphasize that the Banat Mountains, Banat Hills and the karstic regions from the south-western part of Southern Carpathians is a sampling hotspot due to the efforts of STEFAN NEGREA and VICTORIA ILIE with sampling on multiple levels (edaphic, mesovoid shallow substratum and cave systems) and as shown above with multiple types of sampling methods (BABA ET AL., 2019).

In the last two decades this type of studies were carried out even in the urban environment where ION, 2009 sampled individuals from 10 species (5 lithobiomorphs, 1 scolopendromorph and 4 geophilomorphs) directly with tweezers from 3 urban parks. The same number of centipede species (4 lithobiomorphs, 2 scolopendromorphs, 3 geophilomorphs and 1 scutigeromorph) plus 17 species of oniscids and 6 of millipedes were found by GIURGINCA ET AL., 2017 with focus on other urban parks from Bucharest. Direct sampling with tweezers was the sole method used to collect centipedes from a plain forest in the eastern part of the Romanian Plain resulting in 16 species of centipedes (8 lithobiomorphs, 1 scolopendromorph and 7 geophilomorphs), 11 species of Oniscidea and 6 species of millipedes (GIURGINCA & BABA, 2016). The same authors assessed the Myriapoda fauna of Leaota Mountains using pitfall traps with evidence for 7 species of centipedes (4 lithobiomorphs, 1 scolopendromorph and 2 geophilomorphs) 7 species of Oniscidea and 14 species of millipedes (GIURGINCA ET AL., 2015; DOROBĂȚ ET AL., 2017, 2019).

The most recent study on centipedes, targeted Buila-Vânturarița National Park where 12 species of centipedes (8 lithobiomorphs, 2 scolopendromorph and 2 geophilomorphs) were inventoried within 4 habitat types (ION & MURARIU, 2021).

Reviewing the information about the short and long-term sampling allows us to draw several fundamental conclusions regarding sampling deficiencies and the centipede communities from Romania:

– Lithobiomorphs were by large the main order sampled in the case of centipedes but their dominance in these communities is not accurately reflected and it is most probably exaggerated as they are easier to sample and more active in the superficial soil stratum, in the leaf litter and in natural shelters thus being preferentially targeted by most of the sampling techniques;

– Geophilomorphs, on the other side, are obviously undersampled with most of the frequently collected species being large individuals capable of breaching the higher layers of soil and being active on its surface in search for better hunting grounds like *Strigamia* species and *Clinopodes flavidus*, especially when they become adults (the examples occur continuously in the abovementioned studies);

– The proportion of geophilomorphs increased considerably when soil samples were included in the survey and proven actually to be the dominant group on certain sections of soil as GAVA, 2004 emphasized for the durmast oak forests with a remarkable abundance for *Clinopodes flavidus* and *Geophilus flavus*;

– *Lithobius burzenlandicus* was found in high numbers in leaf litter in medium to high elevation localities and we can assume it is the most abundant centipede in the Carpathians; populations decrease at lower altitudes, but it is usually still present.

– Scolopendromorphs can be divided in two categories in Romania: large scarce cryptopids (*Cryptops anomalans* and *Cryptops croaticus*), with some small populations at lower elevation mainly in the southern part of the country and the small abundant cryptopid *Cryptops hortensis*, sampled in huge numbers especially in beech forests, mixed frequently with a few individuals of *Cryptops parisi,* their populations decreasing considerably at lower altitudes.

In Europe the most relevant study using different sampling methods has identified pitfall trapping and extraction of soil samples as best suitable for centipedes in long-term studies on flood plain forests in Czech Republic (TUF, 2015). The same study showed that litter sifting and hand collecting is more suitable for short-term studies, whereas in Slovenian aged beech Dinaric forests pitfall traps alone recorded an impressive 37 species of centipedes in one year of sampling (GRGIČ & KOS, 2009). Other examples include: 13 species of centipedes using only pitfall traps during one year of sampling from inland dunes in eastern Flanders (LOCK & DECONICK, 2001), a two year survey that used also multiple sampling techniques resulted in 22 species of centipedes in beech forests of Ojców National Park in Poland Carpathians (LEŚNIEWSKA ET AL., 2011) and another study concerning this time the western part of the Carpathians (Central Slovakia) lead to the finding of 20 species of centipedes performing only extraction from leaf litter (JABIN ET AL., 2004).

3. MATERIAL AND METHODS

The sampling site is located in the Buzău Land Geopark (Fig.1), where two Romanian eco-regions (the Subcarpathians Bending area and the Eastern Carpathians) overlap. The substratum is represented by sandstone (soft loose and easily detachable) and the dominant type of soil is the brown acidic soils.

Sampling took place in 2017 covering 3 periods (April – May, July – August, September – October) in a perimeter near several artificial caves, namely: Aluniş, Fundătura, Fundul Peșterii, Schitul lui Iosif and Agatonul Nou.

The sampling methods included:

Direct sampling using tweezers – natural shelters (interface between stones and the soil surface or inside and under the decaying tree trunks) were checked; the time allocated to this activity was approximately 30 minutes for each station at each visit.

Sifting leaf-litter with the Winkler sieve wire mesh (0.25 mm^2 apertures) continued by a cone of textile material was used. At each station, 3 samples were taken (a total of 15 at each collection). Samples were sorted approximately 20 minutes immediately after sieving.

Soil samples – using a metal soil extractor, five soil samples from each station were taken at each visit. The soil was sampled at a depth of 10 cm, together with the litter above, on an area of 1/16 m2. After sampling, the soil was collected in plastic bags and subsequently sorted in the laboratory in the first two days after sampling. The time allocated for sorting each soil sample was 30 minutes.

Pitfall traps (Barber traps with 90% ethanol as preserving liquid) – for each site a 25 m² perimeter was established and 5 Barber traps were randomly placed. The Barber traps functioned seasonally (vernal-aestival-autumnal). The plastic containers were retrieved approximately after 30 days.

Regarding the centipedes we tried to adapt the same sampling techniques as Tuf, 2015 which established the main ecological groups of centipedes: larger abundant lithobiomorphs, larger scarcer lithobiomorphs, smaller soil lithobiomorphs, abundant large geophilomorphs and scarcer geophilomorphs.

Sampling sites – type of habitats and vegetation composition and cover (Giurginca et al., 2020):

S1. Aluniş (station 1) (45°24'33.72"N; 26°24'51.60"E) is situated at an altitude of 647.77 m a.s.l. and the sampling took place in a habitat type corresponding to 9130 Asperulo-Fagetum beech forests. The tree layer is represented by *Fagus sylvatica*, few *Carpinus betulus* and *Pinus sylvestris*. The grass layer is reduced. It has strong anthropic influences (domestic animals, organic and inorganic waste) and the pitfall traps were occasionally disturbed by landslides and storms.

S2. Fundul Peşterii (station 2) (45°25'23.68"N; 26°26'19.54"E) is situated at an altitude of 716 m a.s.l. at the limit of the forest. The habitat type corresponds to 9130 Asperulo-Fagetum beech forests having a diverse tree layer with species like *Fagus sylvatica*, *Carpinus betulus*, *Quercus petraea*, *Acer pseudoplatanus*. The shrub layer is also well represented and has variable coverage: *Crataegus monogyna*, *Sorbus aucuparia*, *Rosa canina*, *Corylus avellana*. The grass layer is dominated by species of *Galium odoratum*, *Stellaria holostea*, *Carex pilosa*, *Dentaria bulbifera*.

S4. Schitul lui Iosif (station 3) (45°25'28.88"N; 26°26'20.76"E) is situated at an altitude of 823.5 m a.s.l. The habitat type corresponds to 91V0 Dacian Beech forests (Symphyto-Fagion). The tree layer includes species of *Fagus sylvatica*, *Picea abies* and *Abies alba*. The grass layer is typical to Pulmonario rubrae-Fagetum sylvaticae association and intertwines with patches of moss with species of *Eurynchium striatum*, *Amblystegium serpens*, *Dicranum scoparium*, *Polytrichastrum* sp. The floral composition is completed by: *Acer pseudoplatanus*, *Acer platanoides*, *Sorbus aucuparia*, *Saxifraga cuneifolia*, *Pulmonaria rubra*, *Dentaria* spp.

S5. Agatonul Nou (station 4) (45°25'46.43"N; 26°26'41.41"E) is situated at an altitude of 960.1 m a.s.l. in a mixed forest of beech and spruce, habitat type corresponding to 91V0 Dacian Beech forests (Symphyto-Fagion). The tree layer has three dominant species: *Fagus sylvatica*; *Picea abies* and *Pinus sylvestris*. The grass layer is reduced, the ground being covered mostly by a consistent layer of relatively undecomposed plant material with an abundance of conifer leaves and under it a superficial stratum of soil consisting of a mix of clay and sand.

Schitul Fundătura (station 5) (45°25'33.97"N; 26°26'55.89"E) is situated at an altitude of 715.88 m a.s.l., the floral composition indicates its placement at the interference area between two habitats: 91V0 Dacian Beech forests (Symphyto-Fagion) and 9130 Asperulo-Fagetum beech forests. The tree and the shrub layer diversity are high compared to the other sites. The following tree species are dominant: *Acer platanoides*, *Acer pseudoplatanus*, *Fagus sylvatica*, *Alnus glutinosa*. Beside the younger specimens of the canopy trees, the shrub layer consists mainly of *Corylus avellana* and *Crataegus monogyna*. The grass layer is dense (80 – 100% coverage) and composed of *Urtica dioica*, *Paris quadrifolia*, *Geranium phaeum*, *Stachys sylvatica*, *Sanicula europaea*, *Alliaria officinalis*, *Dentaria bulbifera*, *Dryopteris* spp. *glutinosa*, *Salix alba*, *Cornus sanguinea*, *Sambucus nigra* and *Crategus monogyna*. Unmanaged grass was also present.

Microclimate monitoring

In order to assess the correlation between the edaphic fauna and the environmental parameters, temperature (T°C) and relative humidity (Rh%) was recorded punctually for each site at soil level with a TROTEC BC21 Thermohydrometer at each visit.

Data analysis

We examined differences across sampling techniques and sites using non-metric multidimensional scaling (NMDS) with the Bray-Curtis (B-C) distance index. The NMDS was conducted using the R package “vegan” (OKSANEN ET AL. 2016). First the data matrix of species abundance was standardized using the “total” method (i.e., the abundance was divided by the marginal total) with the function “decostand” within vegan package. The environmental predictors were standardized to have mean zero and unit variance. We ran NMDS using 500 random starts and tested the goodness of fit of the data using the R^2 value and examining the Shepard plot (i.e., the scatter around the regression of the distances between each pair of communities against their original dissimilarities). Significance of differences of communities among techniques and sites was assessed with overall PERMANOVA based on B-C dissimilarities with the function “adonis” within vegan package and pairwise using the function pairwise.perm.manova within RVAideMemoire package (HERVÉ, 2017).

To study responses of the species community to environmental factors we used “bioenv” function, within the extension of “vegan” package, to find the best set of environmental variables and subset of species (i.e., the environmental variables and species, respectively, with the maximum correlation (rank) with community dissimilarities) and then we plotted the best subset of environmental variables as vectors along with the bet subset of species on the NMDS biplots.

We used linear mixed models (LMMs) to test whether the main community feature, i.e., the observed species richness (Sobs) was related to: (i) the technique, (ii) environmental variables and (iii) a combination of technique and environmental variables. In the LMMs the technique and the environmental variables were introduced as fixed effects and sites as random effects. We assessed the relative performance of the models using the selection technique based on Akaike’s information criterion corrected for sample size (AICc: BURNHAM & ANDERSON, 2002; JOHNSON & OMLAND, 2004). We ranked the models and the model with the lowest AICc was used as the reference for calculating the AIC difference (Δi) and the likelihood of a model given the data and model weights (wi). Models within two AIC units of the AICmin were considered competitive and more plausible than others (BURNHAM & ANDERSON, 2002).

To test the performance of the sampling techniques we examined differences using the LMMs for: (1) sampling efficiency by effort, calculated as the ratio between the Sobs and the total number of samples per technique; (2) sampling efficiency by time expended, calculated by dividing the Sobs by the number of minutes expended to apply each sampling technique; and (3) completeness, calculated by dividing Sobs by the value of the most parsimonious species richness estimator. To select the most parsimonious species richness estimator we first calculated four estimators: Chao, Jackknife 1, Jackknife 2 and bootstrap using the function "specpool" within "vegan" package and next we choose the estimator showing the most asymptotic behavior (i.e., the species accumulation curve increases as most species are detected and approaches an asymptote as rarer species are detected) and smallest standard errors. In the LMMs the sampling technique was used as a fixed effect and site as random effect. The completeness was also examined by visual inspection of Mao Tau species accumulation curves of each sampling technique and of pooled data of all techniques. Finally, to identify any taxonomic group-specific pattern across techniques we examined the extent to which each technique detected rare species, i.e., singletons and doubletons. All analyses were performed using R version 3.2.1.

4. RESULTS AND DISCUSSIONS

The results revealed a significant biodiversity: 7 species of Oniscidea, 19 species of Diplopoda, 23 species of Chilopoda, 2 species of Symphyla (Table 1). Additionally, 13 species of Opiliones, 45 species of Araneae, 57 species of Collembola (POPA ET AL., 2018) and 93 species of Coleoptera have been recorded (NITZU ET AL., 2018).

Among the seven species of Oniscidea (141 individuals), *Trichoniscus carpathicus* Tabacaru, 1974 and *Cylisticus brachyurus* Radu, 1951 are endemic, rare species in Romania; both species being recorded for the first time in Buzău Mountains (GIURGINCA ET AL., 2020).

Concerning the 19 species of Diplopoda (533 individuals), seven species are also recorded for the first time in Buzău Mountains: *Propolyxenus trivittatus* (Verhoeff, 1941), *Craspedosoma transsylvanicum* Verhoeff, 1897, *Allopodoiulus verhoeffi* (Jawlovwsky, 1931) *Xestoiulus laeticollis* (Porat, 1889), *Haplophyllum mehelyi* (Verhoeff, 1897), *Ommatoiulus sabulosus* (Linneus, 1758) and *Trachysphaera costata* (Waga, 1857) (GIURGINCA ET AL., 2020).

From 22 species of Chilopoda (459 individuals) 8 species belonging to order Lithobiomorpha (337 individuals), *Lithobius burzenlandicus burzenlandicus* Verhoeff, 1931 (193 individuals) a subspecies mainly known from the Carpathian Mountains and adjacent areas, being the most common – 42.04%. Order Geophilomorpha is represented by 14 species (76 individuals). *Geophilus flavus* (De Geer, 1778) with 29 individuals (6.31%) being the most frequently identified species. We collected only one species from Order Scolopendromorpha – *Cryptops hortensis*, it is also the only one that occasionally takes shelter in the artificial caves.

There are two species of Symphyla with a total of 15 sampled individuals: *Scutigerella orghidani* Juberthie-Jupeau & Tabacaru, 1968 and *Hanseniella nivea* (Scopoli, 1763). Both species are recorded for the first time in Buzău Mountains (GIURGINCA AND BABA, 2017; GIURGINCA ET AL., 2020).

The highest species richness was obtained by combining direct sampling using tweezers with either soil samples or Winkler sieving (45 species), similar results were obtained also by pairing soils samples with Winkler sieving (44 species). The use of pitfall traps alone proved deficient for this groups of arthropods with only 22 species captured (Table 2).

4.1. STATISTICAL ANALYSIS

The Shepard plot showed that original dissimilarities are well preserved in the reduced number of dimensions (Fig. 2). The NMDS analysis reported low stress (R^2= 0.956, stress = 0.151) and showed high clustering of data points by sampling techniques and sites (Fig. 3). PERMANOVA results were significant across all techniques (R = 0.096, P = 0.004) and sites (R = 0.111, P = 0.001). Pairwise comparisons of species communities identified significant differences between all pair of techniques, except between PF and TZ (Table 3). Both Barber traps (PF) and tweezers (TZ) target in particular arthropods active at the soil surface where euedaphic and hemiedaphic species dominate, especially lithobiomorphs and scolopendromorphs in the case of centipedes.

Significant differences of the species communities were found also between all pair of sites except between site 1 and site 2, site 1 and site 3, site 2 and site 3 and site 2 and site 4 (Table 3). This indicates a relative similarity between the sampling sites with minor differences largely due to the specific composition and degree of vegetation cover.

To determine to which extent the species community differences are determined by extrinsic factors we included environmental variables in the NMDS analysis. The best combination of environmental variables included only Rhground (Table 4). Yet the Rhground was not significantly correlated with any of the two principal axes (R^2= 0.015, P = 0.572). These data endorse that the most important abiotic factor that influences the distribution of Oniscidea and myriapods is represented by the degree of relative humidity (VOIGTLÄNDER, 2011).

The best combination of species included 15 species (Table 5) underlying the main composition of the community. Two species: *Lithobius burzenlandicus*

(Lithburz) (R^2= 0.285, P = 0.001) and *Cylindroiulus boleti* (Cylibole) (R^2= 0.107, P = 0.014) were highly correlated with the second axis and *Pachyiulus hungaricus* (Pachhung) was associated with Rhground (Fig. 4) this species population being particularly favored by a high relative humidity. Based on Sobs, the model selection using AICc indicated that two models were more plausible (Table 6). Both models included the sampling technique, while de second-best model included also, Rhground and Thground (Table 6).

The LMMs examining the performance of techniques showed a significant effect of the sampling technique on the efficiency by effort (F [3,78] = 16.890, P < 0.001) and time (F [3,78] = 16.501, P < 0.001) but not for completeness (F [3,78] = 2.094, P = 0.108). The most effective sampling technique both by effort and time was Winker (Fig. 5 and Fig. 6). The most parsimonious species richness estimator for all sampling techniques was found the bootstrap estimator (Table 7 and Fig. 7).

None of the species accumulation curves of each sampling technique or of pooled data for all techniques approached an asymptote (Fig. 8), indicating that more samples are required to detect all the species theoretically expected. Each technique detected several different rare species: PF, two singletons (Harpradu, Lithmuta) and two doubletons (Lithforf, Lithburz); SS, four singletons (Lithforf, Lithluci, Clinrodn, Geopelec) and two doubletons (Litheryt, Lithmuti); TZ, three singletons (Lithluci, Heniilly, Geopelec) and two doubletons (Litheryt, Clinflav) and WK, three singletons (Litheryt, Lithluci, Clinflav) and on doubleton (Heniilly).

5. CONCLUSIONS

Little is known about the efficiency of the sampling techniques used to estimate the diversity patterns of oniscids and myriapods and how environmental factors influence those patterns.

In this study we tested the performance of four techniques (direct collection using tweezers, sifting leaf-litter with the Winkler sieve, soil samples and Barber traps) to evaluate differences in Oniscidea and myriapod community composition and structure and the effects of environmental factors within the Buzau Mountains, in four sites: Fundătura, Fundul Peșterii, Schitul lui Iosif and Agatonul Nou. The results revealed a rich species diversity. Significant differences of myriapod community composition and structure among sampling techniques, except between pitfall traps and direct collection have been found. The environmental factors significantly influencing the oniscids and myriapod community were the interactive effect of altitude and temperature at the ground level followed by the relative humidity at the ground level. Concerning the performance of sampling techniques, we found a significant effect of the sampling technique on the efficiency by effort and time but not for completeness. The most effective sampling technique both by effort and time was Winkler sieving. Thus, for a full inventory of oniscids and myriapod species and to understand the diversity patterns and how environmental factors contribute to patterns in myriapod diversity of Buzău Mountains we recommend a combination of multiple sampling techniques.

The species richness and the interplay among the different ecological groups of Myriapoda and Oniscidea and their interconnectedness with the environmental factors are most efficiently investigated through several collecting methods.

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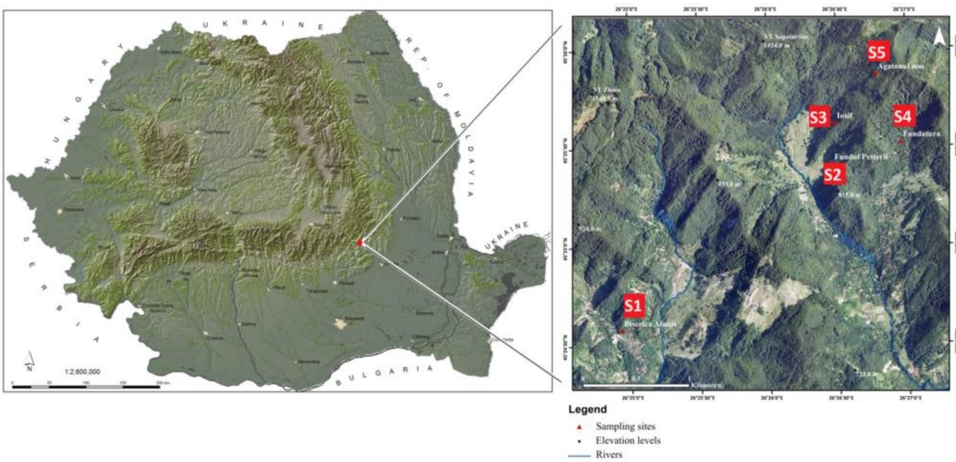

Fig. 1. Map of Romania and the placement of the sampling sites in the Bozioru Mountains.

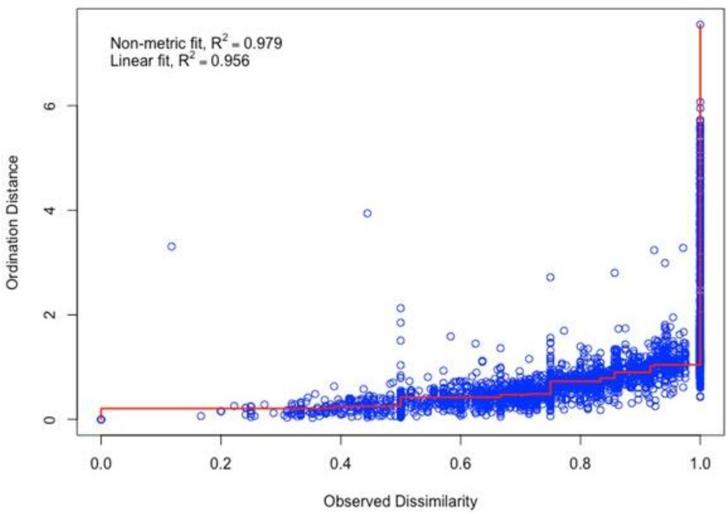

Fig. 2. Shepard plot, the scatter around the regression of the distances between each pair of communities against their observed dissimilarities.

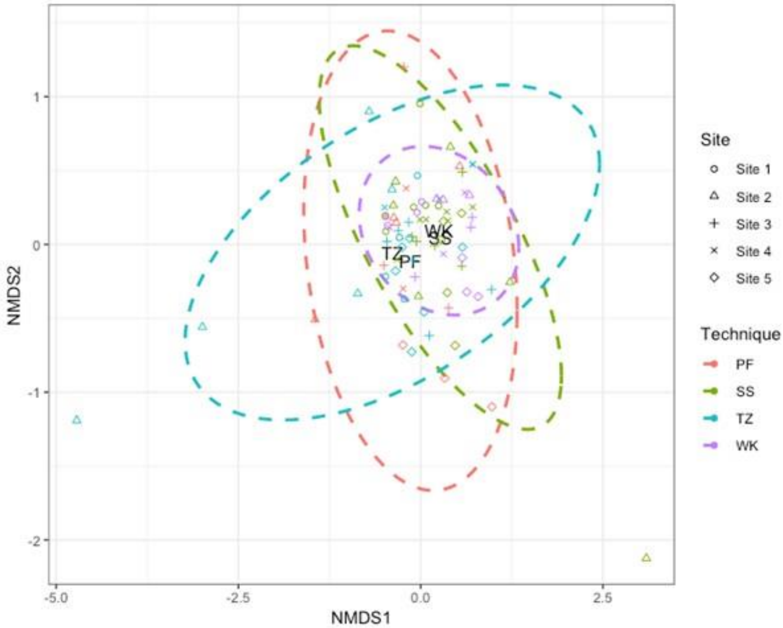

Fig. 3. Non-metric multidimensional scaling (NMDS) plot of samples compositions index for the five sites and grouped by technique: PF – pitfall trap; SS – soil sample, TZ – tweezers and WK – Winkler.

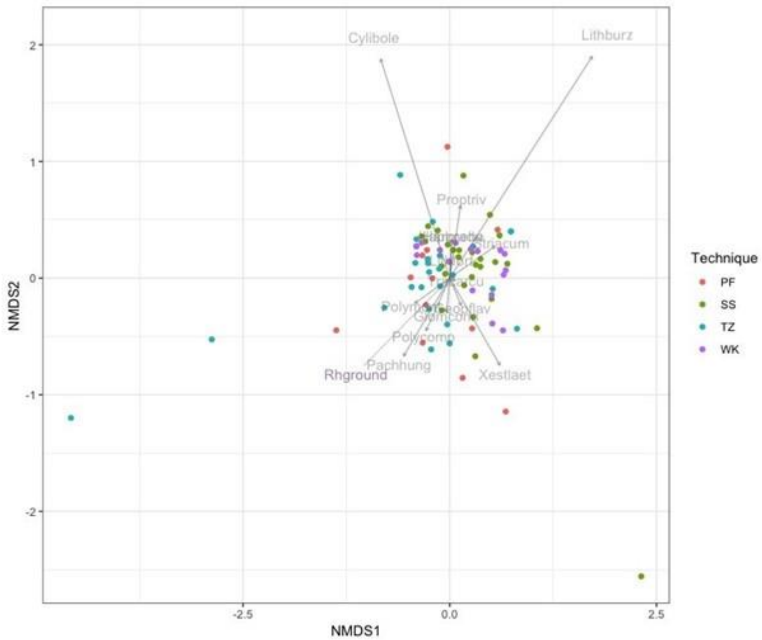

Fig. 4. Non-metric multidimensional scaling (NMDS) plot of species composition and the environmental variable, the relative humidity at ground level (Rhground), judged significant in Table 4 and 5, grouped by technique: PF – pitfall trap; SS – sample soil, TZ – tweezers and WK – Winkler.

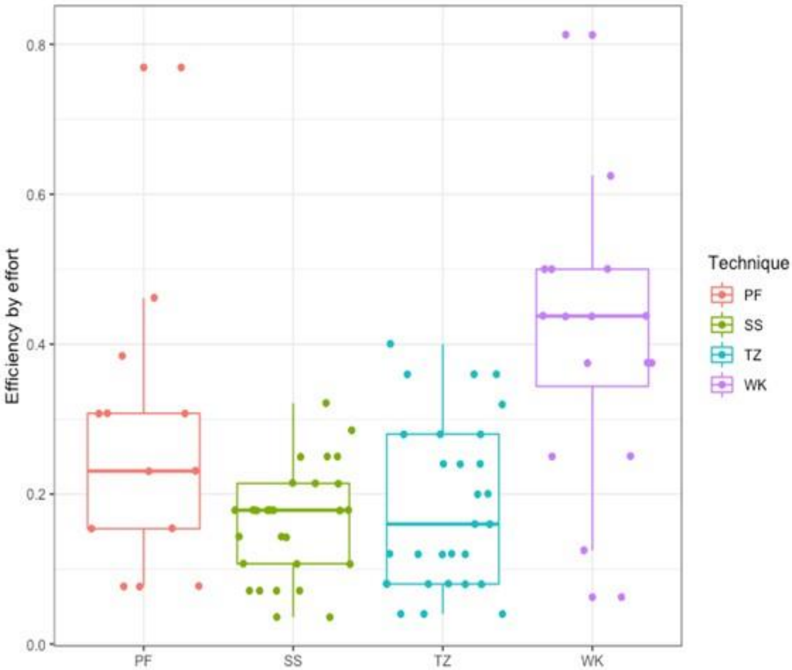

Fig. 5. Mean efficiency by effort values per sampling technique: PF – pitfall trap; SS – soil sample, TZ – tweezers and WK – Winkler.

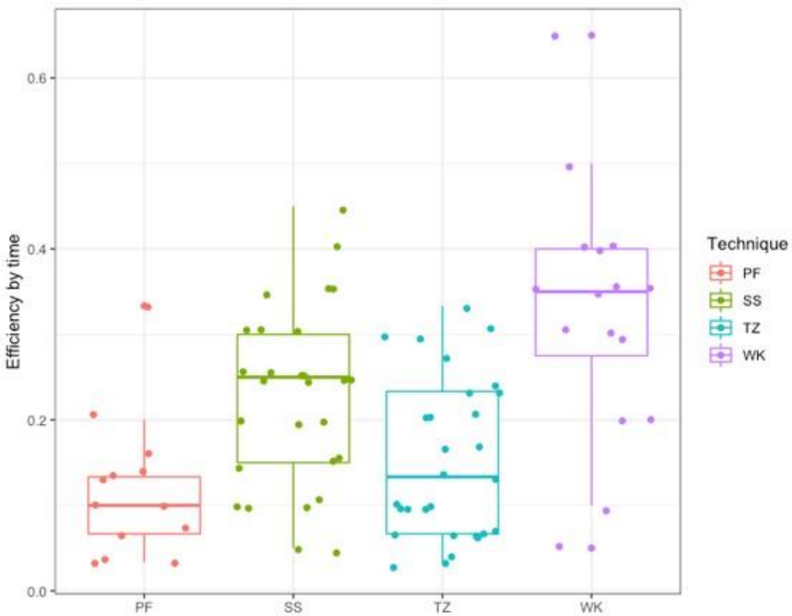

Fig. 6. Mean efficiency by time values per sampling technique: PF - pitfall trap; SS – soil sample, TZ – tweezers and WK – Winkler.

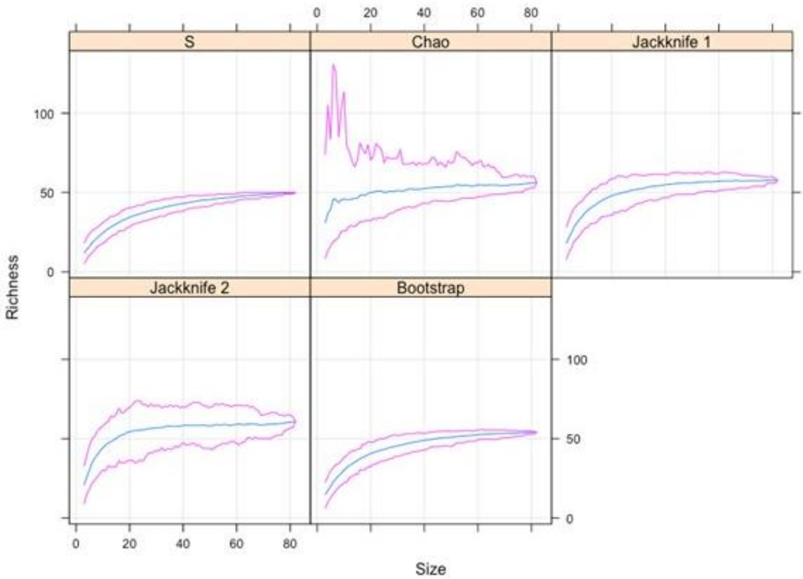

Fig. 7. Mao Tau species accumulation curves per sampling technique: pitfall trap – purple, soil sample – green, tweezers – blue, Winkler – red and for all techniques – grey.

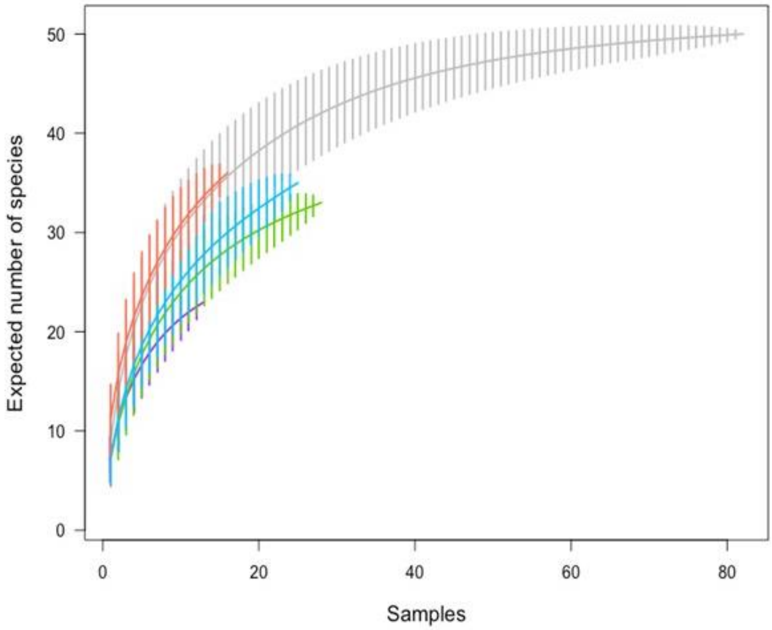

Fig. 8. Species accumulation curves showing their asymptotic behavior for the species richness estimators: Chao, Jackknife 1, Jackknife 2 and bootstrap.

Table 1.

Species distribution according to the sampling site (S1. Aluniș, S2. F. Peșterii, S3. S. Iosif, S4. Agaton, S5. Fundătura) and the number of individuals collected per sampling method (PF – pitfall trap; SS – sample soil, TZ – tweezers and WK – Winkler)

	Locality and type of sampling method																			
	S1. Aluniș				**S2.** F. Peșterii				**S3.** S. Iosif				**S4.** Agaton				**S5.** Fundătura			
SUBORDER ONISCIDEA	SS	WK	TZ	PF	SS	WK	TZ	PF	SS	WK	TZ	PF	SS	WK	TZ	PF	SS	WK	TZ	PF
Ligidium intermedium Radu, 1950	-	-	-	-	-	-	**3**	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoniscus carpaticus Tabacaru, 1974	-	-	-	-	-	-	-	-	-	**1**	-	-	-	-	-	-	**1**	-	-	-
Hyloniscus riparius (Koch, 1838)	-	-	-	-	-	-	**2**	-	**1**	-	-	-	-	-	**1**	-	**1**	-	-	-
Protracheoniscus politus (Koch, 1941)	-	-	-	-	**4**	**1**	-	-	-	-	-	-	-	**1**	-	**1**	**2**	**16**	**4**	**14**
Cylisticus brachyurus Radu, 1951	-	-	**4**	-	-	-	**52**	**15**	-	-	-	-	-	-	-	-	-	-	-	-
Trachelipus arcuatus (Budde-Lund, 1885)	-	-	-	-	-	**4**	**4**	-	-	-	-	-	-	-	-	**2**	-	-	**1**	**1**
Trachelipus rathkii (Brandt, 1833)	-	-	-	-	-	-	**3**	-	-	-	-	-	-	-	-	**1**	-	-	-	-
Number of individuals	-	-	**4**	-	**4**	**5**	**64**	**15**	**1**	**1**	-	-	-	**1**	**1**	**4**	**4**	**16**	**5**	**15**
Number of species	-	-	**1**		**1**	**2**	**5**	**1**	**1**	**1**	-	-	-	**1**	**1**	**3**	**3**	**1**	**2**	**2**
CLASS DIPLOPODA																				
Propolyxenus trivittatus (Verhoeff, 1941)	**1**	-	-	-	-	-	**3**	-	-	**10**	-	-	**1**	-	-	-	-	**1**	-	-
Trachysphaera costata (Waga, 1857)	-	-	-	-	**1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glomeris connexa Koch, 1847	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**	-	**1**	-	**1**

Strongylosoma stigmatosum Eichwald, 1830	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	14	1	22	21
Polydesmus complanatus (Linneus, 1761)	-	1	5	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	2	-
Polydesmus montanus Daday, 1889	1	5	5	-	1	-	-	1	-	-	-	-	1	-	-	-	-	-	5	2
Heterobraueria scopifera Verhoeff, 1898	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Craspedosoma transsylvanicum Verhoeff, 1897	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
Allopodoiulus verhoeffi (Jawlowski, 1931)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Cylindroiulus boleti (Koch, 1847)	12	43	13	1	15	11	8	5	13	-	14	3	16	10	2	4	13	1	30	3
Cylindroiulus luridus (Koch, 1847)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
Megaphyllum projectum Verhoeff, 1894	-	-	-	-	-	-	-	1	2	-	1	-	8	18	-	4	-	11	2	1
Megaphyllum transsylvanicum (Verhoeff, 1897)	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xestoiulus laeticollis (Porat, 1889)	-	-	-	-	14	1	-	-	4	3	1	-	-	-	-	-	21	10	1	-
Haplophyllum mehelyi (Verhoeff, 1897)	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Ommatoiulus sabulosus (Linneus, 1758)	-	-	-	-	-	-	-	-	2	-	1	-	1	-	-	-	-	1	-	1
Pachyiulus hungaricus (Karsch, 1881)	-	1	3	-	-	-	2	1	-	-	3	7	-	-	-	-	-	-	4	2
Unciger transsilvanicus (Verhoeff, 1899)	-	-	-	-	-	-	-	-	-	4	1	1	-	-	-	-	11	-	4	2
Number of individuals	**14**	**61**	**26**	**1**	**39**	**12**	**15**	**8**	**21**	**17**	**22**	**11**	**27**	**28**	**2**	**9**	**65**	**27**	**60**	**48**

Number of species	**3**	**7**	**4**	**1**	**5**	**2**	**4**	**4**	**4**	**3**	**7**	**3**	**5**	**2**	**1**	**3**	**7**	**8**	**8**	**9**
CLASS CHILOPODA																				
Harpolithobius radui (Matic, 1955)	-	**1**	-	-	-	**2**	-	-	-	**1**	-	-	-	-	-	-	-	-	-	**1**
Lithobius erythrocephalus C.L. Koch, 1847	-	-	-	-	-	-	-	-	**1**	-	**1**	**1**	**2**	**1**	-	-	-	-	-	
Lithobius forficatus (Linnaeus, 1758)	-	**2**	**8**	-	-	**1**	**3**	**1**	**1**	-	**4**	-	-	-	-	-	-	**2**	**13**	**3**
Lithobius lucifugus L. Koch, 1862	-	**1**	-	-	-	-	-	-	-	-	-	-	**1**	-	-	-	-	-	**1**	-
Lithobius mutabilis L. Koch, 1862	-	-	-	-	**1**	**4**	**1**	-	-	**2**	**2**	-	**3**	**9**	-	-	**1**	**6**	**2**	**1**
Lithobius muticus C.L. Koch, 1847	**1**	**14**	**3**	-	-	**4**	**3**	-	-	-	-	-	-	-	-	-	**1**	-	**2**	-
Lithobius crassipes L. Koch, 1862	**6**	**9**	**2**	-	**5**	**5**	**2**	-	-	-	-	**1**	-	-	-	**1**	-	-	**1**	-
Lithobius burzenlandicus burzenlandicus Verhoeff, 1931	**5**	**12**	**1**	-	**22**	**22**	-	-	**7**	**11**	**1**	**1**	**25**	**28**	**6**	-	**35**	**10**	**7**	-
Henia illyrica (Meinert, 1870)	-	**1**	**1**	-	-	-	**1**	-	-	-	-	-	-	-	-	-	-	**1**	-	-
Clinopodes flavidus C.L.Koch, 1847	-	**3**	-	-	-	-	**3**	-	**1**	-	-	-	-	-	-	-	-	-	-	-
Clinopodes rodnaensis Verhoeff, 1935	-	-	-	-	-	-	-	-	-	-	-	-	**1**	-	-	-	-	-	-	-
Geophilus electricus (Linnaeus, 1758)	-	-	-	-	-	-	-	-	**1**	-	-	-	-	-	-	-	-	-	**1**	-
Geophilus flavus (De Geer, 1778)	**3**	**1**	**2**	-	-	**1**	**2**	-	**1**	-	**6**	**2**	-	-	-	-	**6**	**1**	**4**	-
Geophilus proximus C.L.Koch, 1847	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3**	**1**	**1**	-
Pachymerium ferrugineum (C.L.Koch, 1835)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**	-
Strigamia acuminata (Leach, 1815)	-	-	-	-	**1**	-	-	-	-	-	-	**1**	-	-	-	-	-	**1**	**2**	**1**

Strigamia transsilvanica (Verhoeff, 1928)	**-**	**1**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Stenotaenia linearis (C.L. Koch, 1835)	**1**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**2**	**1**	**-**	**-**	**2**	**1**	**-**	**-**	**1**	**-**	**-**	**-**
Schendyla walachica Verhoeff, 1900	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**1**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
*Schendyla carniolensis*Verhoeff, 1902	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**2**	**-**	**-**	**-**	**2**	**-**	**-**	**-**
Schendyla tyrolensis Attems, 1895	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Cryptops hortensis (Donovan, 1810)	**-**	**2**	**2**	**-**	**1**	**-**	**4**	**-**	**2**	**4**	**4**	**-**	**5**	**4**	**-**	**1**	**1**	**15**	**-**	**1**
Number of individuals	**16**	**47**	**21**	**-**	**31**	**39**	**19**	**1**	**18**	**20**	**19**	**6**	**42**	**43**	**6**	**2**	**50**	**37**	**35**	**7**
Number of species	**5**	**11**	**9**	**-**	**6**	**7**	**8**	**1**	**9**	**6**	**7**	**5**	**9**	**5**	**1**	**2**	**8**	**8**	**12**	**5**
CLASS SYMPHYLA																				
Scutigerella orghidani Juberthie-Jupeau & Tabacaru, 1968	**3**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**4**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Hanseniella nivea (Scopoli, 1763)	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**1**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Number of individuals	**3**	**2**	**-**	**-**	**1**	**-**	**-**	**-**	**2**	**1**	**1**	**-**	**5**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Number of species	**1**	**1**	**-**	**-**	**1**	**-**	**-**	**-**	**1**	**1**	**1**	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Table 2.

Total number of species (Oniscidea, Diplopoda and Chilopoda) identified using each method and combining two methods

	Soil samples	Winkler sieving	Tweezers	Pitfall traps
Soil samples	36	44	45	42
Winkler sieving	-	35	45	39
Tweezers	-	-	36	39
Pitfall traps	-	-	-	22

Table 3.

Results of MANOVA pairwise comparisons of species communities among sampling techniques (PF – pitfall trap; SS – sample soil, TZ – tweezers and WK – Winkler) and sites

Technique				
	PF	SS	TZ	
SS	0.048	-	-	
TZ	0.740	0.048	-	
WK	0.048	0.048	0.048	
Site				
	Site 1	Site 2	Site 3	Site 4
Site 2	0.120	-	-	-
Site 3	0.114	0.120	-	-
Site 4	0.400	0.120	0.040	-
Site 5	0.400	0.040	0.040	0.067

Table 4.

The best combination of environmental variables with the maximum (rank) correlation with community dissimilarities: Rhground – relative humidity at soil level (%), Tground – air temperature at ground level (^{0}C), Alt – altitude a.s.l. (m)

Environmental variables	Similarity score
Rhground	0.060
Tground	0.040
Alt + Tground	0.022
Tground + Rhground	0.020
Alt + Tground + Rhground	0.012
Alt + Rhground	0.025
Alt	0.052

Table 5.

The 10 best combinations of species with the maximum (rank) correlation with community dissimilarities

Species	Similarity score
Lithforf + Lithcras + Lithburz + Geopflav + Stenline + Schewala + Cryphort + Polygerm + Polymont + Cylibole + Megatran + Haplmehe + Ommasabu + Pachhung + Hansnive	0.843
Lithforf + Lithcras + Lithburz + Geopflav + Stenline + Schewala + Cryphort + Polygerm + Polymont + Cylibole + Megatran + Haplmehe + Pachhung + Hansnive	0.841
Lithforf + Lithcras + Lithburz + Geopflav + Stenline + Schewala + Cryphort + Polygerm + Polymont + Cylibole + Haplmehe + Pachhung + Hansnive	0.839
Lithforf + Lithcras + Lithburz + Geopflav + Stenline + Schewala + Cryphort + Polygerm + Polymont + Cylibole + Pachhung + Hansnive	0.836
Lithforf + Lithcras + Lithburz + Geopflav + Stenline + Schewala + Cryphort + Polygerm + Polymont + Cylibole + Pachhung	0.831
Lithforf + Lithcras + Lithburz + Geopflav + Stenline + Cryphort + Polygerm + Polymont + Cylibole + Pachhung	0.825
Lithforf + Lithcras + Lithburz + Geopflav + Stenline + Cryphort + Polymont + Cylibole + Pachhung	0.819
Lithforf + Litheuxi + Geopflav + Stenline + Cryphort + Polymont + Cylibole + Pachhung	0.811
Lithforf + Litheuxi + Geopflav + Cryphort + Polymont + Cylibole + Pachhung	0.801
Lithforf + Litheuxi + Geopflav + Stenline + Cryphort + Cylibole + Pachhung	0.801

Table 6.

Akaike statistics for model including the observed species richness, the sampling technique and the environmental variables (Rhground – relative humidity at soil level (%); Tground – air temperature at ground level (ºC); AIC (Akaike's Information Criterion) differences (ΔAICc) and Akaike weights (wi) were used to rank models relative to the best model (minimum AIC). K – number of parameters; LL – log likelihood

Model	Covariates	K	AIC_c	ΔAIC_c	w_i	LL
Mod1	Technique	6	373.25	0.00	0.51	-180.63
Mod5	Tground+Rhground+Technique	8	373.35	0.09	0.48	-178.67
Mod6	Month	8	382.1	8.85	0.01	-183.05
Mod3	Rhground	4	385.63	12.38	0.00	-188.82
Mod2	Tground	4	386.61	13.36	0.00	-189.31
Mod4	Tground+Rhground	5	387.37	14.11	0.00	-188.68

Table 7.

The observed species richness (Sobs) and the mean and the standard error (SE) of the species richness estimators: Chao, Jackknife 1, Jackknife 2 and bootstrap per sampling technique; PF – pitfall trap; SS – sample soil, TZ – tweezers and WK – Winkler; SE – standard error; N – number of samples

Technique	Sobs	Chao	Chao SE	Jackknife 1	Jackknife 1 SE	Jackknife 2	Bootstrap	Bootstrap SE	N
PF	23	28.769	4.821	32.231	3.988	34.487	27.551	2.859	13
SS	33	39.888	5.713	42.643	3.351	45.669	37.761	1.928	28
TZ	35	50.680	11.265	48.440	4.744	56.030	41.052	2.765	25
WK	36	58.578	15.083	51.938	7.111	61.912	42.924	3.660	16