

# Exceptional Invertebrate Diversity in a Scree Slope in Eastern Spain

Alberto Jiménez-Valverde<sup>1,2\*</sup>, José D. Gilgado<sup>2</sup>, Alberto Sendra<sup>2</sup>, Gonzalo Pérez-Suárez<sup>2</sup>, Juan J. Herrero-Borgoñón<sup>3</sup> and Vicente M. Ortuño<sup>2</sup>

<sup>1</sup> Departamento de Biogeografía y Cambio Global. Museo Nacional de Ciencias Naturales. Madrid (Spain).

<sup>2</sup> Grupo de Investigación de Biología del Suelo y de los Ecosistemas Subterráneos. Departamento de Ciencias de la Vida. Facultad de Biología, Ciencias Ambientales y Química. Universidad de Alcalá. Alcalá de Henares. Madrid (Spain).

<sup>3</sup> Departamento de Botánica. Facultad de Ciencias Biológicas. Universidad de Valencia. Burjassot. Valencia (Spain).

\* Corresponding author: [alberto.jimenezv@uah.es](mailto:alberto.jimenezv@uah.es), [alberto.jimenez.valverde@gmail.com](mailto:alberto.jimenez.valverde@gmail.com)

**Abstract** In this study, the invertebrate fauna of a colluvial Mesovoid Shallow Substratum (MSS) in Eastern Spain was characterized, and the importance of the MSS as refuge for endemic and rare species was assessed. Ten pitfall traps were buried up to 1 meter deep inside multiperforated PVC tubes across a stone debris. Several environmental variables were measured in each sampling point. The completeness of the inventory was assessed, and different diversity patterns - variation in abundance, species richness and species composition - were analyzed. 4150 individuals and 164 species were captured, most of them arthropods. The number of individuals and species varied considerably among traps. Half of the species were represented by one or two individuals, and neither the species accumulation curves nor the curves for the non-parametric estimators showed any sign of stabilizing. Individuals of abundant species aggregated in one or a few traps. No consistent diversity patterns were found, except that distance among traps partially explained the similarity in species composition when considering only the most abundant species. This is a very rich but uneven assemblage that shows a high degree of species turnover between traps. One sixth of the species can be classified as either troglobionts or troglophiles, but there is a significant presence of edaphic species. Obtaining complete fauna inventories in the MSS has proven to be a challenging task. The study of the MSS reveals important faunistic information, providing new taxa to science as well as interesting records of poorly known species.

**Keywords:** invertebrates, hypogean, Mesovoid Shallow Substratum, subterranean diversity, troglophile

## Introduction

The Linnaean and Wallacean shortfalls, i.e. the lack of knowledge of *how many* species exist on earth and *where* do they live (Lomolino and Heaney 2004), are important problems that hamper fully understanding the real dimension of the now widely recognized biodiversity crisis or "sixth mass extinction" (Dirzo and Raven 2003; Whittaker et al. 2005; Barnosky et al. 2011; Ceballos et al. 2015). The magnitude in the lack of knowledge is not homogeneous among taxonomic groups, geographic regions or ecosystems (Brown and Lomolino 1998). Invertebrates, despite being disproportionately diverse, have traditionally received less attention than vertebrates. For instance, it is estimated that the number of Mollusca species (the second most diverse animal phylum) yet to be discovered could double the number of the known ones (Lydeard et al. 2004). Arthropoda, the most diversified phylum, is probably the most unknown (Grimaldi and Engel 2005) and is the dominant taxon in the subterranean environment (Bellés 1987; Gibert and Deharveng 2002; Culver et al. 2006; Reboleira et al. 2011), providing a high number of rare, endemic and relict species (Ortuño 2002; Ortuño and Martínez-Pérez 2011). Furthermore, our knowledge about the biodiversity of the subterranean domain is scarce and shows strong geographic biases (Culver and Holsinger 1992; Gibert and Deharveng 2002; Culver et al. 2013). The technical difficulties involved in sampling subterranean environments, and the geographical distribution of researchers are probably two of the main reasons that have contributed to this scarce knowledge (Jiménez-Valverde and Ortuño 2007).

The Mesovoid Shallow Substratum (originally Milieu Souterrain Superficiel, or MSS; Juberthie et al. 1980) refers to the shallow subterranean habitat (SSH; Culver and Pipan 2014) formed by the network of cracks and small voids that can be found in the fragmented bedrock horizon (Gers 1992), in colluvial (Juberthie et al. 1980) and alluvial deposits (Ortuño et al. 2013), or in volcanic scoria (Oromí et al. 1986). The coluvial MSS is formed in sloping grounds due to the fragmentation and acumulation of rocks resulting from the erosion of rocky outcrops. It can either be covered by a soil layer, or on the contrary, it can be exposed to the surface as in the case of scree slopes. In any case, the MSS is a confluence habitat for epigean, endogean and hypogean species (Gers 1998; Ortuño et al. 2013; Nitzu et al. 2014) due to three main characteristics: the existence of a dark crevice network; the lower extreme temperatures than outside along with the very high relative humidity (Pipan et al. 2011; Ortuño et al. 2013); and the bi-directional flux of material from superior as well as from inferior layers (Gers 1998). In fact, the MSS' ecological and evolutionary importance is due to the large presence of invertebrate species, including relict and endemic species; some of them are troglobian arthropods (Gers 1992; Nitzu et al. 2010; Pipan et al. 2011; Pipan and Culver 2012) which probably evolved in this subterranean habitat (Uéno 1987; Růžička 1999a). Given its physical characteristics, the MSS plays an important role as climatic refuge and may act as biogeographic corridor, which raise important conservation implications (Culver 1982; Růžička 1993; Hernado et al. 1999; Růžička et al. 2012; Ortuño et al. 2013).

Most studies on MSS have been carried out in mountainous and alpine regions with temperate climate in Central and Eastern Europe (e.g., Juberthie et al. 1980, 1981; Juberthie and Decu 1994; Gers 1992, 1998; Nitzu et al. 2006, 2014; Giachino and Vailiati 2010; Růžička 2011; Rendoš et al. 2012), in Japan (e.g., Uéno 1980, 1981, 1987), and also in volcanic regions such as the Canary Islands (e.g., Oromí et al. 1986, Pipan et al. 2011). In the Mediterranean region and in general, in areas of gentle relief, the MSS usually constitutes a mosaic of isolated patches due to the formation of silted clayey areas (Juberthie and Decu 1994). This characteristic raises methodological sampling challenges, which together with other causes already mentioned (see above) has probably hampered the systematic study of the MSS (Růžička and Klimeš 2005); proof of this is the lack of thorough studies in areas such as the Iberian Peninsula, and the ones that exist have focused on searching for flagship species almost only based on taxonomic interests (see, for instance, Toribio and Rodríguez 1997; Ortuño and Toribio 1994; Fresneda et al. 1997; Carabajal et al. 1999; Faille et al. 2012; but see Galán 2001 and Ortuño et al. 2013). Although it seems that the interest in the study of the faunal assemblages of the MSS has increased in the last years (e.g. Nitzu et al. 2010, 2014; Pipan et al. 2011; Růžička 2011; Rendoš et al. 2012), the absence of knowledge is overwhelming, and in the case of the Iberian Peninsula, it still is in a very early stage.

The three aims of this study are (1) to assess the reliability of the invertebrates inventory of a Mediterranean colluvial MSS in Eastern Spain; (2) to characterize the diversity and structure of the assemblage; (3) to evaluate the importance of this habitat as refuge for endemic and rare species. Although there is still much

to be studied and discovered, the eastern part of the Iberian Peninsula, and especially the reliefs of the Valencia region, harbors more than 120 known hypogean invertebrate species (mostly arthropods; Sendra et al. 2011). These numbers represent about 40% of the total endemic fauna of the region (Domingo et al. 2006) and include ecogeographic and phylogenetic relicts (Ortuño et al. 2004) which have survived in the underground of the so-called “Betic Peri-plateau karstic arc” (Ortuño and Gilgado 2010). We expect to find a rich and diverse assemblage composed by both hypogean and troglophile species as well as by edaphic ones. We also expect to report rare and poorly-known species, and to find new species to science. To our knowledge, this study provides the most comprehensive characterization of the invertebrates assemblage inhabiting an MSS published up to date.

## **Materials and methods**

### Study site

The study was carried out in Sierra de Aitana, a mountain massif located in the province of Alicante, forming part of the Prebaetic Mountain range (Fig. 1A, B). The sampling points were specifically located in an area of colluvial debris in a hillslope at the base of a peak called Peña Cacha (Fig. 1C, D). Sierra de Aitana was chosen for this study as it is the highest altitude in Alicante (1558 m) and is one of the best examples of high Mediterranean mountains. It is also located in an area of great interest from a biogeographic viewpoint (Marco 2001) where typical Eurosiberian species (such as *Taxus baccata* L. or *Rhamnus alpinus* L.)

find refuge, as well as, several baetic endemic species (such as *Genista longipes* Pau or *Vella spinosa* Boiss.) reach their northern range limits. The sampling location suffers from annual climatic extremes, reaching over 30°C in summer and supporting snow cover in winter (in fact, these mountains have harbored snow wells for commerce during the sixteenth to the nineteenth century; Vicedo and Ramírez 2004). The colluvial debris appears in the form of cone-shaped deposits (colluvial fan) in its highest part (Fig. 1D) at the base of the cliff, or as simple screes, exposed in most part but occasionally covered by a layer of soil with scarce vegetation. *Salvia lavandulifolia* subsp. *mariolensis* (Figuerola) Alcaraz and De la Torre, *Erinacea anthyllis* Link and *Hormathophylla spinosa* (L.) P. K pfer are the dominant shrub species in the study area; the ivy *Hedera helix* subsp. *rhizomatifera* McAllister is dominant in the debris together with the grasses *Festuca* spp. and *Arrhenatherum* spp., and moss communities. The bedrock is formed by eocenic limestones (Alfaro et al. 2006); their fragmentation and erosion formed the debris during the coldest periods of the Quaternary (Marco 2000).

#### Fauna sampling

Ten sampling points were placed across the colluvial debris in an attempt to represent the environmental diversity of the area (plant cover, presence of soil, distance to cliff, etc.). They were between 1280 and 1320 m of altitude and separated by at least 10 m from one another (mean distance between sampling points  $\pm$  SD = 66.83  $\pm$  45.49). A subterranean sampling device (SSD) was installed in each sampling point. These devices are designed to collect

specimens between 25-100 cm (approx.) deep. A SSD is a multi-perforated PVC cylinder in which a pitfall trap is lowered into the tube and filled with 1,2-propanediol and a solid bait (very strong-smelling cheese) (Fig. 1 E, F); the SSD also holds a thermometer inside, just beneath the top cover of the cylinder. The design of the SSD was based on the models described by Gers (1992), Owen (1995) and López and Oromí (2010), and it is fully described in Ortuño et al. (2013). The cylinders were buried on 25<sup>th</sup> March 2011 and they were baited four months later in order to avoid a possible digging-in effect; the SSDs operated for three months, from 25<sup>th</sup> July 2011 to 28<sup>th</sup> October 2011. Two SSDs were broken during this period. Specimens were sorted in the laboratory, preserved in ethanol 70% and labeled. Identification was done by the authors and by a broad range of specialists (see Acknowledgements). Specimens collected are deposited in the zoological collection of the University of Alcalá.

#### Environmental variables

For each sampling point, five variables were measured (Table 1): distance to cliff, slope, plant cover (%), stoniness, and temperature inside the SSDs. Plant cover inside a 3 m radius circle and centered in each SSD was visually estimated. Stoniness was visually estimated using a five-category score, based on profile photographs (Fig. 2). Plant cover and stoniness were both estimated by two or more of the authors, and in cases of discrepancies, a consensus was established. Temperature inside the SSDs was measured every hour (the thermometer of SSD1 was broken).



## Data analysis

Species accumulation curves, which represent the variation in the accumulated species richness as a function of sampling effort (Colwell and Coddington 1994; Soberón and Llorente 1993), were raised for the eight SSDs altogether and for each of the SSDs. In the first case, both a sample-based as well as an individual-based curve was built, whilst in the second case only individual-based curves were made (see Gotelli and Colwell 2001). Samples (or individuals) were randomized 100 times and non-linear regression was used to fit the Chapman-Richards (CR) model to the curves. The CR function has the form  $y = a((1 - \exp(-bx))^c)$  where  $y$  is the accumulated species richness and  $x$  is the sampling effort. This model was chosen because it has an asymptote (i.e., it makes possible the extrapolation of species richness) and it passes through the point (0,0), which are desirable properties (Thompson et al. 2003), and because it has previously shown good performance in terms of fit and extrapolation accuracy (Flather 1996; Thompson et al. 2003; Jiménez-Valverde et al. 2006). During the course of the analysis it was noted that the fit of the model was very sensitive to subtle variations in the shape of the accumulation curve, so the generation of the accumulation curves and the fit of the CR models were done 100 times in each case, and the mean asymptote values were calculated. Three nonparametric, incidence-based, species richness estimators were also calculated for the complete debris assemblage and for each SSD: Chao2, first-order Jackknife (Jack1) and second-order Jackknife (Jack2). These nonparametric estimators have shown good performance in previous studies (Chiarucci et al. 2003; Hortal et al. 2006).

Spearman correlations were performed between the environmental variables - plant cover, stoniness, distance to cliff, slope and mean, minimum and maximum temperature - and the value in each SSD of: observed species richness ( $S_{obs}$ ), rarefied species richness ( $S_{raref}$ ), Chao2, Jackk1, Jackk2, CR asymptotic estimate, and number of individuals ( $N_{ind}$ ).

The original community matrix was filtered and only those species with 10 or more individuals were retained. Spearman correlations were performed between the environmental variables and the value in each SSD of  $S_{obs \geq 10}$  and  $N_{ind \geq 10}$ . To get an overall value of fauna dissimilarity among the eight SSDs, three multiple-site distances were computed, one based on the Sørensen index ( $\beta_{sor}$ ; it measures beta-diversity and it is dependent on species richness differences), a second based on the Simpson index ( $\beta_{sim}$ ; it measures dissimilarity due to turnover, i.e., it is independent on species richness differences), and the third based on the Nestedness-resultant fraction of Sørensen dissimilarity ( $\beta_{nes}$ ; it measures dissimilarity due to nestedness), in such a way that  $\beta_{sor} = \beta_{sim} + \beta_{nes}$  (Baselga et al. 2007; Diserud and Odegaard 2007; Baselga 2010). Following the same beta-diversity partitioning rationale (Baselga 2010), three distance matrices were computed and mantel tests (Pearson correlations) were used to test for correlations between these matrices and a spatial distance matrix.

To test if there were more co-occurring species than expected by chance, a null model analysis was performed (Gotelli 2000) in which the presence-absence

filtered matrix was randomized 1000 times in such a way that species occurrence totals were maintained and all SSDs were equiprobable (method SIM2 in Gotelli 2000). Then, the observed number of checkerboard units ( $2 \times 2$  submatrices where two species exclude each other, herein  $C_{obs}$ ; Stone and Roberts 1990) was compared with the histogram of frequencies of the simulated number checkerboard units (herein  $C_{sim}$ ) so that  $p(C_{sim} = C_{obs})$  was calculated (Gotelli 2000).

All the analysis were done in the R environment (R Development Core Team 2009) using base functions (`nls` for non-linear regression) and the `vegan` (accumulation curves, non-parametric estimators, rank/abundance models, mantel tests, co-occurrence analysis; Oksanen et al. 2013) and `betapart` (beta-diversity analysis; Baselga et al. 2013) packages.

## Results

In total, 4150 individuals and 164 species were captured (Table 2; see Table S1 for the complete list of species). These numbers do not include the Pseudoscorpions (16 specimens), Acari Non-Oribatida and other non-identified individuals (216 specimens), Geophilomorpha (2 specimens), Lepidoptera (101 larvae), Homoptera (25 specimens) and 37 larvae of Coleoptera, since they could neither be identified nor separated into morphospecies. The phylum Mollusca was marginally represented by one species and two individuals, being Arthropoda the dominant phylum in the inventory. At the species level, there were four taxa that each contributed 10% or more to the total assemblage: Acari

was the most diversified group with 52 species, followed by Araneae (20 spp.), Coleoptera (18 spp.) and Hymenoptera (17 spp.) (Fig. 3A). Collembola was the most abundant group with 1701 individuals, followed by Acari (684 ind.) and Opiliones (583 ind.) (Fig. 3B). 3.0% of the 164 species are troglobionts, and 13.4% of the species can be classified as troglaphiles (see Table S1). At least seven species are new to science (Table S1).

There is a remarkable number of rare species; 50.0% of the species are represented by one (singletons) or two (doubletons) individuals and 37.9% of the species are singletons (Table 2). On the other hand, two species dominate the entire assemblage; the collembola *Lepidocyrtus* n. sp. (Fig. 4; 22.0% of the individuals, see Table S1) and the troglaphile harvestmen *Dicranolasma soerensenii* (Fig. 4; 10.8% of the individuals, see Table S1), although in the last case most of the individual concentrated in just one SSD (see below). The species accumulation curves end while still rising, and the curves for the nonparametric estimators did not show any sign of stabilizing either (Fig. 5; only the Chao2 curve is shown, but the curves for Jack1 and Jack2 showed a similar shape). According to the estimations (Table 2), the percentage of completeness ranges from 45.5% to 67.0% if SSDs are used to measure sampling effort and from 60.2% to 72.6% if individuals are used as samples. The CR function could not be fitted to the sample-based accumulation curve because of constant convergence errors. Extrapolation of the CR model adjusted to the individual-based accumulation curve suggests that doubling the effort would increase the completeness from a mean value of 60.2% to a mean value of 75.0% (204 species would be collected; Fig. 5B).

The number of individuals varies considerably among traps, ranging from 294 to 1040, and shows a strong positive and significant correlation with  $S_{obs}$  ( $r_s = 0.72$ ,  $p = 0.045$ ) which ranges from 28 to 58 (Table 2). There is a high number of singletons and doubletons and a general dominance of one or two species in each SSD (Table 2). The collembola *Lepidocytrus* sp. dominates in SSD1, SSD6 and SSD7; in SSD2 it dominates together with the collembola *Orchesella* sp. and in SSD4 it does together with the acari *Oribatula tibialis*. *Orchesella* sp. dominates in SSD5, *Dicranolasma soerensenii* dominates in SSD3, and the collembola *Gisinurus malatestai* (troglophile) and *Seira* sp. dominates in SSD8 (see Table S1). All the species accumulation curves and almost all the non-parametric curves stop at the very beginning while still rapidly rising even though there is some heterogeneity in the patterns of completeness (Fig. 6). For instance, in the case of SSD3 and SSD4, the nonparametric curves (as before, only the Chao2 curves are shown) seem to have reached a stable behavior (the percentages of completeness range from 65.2% to 85.7% and from 61.4% to 72.9%, respectively; Table 2); on the contrary, SSD2 and SSD7 show rather increasing curves (the percentages of completeness range from 38.2% to 71.2% and from 47.0% to 67.2%, respectively; Table 2).

Only the slope showed significant positive correlations with  $S_{obs}$  ( $r = 0.88$ ,  $p = 0.007$ ), Jack1 ( $r = 0.86$ ,  $p = 0.011$ ) and Jack2 ( $r = 0.79$ ,  $p = 0.028$ ), although the correlations were no longer significant after Bonferroni's correction (number of Spearman correlation tests = 63, 49 with the complete matrix and 14 with the filtered matrix).

From the 164 species, only 46 species had 10 or more individuals (Table 2). Overall, these abundant species were very unevenly distributed among traps, with most of the individuals being grouped in one or a few SSDs (Figs. S1-S3). No significant correlation was found between  $S_{\text{obs} \geq 10}$ ,  $N_{\text{ind} \geq 10}$ , and any of the environmental variables. Dissimilarity among SSDs was high, with a multiple-sites Sørensen index value of 0.62. Most of the dissimilarity was due to the turnover component (Simpson index value of 0.54), whilst the nestedness component was almost negligible (Nestedness-resultant fraction value of 0.09). Both the dissimilarity matrix accounting for overall beta diversity and the dissimilarity matrix accounting for turnover showed positive and significant correlations with the spatial distance matrix ( $r = 0.33$ ,  $p = 0.042$  and  $r = 0.35$ ,  $p = 0.042$  respectively). Finally, the co-occurrence analysis indicated that there was no difference between the number of  $C_{\text{obs}}$  and null expectations.

## Discussion

### Faunistics

The invertebrate fauna in this study belongs to the phyla Mollusca and Arthropoda. The former is represented by just one species with very few individuals, *Suboestophora boscae* (Gastropoda, Pulmonata), whose known distribution is restricted to the southeast of the Iberian Peninsula (Gasull 1975). It has been reported as a sublapidicolous and crevice-dwelling species, and it has also been found in caves (Martínez-Ortí 2006). Yet, the hegemonic group in

the MSS is Arthropoda. Collembola and Acari were the groups that provided the vast majority of individuals; they are probably very important if not the main source of nutrients for the next trophic levels in the MSS. Opiliones, Coleoptera, Araneae and Myriapoda are the second most abundant groups in the debris. With the exception of Opiliones, whose great abundance in our study is due to the concentration of just one species in one SSD, Rendoš et al. (2012) found the same dominant groups in their study of a karstic debris in Slovakia along with Diptera, a group that in our study is scarcely represented. Acari is the most diversified group, followed by Coleoptera, Araneae and Hymenoptera, three taxa that have been previously reported as highly diverse in the MSS (Pipan et al. 2011; Ortuño et al. 2013) and other rocky habitats (Růžička et al. 2010).

Overall, the arthropod fauna inhabiting the colluvial MSS is very geophile, hygrophile, mesothermophilous, and 16.5% of the species can be classified and either troglaphiles or troglobionts. Among the most sensitive species to dryness and temperature variations in the epigeal zone are probably the terrestrial Isopoda; from the three collected species, *Porcellio incanus* is the most abundant with individuals collected in the eight SSDs. This is a well-known troglaphile species from a number of caves in Andalusia and Eastern Spain (García 2013). The Iberian endemism *Armadillidium mateui* (Fig. 4) is also well represented in the MSS; this species was only known to be in a few mountainous localities in the southern Iberian Peninsula (a record from the north of the Iberian Peninsula is probably a misidentification; Ll. García, pers. com.). It is known to live under stones (Cruz-Suárez 1993), and its presence in

the subterranean environment of Alicante clearly responds to its humidity and mild temperatures requirements.

The class Arachnida is also well represented in the MSS with the orders Araneae, Acarina, and Opiliones. Acarina is the most diverse group with 50 species, most of which belong to the families Mesostigmata (9 spp.) and Oribatida (40 spp.). These are free-living taxa that show a wide spectrum of ecological roles from predators to saprophagous and detritophages, thus playing a very important function in the productivity dynamic of the ecosystem (Coleman and Crossley 1996). The small body size and high density that the acari can attain in the soil (for instance, for the species *Oribatula tibialis*, densities of 5300 individuals/m<sup>2</sup> have been reported; see Vasiliu et al. 1993) gives this group a great potential for reaching the MSS. The most abundant and widespread species in the MSS were Oribatida; the holartic species *Oribatula tibialis* and the (semi)cosmopolitan species *Scheloribates laevigatus* and *Hemileius initialis* (see Subías 2004). The holartic Oribatida *Ceratozetes peritus* was also very abundant but it only appeared in one SSD, like *Zercon montanus*, the most abundant Mesostigmata species. *Z. montanus* is a species known to be present in alpine habitats in central Europe (Bregetova 1977) and hygromesophytic habitats in northern Europe (Salmane 1999). Nevertheless, most of the acari species collected in the MSS were represented by a low number of individuals.

Three harvestmen species were very abundant; the troglophile species *Dicranolasma soerensenii* (Fig. 4) and two epiedaphic species typical from



herbaceous habitats, *Odiellus* sp. and the Iberian endemism *Nelima hispana* (Bellés 1987; Prieto 2006). It is worth noting that 91.5% of the individuals of *D. soerenseni* appeared in only one SSD; this aggregation behavior is common in Opiliones (Machado and Macías-Ordóñez 2007).

Two Araneae species are particularly abundant in the MSS, the Agelenidae *Tegenaria levantina* (Fig. 4) and *Eratigena picta*. The former is an Iberian endemism, a troglophile species only known to be in a few localities in the east and northeast of the Iberian Peninsula (Ribera and Barrientos 1986). With this study, the known distribution of *T. levantina* is increased and its troglophile aptness is emphasized. *E. picta* is a widely distributed spider across Europe, closely associated to woodlands and mountainous areas (Barrientos and Sánchez-Corral 2013), and has been recently found in the MSS (see appendix in Ortuño et al. 2013). *Cybaeodes dosaguas* is only known from two specimens collected inside a cave in the province of Valencia (Ribera and De Mas 2015); it is relatively common in the alluvial MSS (the authors, unpublished data). In general, the Linyphiidae are the best represented spiders in the hypogean environment (Bellés 1987; Růžička 1989), with *Lepthyphantes* the most outstanding group (Ribera 1981). It is worth mentioning significant representation in the colluvial MSS of the Dysderidae *Kaemis* n. sp. This genus was only known to be in Italy and Montenegro until it was found in the alluvial MSS of the Valencia region (see appendix in Ortuño et al. 2013). Other species like *Pholcomma gibbum* (Theridiidae), *Tenuiphantes tenuis* (Linyphiidae) and *Zelotes subterraneus* (Gnaphosidae) have been previously reported from the

MSS and scree slopes (e.g. Růžička 1996, 1999b, 2002; Růžička and Klimeš 2005; Růžička and Zacharda 2010).

Ten species of Myriapoda were collected in the MSS; the Callipodida *Cyphocallipus* sp. (endemic genus to the Iberian Peninsula and possibly a new species for science, H. Engoff com. pers.; Fig. 4) was the most abundant species, followed by *Archipolydesmus* n. sp. (Polydesmida). It is interesting to highlight the presence of *Stosatea capolongoi* (Polydesmida), a species that was described from a cave in the southeast of the Iberian Peninsula (Nguyen and Sierwald 2013) and found again in the alluvial MSS (see appendix in Ortuño et al. 2013), all of which suggests that it is a hypogean species. The presence of *Phryssonotus platycephalus* (Polyxenida) in the Iberian Peninsula is confirmed since the only two records that existed of this Mediterranean species for the peninsular territory date back to 1923 (Silvestri 1923). The species *Lithobius (Lithobius) castaneus* (Chilopoda) (Fig. 4) stands out for its great size and predatory behavior; this species is present in southern Europe and has been observed in the epigean environment but also in caves (Matic 1958; Serra 1980; Zapparoli 2009).

The most outstanding Hexapoda group in the MSS, both in terms of number of individuals and species (only surpassed by Coleoptera with 3 more species), is Collembola. The most abundant species are *Lepidocyrtus* n. sp. (Fig. 4), *Orchesella* sp., *Heteromurus nitidus* and *Seira* sp. (Entomobryidae), and *Gisinurus malatestai* (Sminthuridae), all of which have been previously reported from the MSS (e.g., Nitzu et al. 2010; see appendix in Ortuño et al. 2013). Both

*Lepidocyrtus* and *Orchesella* are genera which harbor numerous hemiedaphic species with troglophile habits (Arbea and Baena 2002-2003). *Seira* sp., *Heteromurus nitidus* and *Gisinurus malatestai* are also very abundant species in the MSS but show a noteworthy aggregation behavior, and thus a patchy distribution in the debris. Only one Diplura species was collected, the Circum-Mediterranean *Campodea grassii*, a typical species of wet environments and which is usually found in the subterranean realm (Sendra and Moreno 2004).

Regarding Insecta, four species of Microcoryphia were collected, being the lapidicolous *Lepismachilis* sp. the most abundant species. Orthoptera is a well-represented group in the MSS with two Gryllidae species, *Petaloptila aliena* and *Petaloptila bolivari* (Fig. 4). Both are Iberian endemic species, and *P. bolivari* has been reported to be part of cave wall communities (Barranco 2006, 2012). They have recently been reported as inhabitants of the MSS in the east of the Iberian Peninsula (Ortuño et al. 2013). From the seven Diptera species that were collected, *Megaselia* sp. (Phoridae) and a new Sciaridae species are the two most outstanding species; the genus *Megaselia* is very typical in subterranean environments (Gers 1992; Barranco et al. 2013). The order Coleoptera is represented by 18 species, most of them with a low number of individuals. The most abundant species are *Cryptophagus jakowlewi* (Fig. 4) and *Cryptophagus denticulatus* (Cryptophagidae), two widely distributed, palaeartic, semicosmopolitan, fungivorous beetles (Otero 2013). The presence of *Stenus* sp. (Staphylinidae) in the MSS is probably due to its predatory life style and wandering habits since they are diurnal exophthalmos beetles and do not belong to the subterranean realm. Nevertheless, they are typical of epigean

habitats with a high degree of humidity (Gamarra and Outerelo 2009). Just two individuals of the hypogean and predatory species *Trechus* cf. *martinezi* (Fig. 4) were collected. Although this species does not show troglobiomorphic characters, they exclusively inhabit the MSS and caves (Ortuño and Arillo 2005; Ortuño 2006). *Leistus* (*Pogonophorus*) *puncticeps* appears only with one individual but it has been collected in other colluvial debris in the area (V. M. Ortuño pers. obs.); it is an example of Eurosiberian species (Serrano 2013) that in the Valencia region finds suitable habitat in the MSS.

### Diversity patterns

Despite the intense sampling effort, the results strongly suggest that the inventories are far from complete. The species accumulation curves for the eight traps altogether end while still rising, indicating that more species are yet to be added to the inventory, and the same happens for each SSD separately. Another symptom of inventory incompleteness is that the curves of the nonparametric estimators – which, in order to be useful, are expected to reach a plateau sooner than the species accumulation curves (Gotelli and Colwell 2001) – do not show any signals of stabilization either. The high number of singletons and doubletons, and the uneven structure of the assemblages, means that a big sampling effort needs to be made to obtain reliable inventories (Gotelli and Colwell 2001; Thompson and Withers 2003; Thompson et al. 2003). The fact that none of the accumulation curves show signs of reaching a mesa implies that richness estimations must be taken with extreme caution (Gotelli and Colwell 2001; Melo and Froehlich 2001; Thompson et al. 2003; Thompson and

Thompson 2007). Roughly, it could be ventured that between half and a quarter of the species are still to be captured, and that doubling the sampling effort – in terms of number of collected individuals – would suppose only a subtle increase in the number of new species. This is because the addition of new species to the inventories is very slow given the high number of rare species.

The presence of a high number of rare species – in terms of number of individuals – in species-rich inventories is not unusual when dealing with hyperdiverse taxa such as arthropods (Gotelli and Colwell 2001). This situation accentuates in the MSS, where the density and richness of invertebrate species tends to be low and to decrease with depth (Růžička 1996; Růžička and Klimeš 2005), presumably due to resource limitations (Gers 1998; Rendoš et al. 2012). Percentages of singletons and doubletons around 50% (as reported in this study) and usually higher, have been previously found in other arthropods inventories in rock debris [Coleoptera: Růžička 2000 (47%) and Rendoš et al. 2012 (63%); Araneae: Růžička 1989 (63% and 46%), Růžička 1996 (66%) and Růžička and Zacharda 2010 (65%); Arachnida: Růžička et al. 1995 (59%)]. The incompleteness of the inventories may be enhanced by the presence of not permanent resident edaphic species which, despite their transitory condition, are key elements of the interaction network in the MSS (Gers 1998; Pipan et al. 2011; Nitzu et al. 2014); this fact complicates the delimitation of the sampling universe in the subterranean realm. It should also be noted that the inventory provided here is just a snapshot of three months and a complete annual survey remains to be explored to see how it improves the inventory.

The incompleteness of subterranean inventories hampers the exploration of community patterns (Schneider and Culver 2004). This could explain the inability of any of the environmental variable to account for the variation in the number of species (observed and estimated) and individuals among traps. However, given the direct relationship that was found between  $S_{obs}$  and number of individuals (another possible indication that sampling effort has not been enough),  $S_{raref}$  (density of species; Gotelli and Colwell 2001) was calculated, as well as  $S_{obs \geq 10}$  and  $N_{ind \geq 10}$ ; not even with these parameters was it possible to find an influential environmental factor.

To explore community patterns it was necessary to eliminate the huge amount of low-abundant species since they may lead to an artificial high level of species turnover between SSDs (Hulcr et al. 2008). Likewise, they may have dramatic effects in the co-occurrence analyses since, if the number of species vary between sites, a high number of singletons could unavoidably yield a high number of checkerboard units in randomized matrices, thus increasing  $C_{sim}$  with respect to  $C_{obs}$ . By just considering species with ten or more individuals, a remarkable level of species turnover is observed. Many species are dominant in one or a few traps and are absent in others traps. This high heterogeneity in species composition has been previously noted in the MSS at a regional scale (among locations; Růžička and Klimeš 2005), and also at a local scale (among traps; Růžička and Klimeš 2005; Giachino and Vailati 2010) such as in the present study. Several variables have been previously proposed as possible factors that condition the biodiversity in the MSS. Microclimate, and more specifically ice formation and temperature, are supposed to be key factors

determining the presence of certain species (Růžička 1989; Růžička et al. 1995; Růžička and Klimeš 2005; Růžička and Zacharda 2010; Nitzu et al. 2014). Other important variables are the density of vegetation cover, the degree of soil development, and in general, the amount of flow of organic resources that comes from the upper layers (Gers 1998; Rendoš et al. 2012; Nitzu et al. 2014). However, in this study we did not find any significant effect on the biodiversity parameters of any of the environmental variables that were considered. No obvious co-occurrence patterns among the abundant species were found either. Nevertheless, we found that species composition is moderately conditioned by spatial distance, i.e. the closer the traps are, the more similar the assemblages are. This is a common pattern found in many other studies (e.g., Genner et al. 2004; Steinitz et al. 2006; Baselga and Jiménez-Valverde 2007), which is the consequence of spatial autocorrelation, and lastly, a product of environmental gradients and dispersal constraints (Nekola and White 1999). In the debris, there may be some environmental variability which we could not measure but is conditioning the composition of the assemblages, and the strong aggregation of conspecific individuals. For instance, Gers (1998) noted how the spatial variation in water flow through the MSS conditioned the amount of food which in turn determined the pattern of arthropod community structure. Kühnelt (1957) mentioned the scarce homogeneity in the soil fauna at a very local spatial scale due to attraction and pure demographic processes such as a rapid reproduction, which may cause close samples to be very different in terms of species composition. Given the close relationship between the soil and the MSS, these causes may also be acting in the subterranean realm. Regardless of the reasons; the high degree of heterogeneity and species turnover are

factors that, together with the high porportion of rare species, hinder the possibility to get complete and reliable inventories in the MSS.

## Conclusions

From an ecological and evolutionary perspective, the MSS is a habitat of high relevance which has remained ignored and unstudied for a long time (Pipan et al. 2011; Ortuño et al. 2013;). This is especially true for stone debris, an ecosystem with very special microclimate conditions that promote the presence of a rich and specific arthropod fauna (Růžička 1993; Růžička et al. 2012; Nitzu et al. 2014). This habitat typically suffers from little human influence (Růžička 1993), and both in Europe and in Spain, several natural habitat types of Community Interest are located on stone debris (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora - The Habitats Directive; Bartolomé et al. 2005). Most of the existing knowledge about the invertebrate fauna of stone debris comes from temperate regions and from studies focused on restricted taxonomic groups, whereas, scree slopes in the Mediterranean area have long been ignored. Here, we provide the first comprehensive study about the entire invertebrate community in a Mediterranean debris stone. We have shown that the assemblage is almost entirely formed by arthropod species, that it is an extremely rich but uneven community, and that there is a high dominance of rare (i.e., low abundance) species. Although there are hypogean and troglophile species, the presence of edaphic species is significant, highlighting the close relationship that exists between soil and subterranean environments (Giachino and Vailati 2010; Nitzu



et al. 2014). Despite the apparent homogeneity of the habitat, there must be a high degree of heterogeneity as judged by the significant species turnover existing between traps. The low density attained by most of the species and the lack of homogeneity between close samples makes obtaining complete fauna inventories quite a challenging task. As already pointed out by others (e.g., Růžička 1990, 1993), we have shown that the thorough study of scree slopes can reveal important faunistic information, potentially providing new taxa to science, enlarging the geographic ranges of many species and providing interesting records of geographically restricted and poorly known species.

### **Online Resources**

**Table S1.** Complete list of species collected in the colluvial MSS.

**Figures S1-S3.** Abundance in each SSD for species with 10 or more individuals.

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**Table 1. Environmental variables measured for each sampling plot.** SSD, subterranean sampling device. See main text for details on how the different variables were measured. The thermometer of SSD1 was broken.

<b>SSD</b>	<b>Distance to cliff (m.)</b>	<b>Slope (°)</b>	<b>Plant cover (%)</b>	<b>Stoniness</b>	<b>T<sup>a</sup> (mean)</b>	<b>T<sup>a</sup> (max.)</b>	<b>T<sup>a</sup> (min.)</b>
1	3.6	35	25	4	-	-	-
2	8.0	34	10	3	15.7	28.1	9.1
3	31.3	40	15	2	16.7	26.5	11.1
4	10.7	27	3	1	18.4	29.5	9.7
5	23	15	50	5	17.8	24.5	11.2
6	14.5	21	20	3	18.3	28.1	9.7
7	30	23	45	3	18.8	29.6	10.7
8	18.2	2	10	2	19.0	31.3	10.7

**Table 2. Species richness values for the complete inventory (total) and for each subterranean sampling device (SSD).** Number of samples (either number of SSDs or individuals), observed species richness ( $S_{obs}$ ), rarefied species richness ( $S_{raref}$ ), number of species with 10 or more individuals ( $S_{obs \geq 10}$ ), number of individuals considering only those species with 10 or more individuals ( $N_{ind \geq 10}$ ), singletons, doubletons, asymptote of the Chapman-Richards model (CR; mean $\pm$ sd and min/max values are shown), and non-parametric estimators of species richness Chao2, first-order Jackknife (Jack1) and second-order Jackknife 2 (Jack2). For the complete inventory, two measures of sampling effort were used: number of SSDs and number of individuals ( $N_{ind}$ ). The CR model could not be fitted to the complete inventory when SSDs were used as a measure of sampling effort.

	TOTAL		SSD							
	SSDs	$N_{ind}$	1	2	3	4	5	6	7	8
<b>Samples</b>	8	4150	366	498	1040	718	424	452	358	294
<b><math>S_{obs}</math></b>	164	164	49	50	58	51	42	48	47	28
<b><math>S_{raref}</math></b>	-	-	44.7	40.1	37.3	36.2	36.2	41.2	42.7	28.0
$S_{obs \geq 10}$	46	46	23	25	31	30	21	30	26	18
$N_{ind \geq 10}$	3862	3862	313	463	983	677	387	426	330	283
<b>Singletons</b>	62	62	20	22	19	19	17	17	23	12
<b>Doubletons</b>	20	20	8	3	7	7	4	7	5	4
<b>CR</b>	-	272.2 $\pm$ 16.63 (239.5/319.9)	63.8 $\pm$ 1.71 (60.4/69.8)	70.2 $\pm$ 3.65 (64.7/82.0)	67.7 $\pm$ 1.30 (65.2/71.6)	83.1 $\pm$ 7.63 (70.2/111.3)	60.3 $\pm$ 3.56 (53.0/71.6)	56.0 $\pm$ 1.05 (53.9/59.6)	80.9 $\pm$ 6.81 (68.2/101.2)	48.0 $\pm$ 7.06 (39.3/86.9)
<b>Chao2</b>	360.6	260.1	74.0	130.7	83.8	76.8	78.1	68.6	99.9	46.0
<b>Jack1</b>	245.4	226.0	68.9	72.0	77.0	70.0	59.0	65.0	69.9	40.0
<b>Jack2</b>	301.0	268.0	80.9	90.9	89.0	81.9	71.9	74.9	87.8	47.9

**Figure 1. Sampling location and subterranean sampling device.** A, B: location of the study area in the Iberian Peninsula. C: location of the sampling points across the stone debris (image source: Instituto Geográfico Nacional de España). D: the stone debris. E, F: details of a subterranean sampling device (SSD, see main text for details).

**Figure 2. Examples of profile photographs used to measure stoniness.** From (A) very high stoniness (= 5 in Table 1) to (E) very low stoniness (= 1 in Table 1).

**Figure 3. Taxa representation.** Percentages of (a) number of species and (b) number of individuals for each taxa found in the MSS. In cases where the percentage was <1%, it was omitted to improve chart clarity.

**Figure 4. Examples of representative species collected in the MSS.** A, *Armadillidium mateui* Vandel, 1953 (Isopoda, Armadillidiidae); B, *Dicranolasma soerensenii* Thorell, 1876 (Opiliones, Dicranolasmatidae); C, *Tegenaria levantina* Barrientos, 1981 (Araneae, Agelenidae); D, *Lithobius (Lithobius) castaneus* Newport, 1844 (Lithobiomorpha, Lithobiidae); E, *Cyphocallipus* sp. (Callipodida, Dorypetalidae); F, *Lepidocyrtus* n. sp. (Collembola, Entomobryidae); G, *Petaloptila bolivari* (Cazurro, 1888) (Orthoptera, Gryllidae); H, *Cryptophagus jakowlewi* Reitter, 1888 (Coleoptera, Cryptophagidae); I, *Trechus* sp. (cf. *martinezi*) (Coleoptera, Carabidae).

**Figure 5. Species accumulation curves for the complete inventory.** Species accumulation curves (lower thin curves) and curves for the Chao2 richness estimator (upper gross curves) for the complete inventory using (a) subterranean sampling devices (SSDs) or (b) individuals as sampling effort units. 95% confidence intervals are shown as stripped lines. In (b), the Chapman-Richard (CR) model is fitted to the species accumulation curve and extrapolated to double the number of individuals actually observed (gross gray line); the horizontal line marks the mean asymptote of the CR functions fitted to 100 accumulation curves (see main text for details).

**Figure 6. Species accumulation curves for each subterranean sampling device.** Species accumulation curves (lower thin curves) and curves for the Chao2 richness estimator (upper gross curves) for each of the subterranean sampling devices (SSDs) using individuals as sampling effort units.