RESEARCH ARTICLE

Effects of macro and micro-environmental factors on the species richness of terrestrial tardigrade assemblages in an Iberian mountain environment

Noemí Guil · Joaquín Hortal ·
Sara Sánchez-Moreno · Annie Machordom

Abstract Tardigrade communities are affected by micro and macro-environmental conditions but only micro-environmental variables, and altitudinal gradients have been studied. We review previous reports of altitudinal effects and evaluate the influence by interacting macro- (climate, soils, biome, and others) and micro-environmental (vegetation, moss and leaf litter) factors on tardigrade assemblages at the Sierra de Guadarrama mountain range (Iberian Central System Mountains, Spain). Terrestrial tardigrade assemblages were sampled using standard cores to collect leaf litter and mosses growing on rocks. General Linear Models were used to examine relationships between Tardigrada species richness and abundance, and macro- and micro-environmental variables (altitude, habitat characteristics, local habitat structure and dominant leaf litter type, and two bioclimatic classifications). Variation partitioning techniques were used to separate the effects of altitude and habitat variation, and to quantify the independent influences of climate and soil, vegetation structure and dominant type of leaf litter. Altitude shows a unimodal relationship with tardigrade species richness, although its effect independent of habitat variation is negligible. The best predictors for species richness were bioclimatic classifications. Separate and combined effects of macro-environmental gradients (soil and climate), vegetation structure and leaf litter type are important determinants of richness. A model including both macro- and micro-environmental variables explained nearly 60% of tardigrade species richness in micro-scale plots. Abundance was significantly related only to soil composition and leaf litter type. Tardigrade abundance was not explained by macro-environmental gradients analysed here, despite a significant correlation between abundance and richness.

N. Guil (✉)
Zoological Museum, Natural History Museum, University of Copenhagen, Universitetsparken 15, Copenhagen DK-2100, OE, Denmark
e-mail: nguillopez@gmail.com

J. Hortal
NERC Centre for Population Biology, Division of Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK

J. Hortal · A. Machordom
Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain

S. Sánchez-Moreno
Departamento de Protección Vegetal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain

Introduction

Large-scale environmental factors influence communities of microscopic invertebrates. For example, soil nematode species richness in European grasslands varies according to climatic gradients (Ekschmitt et al. 2001), and moss-dewelling bdelloid rotifers show strong effects of habitat filtering in spite of their cosmopolitan distributions (Fontaneto and Ricci 2006; Fontaneto et al. 2006). Microscopic invertebrate distribution and communities are highly influenced by interacting macro- (climate, soils, and biome) and micro-environmental (vegetation, moss and leaf litter) factors. For example, while each plant community provides different micro-habitats for invertebrates (Richardson et al. 2005), the structure and composition of these communities vary according to changes in climate, soil, and other major environmental variables (e.g., Lobo et al. 2001). Due to this, separating the effects of macro- and micro-environmental factors on microscopic invertebrate communities presents several difficulties.

In spite of their important roles in food webs (e.g., Sohlenius et al. 2004) and ubiquitous distribution (Nelson 1995), tardigrades are one of the forgotten groups when biodiversity is discussed, so research on their diversity patterns has been encouraged (e.g., Wilson 2002). These animals, known as water bears (Goeze 1773), are symmetric bilateral animals (Nelson 1982). Active tardigrades are found in interstitial water of marine or freshwater sediments, in soils, and in aquatic or terrestrial algae, lichens, mosses and liverworts (Ramazzotti and Maucci 1983; Dewel et al. 1993). Many species are able to enter into a latency state under unfavourable conditions (Nelson 1982).

Tardigrade distribution is commonly thought to depend mainly on micro-environmental habitat conditions. Nevertheless, ecological studies on tardigrades are scarce, mostly limited to the study of altitudinal variation (Dastych 1980, 1988; Kathman and Cross 1991). Previous studies report conflicting results, finding positive, negative or null relationships between altitude and tardigrade species richness (Bartŏs 1939; Rodríguez Roda 1951; Bertrand 1975; Nelson 1975; Dastych 1980, 1985, 1987; Beasley 1988; Dastych 1988; Kathman and Cross 1991; Utsugi et al. 1997; Guidetti et al. 1999; Nichols 1999; Collins and Bateman 2001). Almost none of them have involved quantitative sampling or multivariate analytical techniques, so these inconsistencies may be due to the incomplete representation of the altitudinal gradients or the latitudinal variation in climatic conditions (see Rahbek 2005; Nogués-Bravo et al. 2008).

In this paper, we investigate the relationship between macro- and micro-scale environmental variables, and species richness and abundance of tardigrade assemblages in a heterogeneous mountain landscape (Sierra de Guadarrama, central Iberia Peninsula). We used faunistic data from a standardized survey of terrestrial tardigrades, and environmental data from field observations and GIS. Specifically, we (i) study the effects of several macro-environmental factors (climate and soil characteristics, and biome structure) and micro-environmental factors (habitat characteristics, such as moss and leaf litter type and availability) on species richness and abundance of tardigrade communities, from different landscape types and in a regional scale; and (ii) characterize the separate influence of altitude and other environmental variables on tardigrade communities.

Methods

Study area

Our study was carried out in the southern slope of the Sierra de Guadarrama, which belong to the Sistema Central, located in the centre of the Iberian Peninsula (approximately 40°50′ N, 3°50′ W) (Fig. 1). Sierra de Guadarrama is approximately 2,800 km^2 in extent, ranging from minimum elevations of 750 m in the south and 850 m in the north, to a maximum altitude of 2,430 m at the Peñalara peak. Due to its unique geographic situation, this mountain chain has acted as a barrier, corridor and refuge during the Quaternary climate changes (e.g., Franco Múgica et al. 1998). A large proportion of the Iberian fauna is found in this mountain range as a consequence of its complex biogeographic history and its diverse landscapes and habitats (Chefaoui et al. 2005; Hortal and Lobo 2005).

Biological data

Faunistic data come from Guil's survey (Guil 2004, 2008). Briefly, quantitative samples were taken at 72
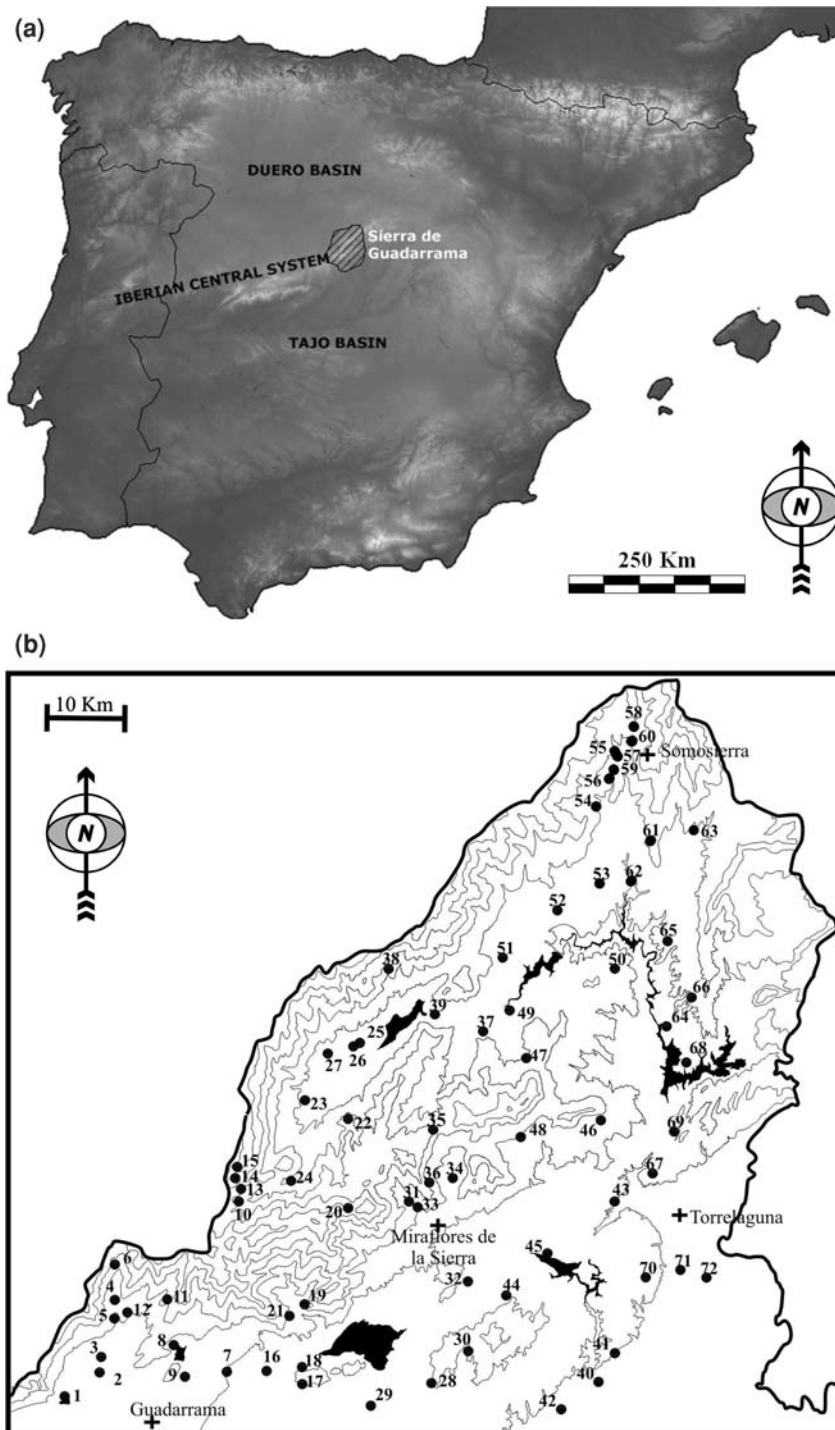
Fig. 1 a Map of the Iberian Peninsula showing location of Sierra de Guadarrama mountain range, as well as of Iberian Central System mountain chain and Duero and Tajo basins. Greyscale shows the elevation above sea level, from dark grey (low altitudes) to white (high altitudes). **b** Sierra de Guadarrama map, showing the location of the 72 sampling sites. Bold line is the northern Comunidad de Madrid administrative border, thin lines are equidistant altitude curves, intervals each 200 m, and medium black areas are reservoirs

stations (Fig. 1B, coded 1 to 72 herein, following Guil 2008) over an elevation range from 680 to 2,240 m. Sampling stations were chosen by a mixed spatially stratified-random procedure to ensure a full coverage of environmental variation and habitat heterogeneity within the altitudinal gradient. Samples were taken in spring and autumn to cover the two seasonal peaks of tardigrade populations (Nelson et al. 1987). Based on previous experience, sampling these two periods has proved sufficient to represent most of the annual variation in the studied communities. During each sample period, we collected two samples per station: one in rock mosses (MR) and the other in leaf litter (LF). A total of 288 samples were taken: 144 in April–May, 2001 (spring) and 140 in September–November, 2001 (autumn). Four autumn stations (numbers 10–12 and 16) were not sampled in September 2001 because of deep snow cover; these stations were sampled in September 2002 instead. Samples were taken with a square core of 3 × 3 cm (hereafter, abundances refer to numbers per 9 cm^2), collecting the entire moss depth (about 2–3 cm) in the case of MR, and up to 3 cm depth in the case of LF. Surface area was consistent across all samples, but sample depth varied slightly. We explicitly examined the effect of dried mass of mosses and leaf litter on observed richness and abundance to account for this potential bias (see variable details below).

Sediment was passed through a 80 μm sieve and examined under a stereoscope to isolate all tardigrades found (see Guil 2008 for more details). A standardized procedure was used to fix (with Carnoy liquid; 3 parts ethanol 96°/97°: 1 part acetic acid), and mount specimens (in Faure mounting medium; Ramazzotti and Maucci 1983). Tardigrade identification followed Guidetti and Bertolani (2005). All specimens were placed in the invertebrate collection of the Museo Nacional de Ciencias Naturales (CSIC, Madrid, Spain). Tardigrade assemblage composition was similar in spring and autumn, and no exclusive communities of rock mosses (MR) or leaf litter (LF) were identified (i.e., there were no significant differences in the species gathered in both samples; Guil 2004). Therefore, data (spring and autumn, and MR and LF) for each sampling station were pooled for analysis, each station yielding a single data point (field plot *sensu* Hortal 2008), assuming that these four samples correspond to different aspects of the same assemblage of terrestrial tardigrades. Hence, the species richness (S_{tard}) and abundance (N_{tard}) of tardigrades was calculated for each one of these field plots, pooling the data from both substrates and both seasons into a single data point. In the case of moss and leaf litter mass, we summed the measurements for each variable across the two seasons. All the analyses quoted below were made on the resulting 72 data points, which are thought to represent the whole terrestrial Tardigrada assemblage found in these field plots all year round.

Environmental data

Landscape characteristics and leaf litter and moss species identifications were made during field surveys and sample processing. Additional data were obtained from a GIS database at 1-km^2 resolution (Hortal 2004; see former applications and detailed descriptions in Hortal and Lobo 2005; Chefaoui et al. 2005; Hortal et al. 2008a). In total, we used 23 variables that describe environmental variation according to five different factors, including topography, macro-environmental gradients (Env: climate and soil characteristics), macro-scale biome structure (Biome) and micro-environmental factors (moss and leaf litter type and availability):

– *Topography*. Two Digital Elevation Models with 1-km^2 and 1 ha spatial resolution, respectively (DEM; Clark Labs 2000) were processed in GIS (*Idrisi 32*; Clark Labs 2001) to obtain four variables: ALT (mean altitude), SLOPE (mean slope), ASPECT (mean aspect, measured as arc distance to North in degrees), and DIV_ASP (aspect diversity in the 1-km^2 cell, calculated from the 1 ha cells).
– *Climate*. 30-year monthly data of precipitation and temperature for 41 stations of Central Iberia (MAPA 1986) were interpolated to obtain monthly maps. We extracted and aggregated these data to obtain seven variables: PANN (mean annual precipitation), PSUM (summer precipitation), PAMP (range of monthly precipitation), TMED (median temperature), TMAX (maximum monthly temperature), TMIN (minimum monthly temperature) and TRNG (range of monthly temperatures).

- *Soil characteristics*. We used 2 maps to create 2 variables of substrate variation. A lithology map (LITO) was reclassified from a regional atlas (ITGE 1988) into four categories (Granite, Gneiss, Acid deposits, and Basic deposits). Four categories of soil type (SOIL) were extracted from the FAO Soil Map of the World (FAO 1988): Distric Cambisols [Cmb_Distric], Humic Cambisols [Cmb_Humic], Calcareous Cambisols [Cmb_Calcar] and Eutric Cambisols [Cmb_eutric].

- *Biome structure*. Two variables described two scales of habitat structure. LANDSC measured large scale characteristics of the landscape surrounding each point; a 3-category map was obtained by reclassifying and enlarging the 250-m European CORINE Land Use/Land Cover map (EEA 2000), into closed (i.e., forests), semi-open (i.e., shrubs, "dehesas" [open habitat covered by scattered trees] and other open forests, and mixed landscapes), and open biomes (i.e., pastures, extensive cultivations). VGSTR described vegetation structure taken at each sampling point (5 categories; pastures and bare rock, shrubs, 'dehesas', open forests and closed forests).

- *Leaf litter and moss*. Six variables accounted for the diversity of leaf litter and moss present at each sampling site (in an approximate area of 100 m^2 around the sampling plots). LEAF accounted for the most abundant type of leaf litter, classified in four categories: conifer, broad, xerophilous (*Quercus ilex*, *Cistus* shrubs, and similar), and no leaves (i.e., grass, bare rock or soil, and occasionally, small pieces of mosses). The other five variables reflected the amount of substrate collected at each sample: moss mass in spring (DW_RMS) and moss mass in autumn (DW_RMO), leaf litter mass in spring (DW_LFS) and leaf litter mass in autumn (DW_LFO), and the sum of these four quantities (DW_TOT). The mass of each sample was obtained by drying and weighting moss and leaf litter samples after isolating all tardigrade individuals.

In addition, each station was assigned to a category of two maps of bioclimatic land classifications, BCM_ALLUE (Allué Andrade 1990) and BCM_RIVAS (Rivas-Martínez 1987). Bioclimatic classifications are commonly used in field studies, survey design, planning and management. The two classifications used here are commonly used in Spain, and are intended to summarize the distribution of soil and climate gradients in accordance to the variation of plant communities within the country. The studied localities were a representative sample of the categories of both classifications present in the studied area, which include several Nemoromediterranean, Oroborealoid and Oroarticoid classes in the case of BCM_ALLUE, and several series (equivalent to subcategories) included in the Mesomediterranean, Supramediterranean, Oromediterrarean and Crioriomediterranean domains in the case of BCM_RIVAS. Present in the study area were the Nemoromediterranean, Oroborealoid and Oroarticoid classes of BCM-ALLUE, and the Meso-, Supra-, Orom- and Crio- domains of the BCM_RIVAS. These categories characterise a distinct climatic gradient determined by topography, ranging from warm and relatively dry temperate Mediterranean environments (Mediterranean in BCM_ALLUE and Mesomediterranean in BCM_RIVAS) to cold and wet sub-alpine environments (respectively Oro- and Crio- in these classifications)." See Allué Andrade (1990) and Rivas-Martínez (1987) for further information on the rationale and construction of these classifications, and http://www.globalbioclimatics.org/ for an introduction and general overview.

Statistical analyses

We used generalized regression techniques of General Linear Models (McCullagh and Nelder 1989; Nicholls 1989; Crawley 1993) to explore the relationships between Tardigrada species richness and abundance, and the predictors listed above. All continuous predictors were standardized to 0 mean and 1 Standard Deviation to avoid spurious weighting of some variables due to differences in the scale of measurement. We assumed a Poisson distribution for both S_{tard} and N_{tard} and a logarithmic relationship between dependent variable and predictor (independent) variables, as recommended for variables based on counts (Crawley 1993). The fit of both variables to the Poisson distribution was confirmed by a visual examination of the histograms of their frequency distributions. We used the change in deviance from a null model to determine the percentage of variation in S_{tard} and N_{tard} explained by each predictor, determining its significance by an F test (McCullagh and

Nelder [1989](ref)). We also included linear and quadratic functions of each continuous variable in a backward stepwise regression analysis (StatSoft [2001](ref)), removing non-significant terms ($P > 0.05$) in each model. This way, we accounted for possible non-linear relationships between richness and/or abundance and the significant environmental predictors (cf. Austin et al. [1996](ref); Lobo et al. [2001](ref); Hortal et al. [2008b](ref)). All GLM analyses were conducted in STATISTICA package (StatSoft [2001](ref)).

Environmental variables are often collinear, which makes it difficult to identify relationships between biodiversity and different environmental predictors (Mac Nally [2000](ref); Lobo et al. [2001](ref); Hawkins et al. [2003](ref); Hortal et al. [2008b](ref)). To minimize the spurious effects of collinearity, we performed a two-step analysis to study the influence of the factors described above on Tardigrada assemblages (see Hortal et al. [2008b](ref) for a detailed explanation of the rationale). On the first step, we developed separate models for four groups of variables: (i) altitude (*Alt*), (ii) macro-environmental conditions (*Env*; including climate and soil characteristics), (iii) landscape and vegetation structure (*Biome*), and (iv) leaf litter and moss characteristics (*Leaf*, a surrogate for some micro-environmental conditions). Models for each factor were constructed by a backward stepwise GLM, where all predictors pertaining to each factor were included in a preliminary model, from which the non-significant ones were eliminated in consecutive steps.

On the second step, we used variation-partitioning techniques to quantify the relative importance of these factors on species richness (see Lobo et al. [2001](ref); Rodríguez et al. [2006](ref); Hortal et al. [2008b](ref)). Variation partitioning analyses used partial regressions to separate the independent effects of each factor, identifying the amount of variation in the dependent variable that can be attributed solely to each one of these factors, and the amount of explained variation that is shared by their different combinations (Borcard et al. [1992](ref); Legendre and Legendre [1998](ref); Mac Nally [2000](ref)). Here, each one of the predictor variables pertaining to a factor was regressed against all predictors from the rest of the studied factors, and the partial regression terms for each variable are obtained as the Pearson residuals from these regressions. In the case of categorical variables, each significant category was converted into a new binary (presence or absence) dummy variable, and a partial logistic regression was carried out for each of these new variables. We used these new partial regression terms to perform two different variation partitioning analyses. In the first one, we calculated the variation in S_{tard} explained by altitude that is independent from the remaining variables. In the second, we analyzed in more detail the relationships between species richness and the other three factors (climate and soil characteristics, macro-scale biome structure, and moss and leaf litter type and availability), partitioning richness variation into eight components: three quantifying the separate effects of each factor, four accounting for their collinear effects (both by pairs and altogether), and a component quantifying the unexplained variation. The amount of variation explained by the independent effect of each factor and by their joint influence was inferred by solving a system of equations (see Lobo et al. [2001](ref)). A step-by-step description of our implementation of variation partitioning analyses using both quantitative and categorical explanatory variables is available as supplementary material in Hortal et al. ([2008b](ref)).

We evaluated the presence of spatial autocorrelation in species richness and abundance values by comparing the spatial autocorrelation in both the original data and the residuals of the models (see Diniz-Filho et al. [2003](ref); Hawkins et al. [2003](ref)). We generated correlograms of both raw data and model residuals using Moran's I coefficients on 10 lags of 5 km width each (from 0 to 50 km), using GS + software (Gamma Design [2001](ref)). If autocorrelation was present in the original richness or abundance data, but no autocorrelation remained in model residuals it could be assumed that the predictors in the model account for the spatial structure in the original data. Conversely, any spatial autocorrelation remaining in residuals indicated that predictor variables used in the model do not describe adequately the spatial pattern in richness/abundance, and that spatially structured variables not included in our analyses may be affecting richness or abundance patterns (Diniz-Filho et al. [2003](ref)).

Finally, we performed Spearman non-parametric correlation analyses to assess if the differences in the altitude showing the maximum species richness in former studies were related to the latitude of the studied localities. Data were obtained from 10 published studies that found maximum richness at

altitudes ranging from 50 to 2,310 m and latitudes from 34.5 to 78.5° N.

Results

A total of 11,019 tardigrades from 48 species were collected in the survey. Species richness ranged from 1 to 20 species per sampling station, and abundance from 1 to 1,102 individuals per 9 cm^2. Dried mass of moss and leaf litter samples had no significant effects, neither on tardigrade richness, nor on abundance. Since all differences in dried mass would have come from differences in sample depth (surface was limited by the square core), and we have demonstrated no significant effects, we can assume equivalent size in samples for these analyses.

Species richness and abundance were positively correlated ($r = 0.428$; $N = 72$; $P < 0.001$). In spite of this, while S_{Tard} showed a pattern of spatial dependence, with significant positive autocorrelation at the first lag class (from 0 to 5 km) and significant negative autocorrelation at the last class (from 45 to 50 km), N_{Tard} contained no spatial structure (not shown).

A number of variables presented significant relationships with species richness (Table 1). The most important single predictor was the predominant type of leaves of the vegetation (LEAF), a micro-environmental variable. Several macro-environmental variables were significant, such as landscape structure (LANDSC), the two bioclimatic classifications (BCM_RIVAS and BCM_ALLUE) and the quadratic functions of altitude, maximum temperature, annual range of temperatures and summer precipitation. Leaf litter of broad-leaved trees harboured high species richness, while the litter of xerophilous trees had relatively few species (Fig. 2). Richness was also higher in closed biomes, at both landscape and local scales (i.e., LANDSC and VGSTR), as well as in areas of acid substrate, while it was lower in shrublands and areas with calcareous soils. Precipitation and temperature variables presented positive and negative relationships with S_{Tard}, respectively. The richness gradient saturated before reaching the extreme of the gradient of coldest temperatures and highest summer precipitations (Fig. 2). This was also evident when examining the relationship with the bioclimatic classifications: BCM_ALLUE shows a

gradient from higher richness in Nemoromediterranean areas to lower numbers of species in Oroboreal places, which is depicted more accurately by BCM_RIVAS, where the highest and lowest richness values are found in humid Supramediterranean and dry Mesomediterranean areas of calcareous soil, respectively (not shown).

When considering the whole factors individually, biome structure was the most important, followed by the effects of climate and leaf litter type (Table 1). Altitude showed a significant hump-shaped (quadratic) relationship with species richness, explaining 23.4% of the variation (Table 1 and Fig. 2), but its independent effect on richness was marginal (Table 2). On the contrary, *Env*, *Biome*, and *Leaf* were significantly related to species richness when analyzed separately; yet, macro-environmental variation (*Env*) was the strongest independent effect (Table 3 and Fig. 3). These results evidence a hierarchical effect of the different factors on species richness that modifies the patterns observed on the single-factor analyses depicted in Table 1. Climate and, to a less extent, soil characteristics are the most important effects in favouring the presence of a higher number of species, followed by landscape structure at the macro-scale, and of moss and leaf litter type and availability. A model including all these factors explained nearly 60% of the variation in species richness (Table 3). Model residuals were not spatially autocorrelated.

Despite its significant correlation with richness, Tardigrade abundance presented much weaker relationships with all predictors (Table 4). The strongest predictors were soil type (higher abundance in Humic and Distric cambisols, and lower abundance in Calcareous soils as with STard; Fig. 4) and a bioclimatic classification (BCM_RIVAS); explaining 9.1% of the variation in abundance (leaf litter of xerophilous and coniferous plants contained significantly lower numbers of individuals). Vegetation structures where xerophilous species were more abundant (shrublands and dehesas) also had negative relationships with abundance, while it was higher in pastures and open forests (Table 4).

Finally, there was a significant relationship (Spearman $r = -0.745$; $N = 10$; $P = 0.013$) (data from Table 5 and Fig. 5) between altitudes at which species richness is maximum in different studies and latitude of localities analysed in different studies.

Table 1 Variation of Tardigrada species richness (S_{tard}) explained by the studied predictors (*Expl.Var.*)

Variable	d.f.	Dev	ΔDev	F	Expl.Var.	Sign
Null model	71	130.7				
Topography						
ALT	70	117.8	12.9	7.68	9.89***	+
ALT + ALT^2	69	100.2	30.6	21.06	23.38***	+, −
SLOPE	70	125.2	5.6	3.11	n.s.	
ASPECT	70	129.4	1.3	0.70	n.s.	
DIV_ASP	70	129.5	1.2	0.66	n.s.	
Model (ALT + ALT^2)	69	100.2	30.6	21.06	23.38	
Climate						
PANN	70	115.3	15.4	9.38	11.82***	+
PSUM	70	109.4	21.3	13.63	16.29***	+
PSUM + PSUM^2	69	103.8	26.9	17.89	20.59***	+, −
PAMP	70	109.0	21.7	13.92	16.59***	+
TMED	70	112.6	18.2	11.29	13.89***	−
TMAX	70	107.5	23.2	15.09	17.73***	−
TMAX + TMAX^2	69	103.3	27.4	18.30	20.96***	−, −
TMIN	70	120.3	10.4	6.05	7.96**	−
TRNG	70	107.2	23.5	15.35	17.98***	−
Model (PANN + TMIN + TMED + PAMP)	67	86.6	44.2	34.19	33.79	
Soil characteristics						
LITO	68	121.9	8.9	4.95	6.78**	
Granite						+
Dep_Bas						−
SOIL	68	108.6	22.1	13.83	16.90***	
Cmb_Distric						+
Cmb_Humic						+
Cmb_Calcar						−
Model (SOIL)	68	108.6	22.1	13.83	16.90	
Biome structure						
LANDSC	68	94.1	36.6	26.44	28.00***	
Closed						+
VGSTR	67	109.6	21.1	12.91	16.15***	
Shrubland						−
Closed forest						+
Model (LANDSC + VGSTR)	64	84.5	46.23	35.02	35.36	
Leaf and moss						
LEAF	68	91.2	39.5	29.42	30.20***	
Broad						+
Xerophilous						−
DW_RMS	70	127.7	3.0	1.64	n.s.	
DW_RMO	70	130.2	0.5	0.26	n.s.	
DW_LFS	70	128.5	2.2	1.18	n.s.	
DW_LFO	70	129.5	1.2	0.64	n.s.	
DW_TOT	70	129.2	1.5	0.82	n.s.	
Model (LEAF)	68	91.2	39.5	29.42	30.20	

Table 1 continued

Variable	d.f.	Dev	ΔDev	F	Expl.Var.	Sign
Bioclimatic classifications						
BCM_ALLUE	68	105.2	25.5	16.49	19.52***	
BCM_RIVAS	65	93.4	37.3	25.96	28.54***	

Groups of explanatory variables are given in italics, and variable codes as in text. Deviance (*Dev*) and Change in Deviance (Δ*Dev*) were calculated through the comparison with a null model (with no explanatory variables). The *Sign* of the relationship is provided for continuous variables, and for significant categories of categorical predictors (except Bioclimatic classifications). Quadratic or cubic functions of quantitative variables were selected when they produced a Change in Deviance significant at a 5% level. Only significant quadratic or cubic functions are shown. n.s. means not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Percentage of explained variation by each model is shown in bold numbers. The best model for each factor, selected by means of a backward stepwise GLM, is shown
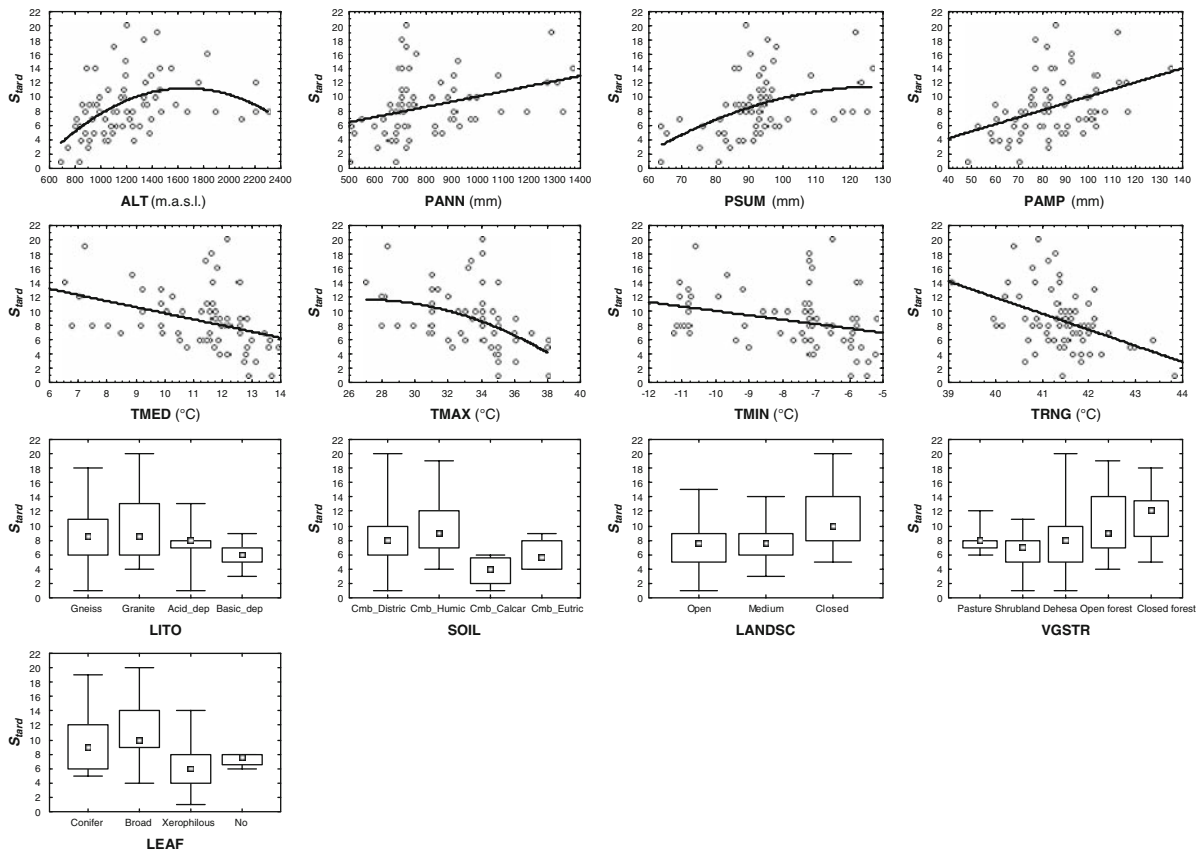
Fig. 2 Relationships between Tardigrada species richness (S_{tard}) and the variables identified as significant in GLM analyses (see Table 1). Continuous predictors are shown as scatterplots, fitted with linear or quadratic functions (depending on the degree of the significant function). Categorical predictors are shown as box and whisker plots, where points are the median S_{tard} value for each category; boxes are second and third quartiles, and brackets are the total range of observations. Variable codes as in text

Discussion

Tardigrade assemblages are generally considered to be strongly influenced by micro-environmental factors, and less by large-scale environmental gradients (e.g., Collins and Bateman 2001). Such belief comes from the well-established "Everything is Everywhere" hypothesis (Baas-Becking 1934), which assumes that microscopic organisms are globally distributed due to their high dispersal potential and

Table 2 Results of partition of the variation of Tardigrada species richness explained by altitude (*Alt*; see Table 1) and other *Explanatory Factors* (*Other*; see functions in Table 1)

Model	d.f.	Dev.	Ch. Dev.	F	Expl.Var.
Null	71	130.7			
Alt (ALT + ALT^2)	69	100.2	30.56	21.06	23.38
raw *Alt*	69	127.9	2.84	1.53	2.17
Other	54	53.8	76.95	77.27	58.86
raw *Other*	54	84.6	46.11	29.42	35.27
Joint effect (Total − raw *Alt* − raw *Other*)					21.76
Total (*Alt* + *Other*)	52	53.3	77.40	75.48	59.21

Other abbreviations as in Table 1

Table 3 Results of partition of variation of Tardigrada species richness explained by effects of *Macro-Environmental variables* (*Env*; i.e., climate + soil characteristics), *Biome structure* (*Biome*) and *Leaf and Moss* (*Leaf*; see Table 1)

Model	d.f.	Dev.	Ch. Dev.	F	Expl.Var.
Env (PANN + TMIN + TMED + PAMP + SOIL)	64	82.8	47.96	37.08	36.69
Biome (LANDSC + VGSTR)	64	84.5	46.23	35.02	35.36
Leaf (LEAF)	68	91.2	39.5	29.42	30.20
Env + *Biome* (a + b + d + e + f + g)	57	63.7	67.02	59.98	51.27
Env + *Leaf* (a + c + d + e + f + g)	61	62.3	68.40	66.96	52.33
Biome + *Leaf* (b + c + d + e + f + g)	61	70.6	60.07	51.87	45.95
Complete model: *Env* + *Biome* + *Leaf*	54	53.8	76.95	77.27	58.86
Partial regressions					
a (res *Env* vs. *Biome* + *Leaf*): rPANN + rTMIN + rTMED + rPAMP + rSOIL	64	112.08	18.64	10.64	14.26
b (res *Biome* vs. *Env* + *Leaf*): rLANDSC + rVGSTR	64	118.6	12.12	6.54	9.27
c (res *Leaf* vs. *Env* + *Biome*): rLEAF	68	120.05	10.66	6.04	8.16

Then, S_{tard} was modelled against these new variables to obtain *a*, *b* and *c*, the pure effects of *Env*, *Biome* and *Leaf*, respectively; *d, e, f, g* are the joint effects of *Env* + *Biome*, *Env* + *Leaf*, *Biome* + *Leaf* and *Env* + *Biome* + *Leaf*, respectively (see Fig. 3). Other abbreviations as in Table 1

their ability of enter dormancy and producing dormant propagules (Fenchel and Finlay 2004; Kellogg and Griffin 2006). Recent studies, however, show that the communities of bdelloid rotifers, another group of microscopic animals also inhabiting mosses, present an effect of habitat filtering at the landscape scale, in spite of having large geographical distributions (Fontaneto et al. 2005, 2006, 2008; Fontaneto and Ricci 2006). Here, habitat filtering means that all sites receive a number of passively dispersed individuals, but the relative suitability of local environmental conditions for each species results in species specific mortality and fecundity rates. Consequently, only a subset of arriving species will establish successful populations. Our results provide similar evidence; a model of macro- and

micro-scale variables explained 60% of the variation of tardigrade species richness in an altitudinal gradient, suggesting that tardigrade communities are strongly affected by habitat filtering according to climate and habitat, which determine to some extent the species that are able to colonize each field plot within the mountain landscape studied (N. Guil, S. Sánchez-Moreno, and A. Machordom, unpublished data).

The origin of altitudinal variations

Tardigrade richness presented a quadratic relationship with altitude, as previously reported by several authors (Rodríguez Roda 1951; Dastych 1987; Beasley 1988; Utsugi et al. 1997; Collins and Bateman
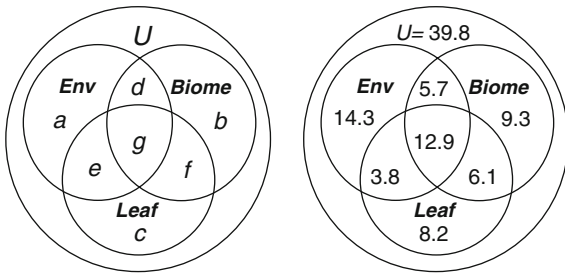
Fig. 3 Variation partition of effects of *Environmental variables* (*Env*; i.e., climate + soil characteristics), *Biome structure* (*Biome*) and *Leaf and Moss* (*Leaf*) over Tardigrada species richness in the 72 sampling sites. Areas *a*,…,*g* in the left drawing represent separate influence over S_{tard} of each one of these three factors, and their joint effects (see text and Table 3). *U* is unexplained variation. A system of equations was applied to obtain the scores for *d*, *e*, *f* and *g*: $[d + e + g = Env - a]$, $[d + f + g = Biome - b]$, $[e + f + g = Leaf - c]$, $[d = (Env + Biome) - (e + f + g) - (a + b)]$, $[e = (Env + Leaf) - (d + f + g) - (a + c)]$ $[f = (Biome + Leaf) - (d + e + g) - (b + c)]$ and $[g = (d + e + g) - d - e = (d + f + g) - d - f = (e + f + g) - e - f]$ (see Lobo et al. 2001; Hortal et al. 2008b)

Table 4 Variation in abundance of Tardigrada individuals (N_{tard}) explained by studied predictors

Variable	d.f.	Dev	ΔDev	F	Expl.Var.	Sign
Null model	71	11886.0				
DIV_ASP	70	11299.5	586.5	3.63	4.93*	–
SOIL	68	10307.3	1578.7	10.41	13.28***	
Cmb_Distric						+
Cmb_Humic						+
Cmb_Calcar						–
VGSTR	67	11275.8	610.2	3.63	5.13*	
Pasture						+
Shrubland						–
Dehesa						–
Open forest						+
Closed forest						+
LEAF	68	10807.0	1079.0	6.79	9.08**	
Conifer						–
Xerophilous						–
DW_RMO	70	11278.6	607.4	3.77	5.11*	–
BCM_RIVAS	65	10587.5	1298.5	7.97	10.92***	

Variable codes as in text. Other abbreviations as in Table 1

2001). The absence of such relationship reported by others (Bartŏs 1939; Nelson 1975; Kathman and Cross 1991; Ito 1999; Nichols 1999) may be explained by the short altitudinal gradient analyzed

in these studies. Altitudes ranging from 1,400 to 1,800 m had the highest tardigrade richness in the Sierra de Guadarrama, with lower number of species at both higher and lower altitudes. A similar pattern was reported by Dastych (1980, 1988) in Poland, Beasley (1988) in New Mexico, and Rodríguez Roda (1951) for the whole continental Spain (highest richness between 1,000 and 2,000 m, and lowest at 0–500 m; Guil 2004). On the other hand, other authors have reported maximum tardigrade richness between 0 and 500 m, falling with increasing altitude (Utsugi et al. 1997 in Japan and Collins and Bateman 2001 in Canada). Our results show that there is a negative relationship between the altitude at which maximum tardigrade species richness occurs and latitude (Table 5 and Fig. 5). These differences could be a result of differences in extent and grain size of the different studies (Rahbek 2005; Nogués-Bravo et al. 2008). Nevertheless, altitude was weakly associated with richness after entering environmental variables into the models, so altitude *per se* is of little or no real importance. To what extent altitudinal patterns detected in species richness are due to micro-habitat variation instead of macro-climatic limitations remains unresolved. This may be a particular challenge for small-sized invertebrate groups, which may be more dependent on habitat variation due to their micro-environmental adaptations.

Environmental and landscape influences on species richness and abundance

Tardigrades undergo passive dispersal (in both cryptobiotic and active states), and their activity depends on moisture conditions. Thus, one of the most important open questions in tardigrade ecology is whether geographic variation in communities is due to the random assemblage of species produced by dispersal processes, or to an actual habitat filtering that determines the species inhabiting each site.

In Sierra de Guadarrama, highest tardigrade richness occurred in closed broad-leaved forests with high precipitation and moderate temperatures. We hypothesize that this kind of landscape provides a highly suitable environment to tardigrades, due to the abundant vegetation and detritus, the presence of other invertebrates that may serve as food sources, and to high moisture levels. Closed forests also offer complex environmental structure, which may provide

Fig. 4 Relationships between Tardigrada abundance (N_{tard}) and variables identified as significant in GLM analyses (see Table 4). Continuous predictors are shown as scatterplots, fitted with linear functions. Qualitative predictors are shown as box and whisker plots, where points are the median N_{tard} value for each category, boxes are second and third quartiles, and brackets are total range of observations. Variable codes as in text
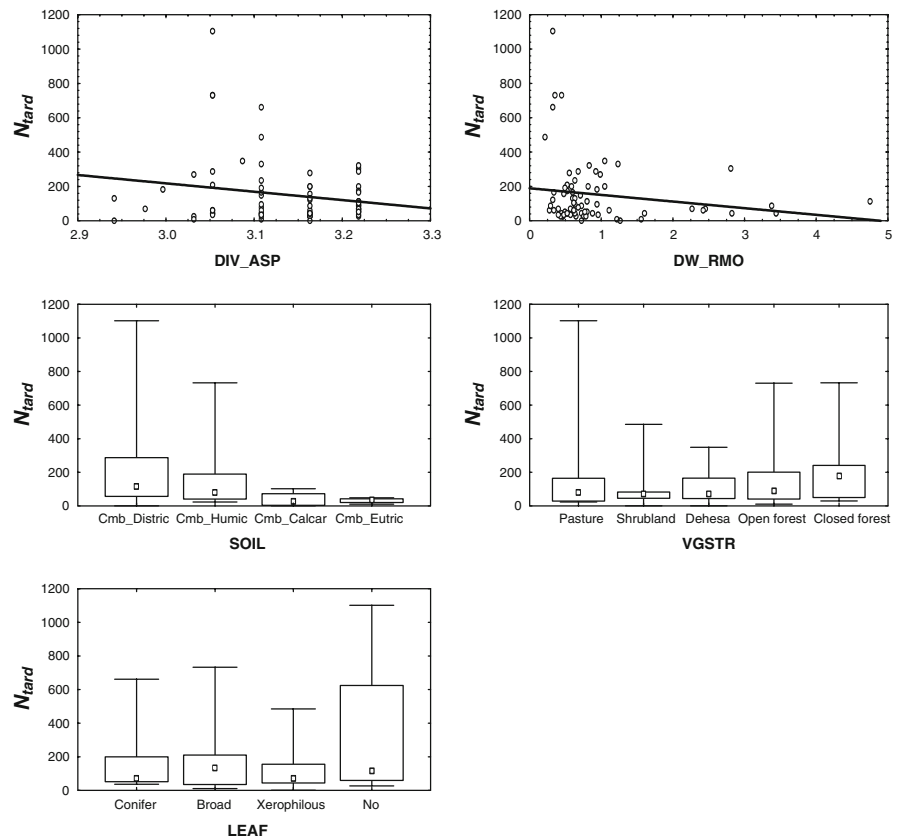

Table 5 Studies including data about the relationship between terrestrial Tardigrada species richness and/or distribution and altitude

Place	Latitude (°N)	Alt.Interv. (m)	Alt. S_{max} (m)	Reference
Spain	40.5	0–2,000	1,500	Rodríguez Roda 1951
Roan Mountain, Southern Appalachians; Tennessee, USA	35	1,219–1,647	1,525	Nelson 1975
Mont Aigoual; France	44.1	–	1,300	Bertrand 1975
Poland	52.5	1,100–2,474	1,450	Dastych 1980
Spitsbergen; Svalbard archipelago	78.5	0–above 1,000	–	Dastych 1985
Poland	52.5	0–2,480	–	Dastych 1987, 1988
New Mexico	34.5	1,524–3,658	1,829	Beasley 1988
Vancouver Island, British Columbia; Canada	49	150–1,525	150	Kathman and Cross 1991
Toyama Prefecture; Japan	36.2	0–2,450	250	Utsugi et al. 1997
Mt. Fuji; Japan	35.5	950–2,380	2,310	Ito 1999
Roan Mountain, Southern Appalachians; Tennessee, USA	35	1,200–1,650	–	Guidetti et al. 1999
Monte Rodinaio and Piane di Mocogno, Modena Apennine; Italy	41	1,200–1,700	–	Guidetti et al. 1999
Newfoundland Island; Canada	55	0–above 1,000	50	Collins and Bateman 2001
Sierra de Guadarrama; Spain	41	680–2,240	1,080	Guil 2004; this study

Alt.Interv. is the altitude interval studied, and Alt. S_{max} the approximate altitude where higher species richness scores were observed
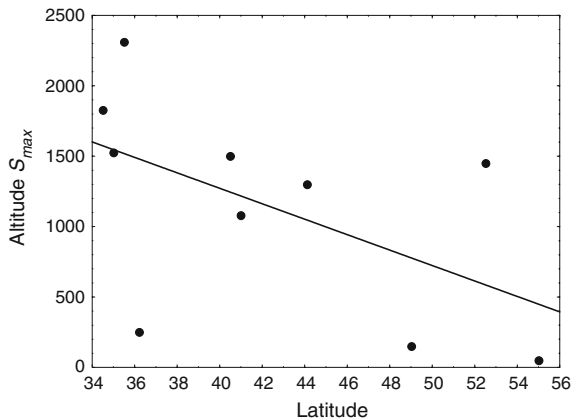
Fig. 5 Relationship between altitude, latitude and maximum species richness found in 10 studies (data in Table 5). Points represent latitude (° N) and altitude (m) coordinates for maximum values of species richness. Continuous line represents the fitted regression function ($S_{max} = 3466.9 - 54.9 \times$ Latitude; see text)

stable conditions for diverse tardigrade species assemblages (Richardson et al. 2005). Our results indicate that leaf litter in these forests from Spanish oak (*Quercus pyrenaica*) and narrow-leaved ash (*Fraxinus angustifolia*) provide the most favourable conditions for the development of complex tardigrade communities.

Few species were found in open areas with xerophilous bushes, high temperatures, high climatic variation, and low precipitation. This kind of habitat provides exposed areas that tardigrades may colonize by passive dispersal. Extreme conditions in such areas are expected to be less suitable to most Tardigrada species. Thus, many individuals found in these open areas could be dispersers that form small populations. In spite of this, these extreme environments host some specific species. One recently described Tardigrada species in the Iberian Peninsula comes from a xerophilous landscape of this region (Guil et al. 2007). This suggests that some species are adapted to hostile environmental conditions, and are therefore exclusive to these landscapes due to habitat filtering processes.

Most variables analyzed here were not related to tardigrade abundance. Only 9.1% of abundance variation is explained by soil type and a bioclimatic classification (while leaf litter of xerophilous and coniferous plants contained significantly lower numbers of individuals, abundance was higher in pastures and open forests; Table 4). Abundance might, in turn,

be mostly dependent on micro-environmental conditions rather than on macro-scale gradients. In spite of this, abundance and richness are positively correlated; so tardigrade community becomes more complex (and abundant) under more suitable macro-environmental conditions. This supports the idea of a hierarchical effect on the assembly of tardigrade communities, where passive habitat filtering is followed by interactions among individuals (N. Guil, S. Sánchez-Moreno, and A. Machordom, unpublished data), and allows for the existence of community-related events (competitive exclusion, facilitation, etc). High abundances were found only at intermediate species richness values (from 5 to 8 species; not shown). This might result from community processes that hinder the dominance (in individuals) of a few single species in highly diverse assemblages. High-abundant, low-diverse assemblages are common in other soil organisms, such as nematodes, that are abundant when opportunistic species dominate (Zelenev et al. 2004). Thus, while tardigrade richness seems to depend largely (but not only) on coarse grain/macro-environmental variables that determine persistence of each species in a given habitat, abundance may depend on microhabitat factors (such as amount of available food, presence of antagonists, etc). Similar patterns in the influence of local micro-habitat type independent from elevation on micro-fauna have been reported elsewhere (Lawton et al. 1987; Richardson 1999; Richardson et al. 2000, 2005; Catterall et al. 2001; Fontaneto et al. 2005, 2006). Interestingly, the best predictors of tardigrade species richness were vegetation structure patterns and bioclimatic classifications, which seem to summarize the complex output of climate, biome and vegetation type, providing highly explanatory (although descriptive) models of richness variation.

Our study is one of the first attempts to understand the ecological and geographic determinants of the diversity of Tardigrada communities. Our results show that large scale variables such as climate or landscape characteristics show strong effects on tardigrade assemblages. Therefore, in spite of their random passive dispersal and their sensitivity to moisture conditions, habitat filtering and community processes must be taken into account when studying tardigrades. We thus hypothesize that the origin and assembly of tardigrade communities is partly determined by the coupled hierarchical effects of external

(i.e., habitat filtering) and internal (i.e., community processes through time) factors. However, further work is needed to test this hypothesis explicitly. Also, little is known about the effects of the factors identified here in other regions and/or extents, nor about the assembly process and the functioning of tardigrade assemblages. We thus encourage other tardigradologists and ecologists to carry out similar studies to increase our limited understanding of the environmental and dispersal processes influencing the structure and composition of tardigrade communities.

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