Deciphering the patterns and drivers of tardigrade diversity along altitudinal gradients

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Abstract

Altitudinal gradients offer a unique opportunity to understand the drivers of species richness, as mountain regions cover vast areas and contribute disproportionately to global terrestrial biodiversity. However, most studies have focused on larger organisms, often neglecting microscopic animals such as meiofauna also in mountain biodiversity research. In this study, we investigated patterns of tardigrade diversity and distribution in the Western Alps (Northern Italy) by compiling an extensive inventory of taxa inhabiting bryophytes. We analyzed 546 bryophyte samples collected across a broad altitudinal gradient and used DNA metabarcoding to characterize tardigrade communities. For each taxon, we gathered functional trait data to assess how species characteristics influence distribution. We then evaluated the effects of macroenvironmental variables (altitude, vegetation type, slope exposition) and microhabitat-level traits (bryophyte biological and structural features) using spatially explicit statistical modeling. We found that species richness decreased with altitude, whereas standardized phylogenetic and functional diversity increased, indicating higher redundancy at lower elevations. Community composition was not driven by specific bryophyte species but rather by general bryophyte functional traits. Our results reveal that tardigrade communities in bryophyte microhabitats are highly heterogeneous, with strong species turnover and prevalent phylogenetic and functional underdispersion. Despite the influence of stochastic processes in shaping their distributions, we show that macroenvironmental variables such as altitude and geographic location drive species turnover, while microhabitat traits govern trait-based community structure. These findings suggest that macroenvironmental gradients shape species distributions, whereas trait-based environmental filtering operates primarily at the microhabitat scale.

Running title: Tardigrade diversity in mountain ecosystems

Keywords: Alps, altitudinal gradient, biodiversity, bryophytes, invertebrates, meiofauna, metabarcoding, Tardigrada.

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Introduction

Altitudinal gradients offer an opportunity to disentangle the causes of broad-scale biodiversity patterns, thanks to their globally replicated nature, and to better understand the threats posed to biodiversity by climate change. Importantly, identifying the main drivers of species richness is a critical task, especially now, in the era of the sixth mass extinction and global environmental change. Biodiversity studies along altitudinal gradients have a long history of research, from the pioneering explorations by renowned scientists such as Alexander von Humboldt; yet, the environmental variables shaping biodiversity remain far from fully understood. A clear interpretation of the biotic and abiotic variables influencing how species richness varies with altitude continues to elude researchers, mostly due to idiosyncratic taxon-specific unique responses (Antonelli *et al.*, 2018; Rahbek *et al.*, 2019a; Dolson & Kharouba, 2024); thus, exploring the drivers of diversity in previously unstudied groups of organisms may provide unexpected and useful insights. Understanding these dynamics is particularly important because mountains cover approximately 25% of the Earth's land area and contribute disproportionately to terrestrial biodiversity. Mountain regions harbor more than three-quarters of the world's vertebrate taxa, many of which are exclusive to these environments (Rahbek *et al.*, 2019b). Therefore, it is not surprising that most studies on the role of altitudinal gradients in shaping biodiversity have focused on relatively large organisms (Rahbek *et al.*, 2019b,a; Dolson & Kharouba, 2024). Comparable research on microscopic animals, such as meiofauna (organisms with body sizes up to 1 mm), remains scarce (Obertegger & Flaim, 2018; Fontaneto, 2019; Morek *et al.*, 2021).

Studies on microscopic animals have long been dominated by the ubiquity paradigm, encapsulated in the 'everything is everywhere' hypothesis (Fenchel & Finlay, 2004; Foissner, 2006). However, recent research has challenged this view, revealing that microscopic animals are not as universally widespread as previously assumed (e.g., Fontaneto, 2019; Morek *et al.*, 2021). Moreover, even if organisms are widely distributed geographically, the 'everything is everywhere' hypothesis suggests that it is the environment that determines which species thrive - 'the environment selects' (O'Malley, 2008). This opens the door for altitudinal gradients in diversity to emerge as a result of environmental variation, also for microscopic animals. Yet, the mechanisms and extent to which environmental predictors select for specific communities or traits in microscopic animals remain largely overlooked (Fontaneto, 2019). While many general ecological patterns, such as species-area and species-energy relationships, are well-established (Andrew *et al.*, 2003; Fontaneto, 2019), their applicability to meiofauna remains unclear (Fontaneto *et al.*, 2006, 2008; Obertegger & Flaim, 2018). Because the community composition of large organisms is easier to characterize than that of microscopic animals, most studies have prioritized research on larger organisms (Green & Bohannan, 2006; Nemergut *et al.*, 2011). Currently, the availability of comprehensive data on the functional traits of microscopic animals, including meiofauna, is extremely limited (Obertegger & Flaim, 2018; Martínez *et al.*, 2025). This issue is further exacerbated by the poorly resolved taxonomy of many microscopic taxa, which are often highly cryptic (Fontaneto *et al.*, 2015; Schenk & Fontaneto, 2020).

Regarding altitudinal gradients, the largely linear relationship between altitude and temperature has led to the consideration of altitudinal gradients as convenient approximations of global biodiversity patterns across latitudes, particularly for understanding the effects of climate change on organisms (Høye *et al.*, 2018). This subject can be approached from a relatively straightforward and widely used perspective: species richness and species composition, defined as the count and identification of species within a given area. However, these measures should be complemented by a careful examination of (phylo)genetic diversity, which accounts for the evolutionary relationships among taxa (Webb *et al.*, 2002), as well as detailed ecological features of the (micro)environment and the functional traits of species within a community (Mace *et al.*, 2003). Mountainous areas, with their altitudinal gradients, provide a highly heterogeneous environment on a small spatial scale, making them ideal for designing natural experiments (Albrecht *et al.*, 2021). The complex climatic characteristics, such as thermal variability, of rugged mountain regions differ fundamentally from those of lowland areas, offering an exceptional setting for high-resolution studies of the distribution patterns of microscopic animals (Bale *et al.*, 2002; Erschbamer *et al.*, 2009; Zawierucha *et al.*, 2015).

One of the most charismatic groups of meiofauna is tardigrades. These aquatic microscopic metazoans range in size from approximately 50 to 1,200 µm (Nelson *et al.*, 2019), with more than 1,400 species described worldwide (Degma & Guidetti, 2024). They can colonize terrestrial environments, living in the thin film of water surrounding soil particles, especially in mosses and lichens (Nelson *et al.*, 2019). In addition, their ability to enter cryptobiosis (a temporary and reversible suspension of metabolism that allows them to enter a diapause stage and resist unfavorable conditions) enables some species to survive extreme stressors such as desiccation and frost (cryptobiosis; Wright, 2001; Guidetti *et al.*, 2011b). Thanks to their small size and unique metabolic adaptations, tardigrades can inhabit a wide range of environments and climatic conditions (Nelson *et al.*, 2019). These microscopic animals also exhibit diverse morphologies, egg-laying strategies, and diets. Moreover, most known species are found in mosses or lichens, which makes them relatively easy to collect in the field. Despite these attributes, tardigrades have long been neglected or superficially addressed in ecological studies of meiofauna. This neglect stems from limited taxonomic and ecological knowledge, insufficient understanding of their general biology, and the labor-intensive process of analyzing large numbers of samples and individuals. However, these challenges are now being mitigated by advancements in high-throughput approaches, such as DNA metabarcoding. This revolutionary tool for biodiversity studies has recently been demonstrated to be both effective and efficient for tardigrade inventories (Topstad *et al.*, 2021; He *et al.*, 2024; Surmacz *et al.*, 2025).

Although a considerable number of studies on mountainous tardigrades have been conducted, only a few inventories explicitly tested the effect of altitudinal gradients on diversity (e.g., Dastych, 1980, 1988; Guidetti *et al.*, 1999; Guil *et al.*, 2009; Kaczmarek *et al.*, 2011; Zawierucha *et al.*, 2015, 2019). Much of the remaining research on this topic consists of descriptive faunistic investigations of mountainous regions (e.g., Beasley, 1988; Bertolani & Rebecchi, 1996). Importantly, the more robust

studies have demonstrated relationships between altitude and tardigrade richness, community structure, and/or abundance, though the direction of these relationships varies (e.g., positive or negative; Zawierucha *et al.*, 2015). These discrepancies may stem from inadequate sampling designs and/or insufficient altitudinal gradients. Moreover, with recent advances in integrative taxonomy that have helped disentangle cryptic species complexes within meiofauna, many of these earlier studies are likely biased in species identification, as none employed integrative approaches or used genetic data (Obertegger *et al.*, 2014). This suggests that tardigrade ecology, particularly in mountainous environments, requires more comprehensive and detailed studies that account for various confounding biotic and abiotic variables (Zawierucha *et al.*, 2015; Nelson *et al.*, 2020). Additionally, while most studies on tardigrade diversity have focused on mosses and lichens, few have investigated the degree to which tardigrade taxa exhibit substrate specificity (Jönsson, 2003; Meyer, 2006; Young *et al.*, 2018; Nelson *et al.*, 2020; Ramsay *et al.*, 2021). It remains unclear whether certain tardigrade taxa are strongly associated with specific mosses or lichens or if most species are generalists. Rare tardigrade species may, for example, be tied to particular substrates, but the extent of such associations is poorly understood. Studies exploring these relationships have yielded inconsistent and sometimes contradictory results, with associations either absent (Meyer, 2006; Zawierucha *et al.*, 2017) or present (Ramsay et al., 2021).

To elucidate the patterns of tardigrade diversity and distribution in a heterogeneous mountain environment, we employed DNA metabarcoding to compile an extensive inventory of taxa from over 500 bryophyte samples collected in the Italian Alps. We gathered trait data for the identified tardigrade taxa, including (i) body size, (ii) buccal tube width (proxy for diet), (iii) egg-laying strategy, and (iv) pigmentation presence or absence, to assess their influence on species distribution. We investigated the impact of macroenvironmental variables, such as altitude, vegetation type, and slope exposition, on tardigrade diversity. Additionally, we examined the extent to which microecological variables, characterized by the biological and ecological traits of bryophytes, shape the composition of tardigrade communities. Our study provides a comprehensive approach to understanding the drivers of tardigrade diversity in mountainous ecosystems by integrating taxonomic, phylogenetic, ecological, and macro- and microclimatic perspectives.

Materials and Methods

Sample collection and processing

A total of 546 bryophyte samples were collected, each with a diameter of approximately 15 cm. The samples were gathered from a mountainous region in northern Italy (Piemonte) across seven localities (transects), which were distributed between 1.4 and 47.5 km apart. Each transect spanned an altitudinal range of 500 to 1063 m, in total covering altitudes from 305 to 2,486 m asl (Figure 1, Table S1). Fieldwork was conducted during one vegetative season in 2023. During collection, samples were placed in paper envelopes, and relevant data for each sample were recorded in a spreadsheet. These records

included GPS coordinates, sample exposition, vegetation type, and the substrate from which the sample was taken. Detailed collection data for each sample are provided in Supplementary Materials SM1. After field collection, the samples were transported to the CNR-IRSA facility in Verbania, Italy, where they were dried at room temperature if necessary. Subsequently, the samples were transferred to the ISEA PAS laboratory in Kraków, Poland for further analysis.

We followed the recently described tardigrade metabarcoding protocol (Surmacz *et al.*, 2025), which includes sieving the soaked cryptogam sample, isolating DNA from sediment using an extraction kit dedicated to soil, amplifying a fragment of Cytochrome C Oxidase I (COI) gene using highly degenerated primers and deep sequencing of the libraries to mitigate the low primer specificity. In brief, before processing, each bryophyte sample was carefully fragmented by hand and soaked overnight in water in a 0.5-liter plastic beaker. The following day, the samples were sieved through a set of metal sieves with mesh sizes of 500 µm, 250 µm, and 36 µm. Approximately 0.25 g of sediment collected in the finest sieve of each sample was transferred into 1.5 ml Eppendorf tubes using a sterile metal spatula, labelled, and stored in a freezer until further processing. The exact weight of each portion was measured with a laboratory scale. The remaining sediment was washed into a 50 ml Falcon tube using a wash bottle and stored frozen as a backup. After washing, the bryophyte material was dried at room temperature, repacked into new paper envelopes, and used for taxonomic identification. Between the processing of different samples, gloves were changed, and all equipment and the sink were sterilized with 20% bleach.

DNA extraction and library preparation

DNA was extracted from a portion of the frozen sample of about 0.25 g using the DNeasy® PowerSoil® Pro Kit (Qiagen). To transfer the sediment from the Eppendorf tube, the first kit solution was added to the tube, mixed with an automatic pipette, and transferred to the bead-beating tube. Then the manufacturer's protocol was followed with a modification involving preincubation with proteinase K, as described in (Surmacz *et al.*, 2025). Extraction blank samples were included to monitor for potential contamination. The final DNA was eluted in 100 µl of elution buffer and stored in a freezer. For metabarcoding, a fragment of the COI gene was used as the target, amplified using primers optimized for tardigrades (BF2_TardF_2 and BR2; Surmacz *et al.*, 2025). Library preparation was performed using a two-step PCR method. The first PCR amplified the target region using region-specific primers with Illumina overhangs. This reaction involved an initial denaturation step of 5 minutes at 95°C, followed by 30 cycles of 30 seconds of denaturation at 95°C, 90 seconds of annealing at 55°C, and 20 seconds of elongation at 72°C, with a final elongation step of 10 minutes at 72°C. The product of the first reaction was then used in a second, indexing PCR to produce uniquely barcoded libraries. This step employed primer sets containing flow-cell binding domains and unique indices from the Nextera XT Index Kit (FC-131-1001/FC-131-1002) and followed the Illumina protocol (Illumina, 2013). The resulting libraries were sequenced on an AVITI instrument (Element Biosciences, San Diego, CA) using 300-bp

paired-end mode. Both library preparation and sequencing were conducted by a commercial provider, IGA Technology, based in Udine, Italy. The raw sequence reads are deposited in NCBI SRA under accession number PRJNA1216760.

Bioinformatic analysis

The demultiplexed reads were analyzed using a custom pipeline based on vsearch (Rognes *et al.*, 2016). The paired reads were assembled into contigs and quality-filtered using PEAR v0.9.11 (Zhang *et al.*, 2014), using default parameters. Then, the primer sequences were trimmed using Cutadapt 4.6 (Martin, 2011) with default parameters, and contigs without both primer sequences or of incorrect length after trimming (419-424 base pairs) were discarded. The trimmed contigs were dereplicated, denoised, and screened for chimeras using the USEARCH- UCHIME (Edgar *et al.*, 2011). The resulting denoised zero-radius Operational Taxonomic Units were clustered into OTUs with a 97% similarity threshold (Surmacz *et al.*, 2025) to remove potential erroneous variants and the effects of intraspecific variability. Then, the post-clustering curation of the OTU table was done using the R package 'lulu' (Frøslev *et al.*, 2017) with default parameters to remove potential erroneous OTUs. The OTUs' representative sequences were translated into amino acids using the R package 'Biostrings' (Pagès *et al.*, 2023) using translation table 5, and those containing stop codons or indels were removed from the analysis.

To classify the tardigrade sequences, as well as the other eukaryotes, we used the 'Tardi-COI' database (v.02; Surmacz *et al.*, 2025) merged with 'MIDORI2' database (v. GB260; Leray *et al.*, 2022) to achieve a reliable classification of tardigrades and high coverage of outgroups, reducing the probability of false positive classifications as Tardigrada. The taxonomy was assigned by classifying the representative sequences of each OTU by a local BLAST search with parameters *evalue* = 0.00001, *pident* = 85 (Altschul *et al.*, 1990; Camacho *et al.*, 2009) using a top-hit classification method (Hleap *et al.*, 2021) with a widely accepted threshold of 85% sequence identity for phylum-level classification (Clarke *et al.*, 2021; Macher *et al.*, 2024; Surmacz *et al.*, 2025). For OTUs classified as Tardigrada the species-level taxonomy was assigned to OTUs with at least 97% similarity to the reference sequence representing a described species, while in cases when the reference sequence belong to unnamed species or similarity to the reference sequence is lower (85-97%) only genus-level taxonomy was considered (Surmacz *et al.*, 2025). The complete bioinformatics pipeline is provided in Supplementary Material 2.

To analyze the phylogenetic diversity, a phylogenetic tree was constructed based on the representative sequences of recovered tardigrade OTUs, which were aligned using MAFFT v7.520 (Katoh & Standley, 2013). Before partitioning, the alignment was divided into three data blocks, constituting three codon positions in the COI data set. Using PartitionFinder (Lanfear *et al.*, 2017) under the Akaike Information Criterion (AIC), the best scheme of partitioning and substitution models was chosen for Bayesian phylogenetic analysis. Bayesian inference (BI) marginal posterior probabilities were calculated for the COI data set using MrBayes v3.2 (Ronquist & Huelsenbeck, 2003). Random starting trees were used, and the analysis was run for fifteen million generations, sampling the Markov

chain every 1,000 generations. An average standard deviation of split frequencies of <0.01 was used as a guide to ensure the two independent analyses had converged. The program Tracer v1.7 (Rambaut *et al.*, 2018) was then used to ensure Markov chains had reached stationarity and to determine the correct 'burn-in' for the analysis, which was the first 10% of generations. The ESS values were greater than 200, and the consensus tree was obtained after summarizing the resulting topologies and discarding the 'burn-in'. The raw tree is provided in Supplementary Material 3.

Explanatory variables

To examine the variables influencing tardigrade diversity and distribution in mountainous regions, we selected a range of explanatory variables associated with each bryophyte sample, which can be categorized into macroenvironmental and microhabitat variables. The first group pertains to broader environmental conditions and includes altitude, vegetation type, slope orientation, and geographic coordinates. The slope orientation values were derived from the Digital Elevation Model (OpenTopography, 2013) using the R package 'whitebox' (Lindsay, 2016), as aspect values (degrees) converted to the values of eastness and northness, ranging from −1 to +1 by sine and cosine transformations of slope orientation, respectively. The second group of explanatory variables is related to the tardigrade microhabitats (bryophytes) – exposition, substratum, bryophyte species, and their characteristics from the Bryophytes of Europe Traits (BET) dataset (van Zuijlen *et al.*, 2023). The identified bryophytes follow the standardized nomenclature of The World Flora Online Plant List (http://www.worldfloraonline.org/, accessed 2025-01-25). A complete list of the predictors is provided in Table 1, while the list of samples, along with the corresponding data, is available in Supplementary Materials SM.1.

Tardigrade trait data

To examine how functional traits of tardigrades relate to environmental variables, we coded each final OTU for four specific traits: two continuous morphometric traits: (*i*) body size (maximum body length, BL) and (*ii*) buccal tube width (BTW, maximum value); and two binary traits: (*iii*) body pigmentation (presence = 1, absence = 0), and (*iv*) egg-laying strategy (eggs laid within exuviae = 1; eggs laid freely in the environment = 0). Maximum values were used for morphometric traits to better represent adult morphology and minimize bias from immature individuals in source datasets. For OTUs confidently assigned to nominal species (≥95% sequence similarity to described species), BL and BTW values were taken from original descriptions, redescriptions, or taxonomic revisions. In case of trait assignment, we selected a 95% similarity threshold instead of the more commonly used 97% because tardigrade species exhibiting such genetic divergence (between 5–10 % in COI) are often morphologically and morphometrically indistinguishable (Morek *et al.*, 2019; Stec *et al.*, 2022; Brandoli *et al.*, 2024). In such cases, trait values derived from the named species are likely to more accurately reflect the focal OTUs than genus-level means. For OTUs identified only to the genus level, we imputed BL and BTW using genus-level means calculated from up to five species, prioritizing descriptions from similar climatic

regions. BTW values are typically unavailable for heterotardigrades due to the frequent dissolution of their buccal apparatus in mounting media. To overcome this, we selected samples containing the five representative heterotardigrade genera detected in our metabarcoding data. For each genus, we located target taxa and measured the BTW of five large individuals under a microscope. The maximum observed value was then assigned to all OTUs representing that genus. Supplementary Materials SM.4 include (*i*) a list of all OTUs assigned to described species with their coded traits, (*ii*) species and values used to estimate genus-level BL and BTW, and (*iii*) heterotardigrade genera with BTW that was measured de novo. A list of all trait data sources is provided in Appendix 1.

Hypotheses testing

All the statistical procedures, data processing, and visualization were performed using R 4.3.2 (R Core Team, 2023). To check if our sampling was sufficient to describe the regional tardigrade fauna, we visualize a sample-based species accumulation curve calculated using the 'specaccum' function from the R package *vegan* version 2.6-8 (Oksanen *et al.*, 2025). In further analyses, we aimed to test how our predictors shape tardigrade communities along altitudinal gradients at the taxonomic, phylogenetic, and functional levels, including both within-community alpha diversity and beta diversity patterns. Regarding the bryophyte traits, due to the rarity of dendroid life form, the category 'dendroid' of the variable 'life form' was merged with 'weft', forming a category 'weft or dendroid', as both groups have a multi-layer structure and indicate shady habitats (Mägdefrau, 1982). We did not include the selected ecological indicator values (EIVs) for bryophytes in the models because of the large share of missing data (10-30%). Instead, we analyzed Spearman's correlation coefficient between the selected EIVs and bryophyte growth form, binary coded to support the potential ecological findings. After explanatory analyses, we chose a set of independent variables with low multicollinearity (VIF<10), discarding the variable 'eastness'. To test the joint and individual effects of microhabitat and macroenvironmental variables on tardigrade diversity patterns, we created separate models for the two variable groups (for variable explanation, see Table 1).

Alpha diversity:

We tested the effect of environmental predictors along altitudinal gradients on the three levels of alpha diversity (taxonomic, phylogenetic, and functional). As taxonomic diversity, we refer to OTU richness (number of OTUs detected in a sample). For the analysis of phylogenetic diversity, we used the standardized effect size of Faith's Phylogenetic Diversity (Faith, 1992) (standardized phylogenetic diversity) calculated using the R package *picante* version 1.8.2 (Kembel *et al.*, 2010) using 999 permutations. To quantify the functional diversity, we calculated Rao's Q diversity index using the R package *FD* version 1.0-12.3 (Laliberté & Legendre, 2010). The values of BL and BTW were log-transformed due to the skewness and standardized to zero mean and unit variance before the analyses of functional diversity. In addition, to overcome the dependence of functional diversity on taxonomic

diversity, we analyzed the standardized effect size of Rao's Q diversity index (Ricotta *et al.*, 2022), calculated as the difference between the observed Rao's Q and the mean value of the index calculated using randomly permuted communities of the same size, divided by the standard deviation of null models' Rao's Q values, using 999 permutations (standardized functional diversity). The significance of the departures from random assembly was tested using one-tailed Monte Carlo tests: when the observed Rao's Q or Faith's PD were higher or lower than 95% of null communities, we indicated a significant under- or overdispersion.

Firstly, to directly test for the existence of altitudinal trends of the three diversity aspects, we used univariate generalized linear models with altitude as the only predictor. For taxonomic diversity we used a model with negative binomial error structure while for standardized phylogenetic and functional diversity we used models with Gaussian error structure. To get a deeper insight into ecological effects of tardigrade diversity, we fitted generalized linear models with a series of different predictors on microhabitat and on macroenvironmental variables using the R package *spaMM* version 4.5.0 (Rousset & Ferdy, 2014). To model the effect of microhabitat, we used nonspatial models using the formula: Y ~ substratum + rK + gform + lform + size + sample exposition. To model the macroenvironmental variables, we used a spatially autocorrelated model using the formula: Y~ altitude + northness + vegetation type + Matern(1 | Latitude + Longitude). The full models included all microhabitat and macroenvironmental variables and spatial autocorrelation. In the functional and phylogenetic diversity models, we included the OTU richness as an additional predictor to disentangle the effect of environmental predictors from the effect of taxonomic richness on these diversity metrics. Taxonomic diversity was modelled using generalized linear models with negative binomial distribution and all samples (n=546), while standardized phylogenetic and functional diversity were analyzed using generalized linear models with Gaussian distributions, keeping only the samples with at least two OTUs (n=500). We tested the relations between the three levels of alpha diversity using the three formulas (microhabitat model, macroenvironment model, and full model). For all the models, we calculated 95% confidence intervals of models' effects using the 'confint' function from R package *spaMM*. We compared the pseudo-R^2 calculated using the 'pseudoR2' function from the R package *spaMM* and conditional AIC values (cAIC) to evaluate goodness of fit. For taxonomic diversity, we calculated pseudo-R^2 values to evaluate the total variance explained by the models. For phylogenetic and functional diversity, we used pseudo-R^2 to investigate the increase of variance explained relative to the model including OTU richness as the predictor to test the effects of environmental variables, accounting for the confounding effect of taxonomic richness. We also checked all the models for zero-inflation and overdispersion using the 'testZeroInflation' and 'testDispersion' functions from the R package *DHARMa* version 0.4.7 (Hartig *et al.*, 2024), which indicated no significant overdispersion and no evidence that a zero-inflated term was necessary ($p>0.05$).

Beta diversity and trait-environment associations:

We investigated the beta diversity patterns of the three diversity aspects (taxonomic, phylogenetic and functional). For taxonomic diversity, we used Jaccard dissimilarities, for phylogenetic beta diversity we used unweighted Unifrac distances, and for trait dissimilarities, we used Euclidean distances between community means (Lengyel & Botta-Dukát, 2023), as more complex methods would likely be biased due to overrepresented imputed values for means for the genera and the low OTU richness in many samples. To check whether species replacement or nestedness contribute to the observed beta-diversity patterns, we calculated the Jaccard beta diversity and its nestedness and turnover components for taxonomic beta diversity and Unifrac distances for phylogenetic beta diversity using the R package *betapart* version 1.6 (Baselga & Orme, 2012). We did not perform such analysis for the functional beta diversity, as due to the limitations of the trait dataset, we analyzed only patterns of community means. To investigate the overall beta-diversity patterns, we tested the relationships between taxonomic, phylogenetic, and trait dissimilarities using distance-based redundancy analysis (db-RDA) using the 'dbrda' function from the R package *vegan*. Firstly, we created models with altitude as the only predictor, to simply test the effect of altitude on beta diversity. Then, we separately analyzed microhabitat and macro-environmental variables in separate db-RDA models. To include the spatial components in beta-diversity models, we included the distance-based Moran's eigenvector maps as predictors (dbMEM1 and dbMEM2), calculated using the R package *adespatial* version 0.3-24 (Dray *et al.*, 2025) based on the distance matrix calculated using the R package *geodist* version 0.1.0 (Padgham *et al.*, 2025), representing large-scale geographical patterns. To model the effect of microhabitats, we used the model formula Y ~ substratum + rK + gform + lform + size + sample exposition. For macroenvironment models, we used the formula Y~ altitude + northness + vegetation type + dbMEM1 + dbMEM2. The full models included all the microhabitat and macroenvironmental predictors and the spatial predictors. We tested the significance of the predictors in all models (altitude-only, microhabitat, macroenvironment, and full model), performing a permutation test using 'anova.cca' function from the R package *vegan* using 999 permutations. We checked the relative explanatory power of microhabitat and macroenvironmental variables by comparing the values of R^2_{adj} of the three models.

To verify the significant variables shaping tardigrade communities indicated by db-RDA, we investigated the relationships between tardigrade occurrence data and environmental predictors using a multivariate model-based analysis, fitting the generalized linear models with binomial distribution using the 'manyglm' function from the R package *mvabund* (Wang *et al.*, 2012) and testing the significance of the effect of all the predictors (both microhabitat and macroenvironmental) on OTU distribution using 'anova.manyglm' function from *mvabund* R package calculating likelihood ratio deviance, using 999 iterations via probability integral transform residual bootstrap resampling (PIT-trap). We also used the 'anova.manyglm' function to test whether bryophyte species would better predict the tardigrade communities than the general bryophyte trait categories. We achieved this by comparing the two models. In the first model we used the same formula as one used in db-RDA (Y ~ substratum + rK+ gform + lform + size + sample exposition + altitude + northness + vegetation type + dbMEM1 + dbMEM2). In

the second model, instead of bryophyte traits (rK, gform, lform, size), we included bryophyte species (Y ~ substratum + bryophyte species + sample exposition + altitude + northness + vegetation type + dbMEM1 + dbMEM2). We also aimed to investigate the relationships between specific tardigrade taxa and environmental covariates (at first, without linking the responses to tardigrade species traits). Due to a high total OTU richness and prevalence of rare taxa, we did not model the response of each OTU separately, but instead, we tested and visualized the responses of tardigrades with genus-level resolution (presence-absence data of tardigrade genera, based on the respective classification of metabarcoding OTUs). To achieve this, we tested the genus-level tardigrade-environment associations using a single predictive model (multivariate species distribution model) with binomial distribution with L1 (LASSO) penalty, which automatically sets terms in the model that do not explain any variation in species composition to zero by 'traitglm' function from the *mvabund* R package.

Finally, we applied the predictive fourth-corner approach (Brown *et al.*, 2014) to construct a regression model for OTUs' presence as a function of interaction terms between tardigrade trait and environmental variables, using the 'traitglm' function from the *mvabund* R package with a binomial response and LASSO penalty, including all the microhabitat and macro-environmental variables and dbMEMs. To visualize the altitudinal patterns of common species belonging to the most widespread genus, we plotted the spline smoothing functions of univariate logistic generalized additive models (GAM) in the R package *mgcv* version 1.9-0 (Wood, 2011) to model different shapes of altitudinal distributions.

Results

Data summary

The collection of 546 samples from seven transects included 103 bryophyte species representing 62 genera and 11 orders. The most common substrates for the collected bryophytes were rocks (78% of samples), soil (7%), wood (10%), and anthropogenic surfaces (4%). The analysis of ecological indicator values (EIVs) revealed that bryophyte growth form was associated with specific indicator values. Specifically, acrocarpous species were positively correlated with EIVs for light (Spearman's rank correlation test, $n = 471$, $\rho = 0.31$, $p < 0.001$) and negatively correlated with EIVs for reaction/acidity ($n = 470$, $\rho = -0.43$, $p < 0.001$) indicating their preferences for sun-exposed and acidic substrates.

The sequencing of metabarcoding libraries for all the samples generated a total of 204.75 million read pairs, with an average of 375,000 per sample. Among these, 29.33 million read pairs passed all the filtering steps, and 3.23 million were classified as Eukaryotes based on an 85% sequence identity threshold. Among the classified OTUs, the most common phyla (in terms of the number of reads) were Tardigrada (43.77%), Ascomycota (33.37%), Chlorophyta (5.62%), Basidiomycota (4.22%), and Arthropoda (3.55%). The final tardigrade OTU table contained 1.41 million reads, clustered into 169 OTUs representing 11 families of limnoterrestrial tardigrades: Acutuncidae, Adorybiotidae, Calohypsibiidae, Echiniscidae, Isohypsibiidae, Itaquasconidae, Macrobiotidae, Milnesiidae,

Murrayidae, Pilatobiidae, and Ramazzottiidae. None of the OTUs revealed stop codons or indels. Among tardigrade OTUs, 99 OTUs (58.58%) confidently matched reference sequences (≥97% identity) while 70 OTUs (41.48%) matched the reference sequences with lower similarity (85-97% identity). The results suggest no apparent bias in taxa recovery since all the major taxonomic groups of limnoterrestrial tardigrades were successfully detected (Figure 2a), and no sequences representing marine or obligate freshwater tardigrade taxa were found. The blank samples were negative for tardigrades, indicating no contamination. The species accumulation curve (Figure S2.1) reached a plateau, indicating that our sampling intensity was sufficient to characterize the tardigrade fauna in the studied region. Of the detected OTUs, 136 (80.47%) occurred in more than one sample. The most widespread were *Macrobiotus hufelandi* (found in 47.99% of samples), *Dianea sattleri*, and both *Echiniscus merokensis* and *Macrobiotus* aff. *hufelandi* (OTU2 in this study; reference sequence HQ876589), each present in 26.74% of samples. The trait dataset was compiled using raw morphometric values extracted from 97 publications (Appendix 1). In total, we used 169 measurements of body length (BL) and 136 measurements of buccal tube width (BTW), while five maximum BTW values for five heterotardigrade genera were obtained *de novo*. We obtained species-specific trait values only for 54 OTUs assigned to described species by at least 95% similarity, whereas for other OTUs (either assigned to putative species or to sequences not identified to species level), we imputed the genus-specific means. Of all OTUs, 54 (32%) were classified as pigmented tardigrades, while 115 (68%) were scored as lacking pigmentation. Regarding the egg-laying strategy, 105 OTUs (62%) were identified as tardigrades that oviposit eggs in exuviae, and 64 OTUs represented species that lay eggs freely in the environment. The trait dataset revealed three distinct clusters of tardigrade taxa representing three different orders (Figure 2b), indicating a clear link between traits and phylogeny.

Patterns of alpha diversity

The metabarcoding results identified 531 samples (97.25% of the dataset) as positive for tardigrades. The mean taxonomic richness was 6.70 tardigrade OTUs per sample (SD = 4.34). A univariate model with altitude as the sole predictor indicated a negative effect of altitude on taxonomic diversity, expressed as species richness (negative binomial GLM: $p<0.001$; Figure 3, Table S2.1). In contrast, both phylogenetic and functional diversity were positively affected by altitude (LM: $p<0.001$ and LM: $p=0.004$, respectively; Figure 3, Tables S2.2-3).

The GLM results indicated that both microhabitat and macroenvironmental variables affected taxonomic alpha diversity (microhabitat model: pseudo-R^2 = 0.206, cAIC = 2955.4, Table S2.4; macroenvironment model: pseudo-R^2 = 0.282, cAIC = 2839.2, Table S2.5; full model: pseudo-R^2 = 0.388, cAIC = 2721.9). In the full model, taxonomic diversity was negatively affected by altitude and positively affected by rock substrate, shoot size, and pleurocarpous growth form (Figure 4, Table S2.6).

Phylogenetic clustering was significant in 166 of the 500 samples with at least two OTUs (33.2%; one-tailed Monte Carlo test, $p < 0.05$), while phylogenetic overdispersion was detected in six

samples (1.2%; one-tailed Monte Carlo test, $p < 0.05$). Standardized phylogenetic diversity was also influenced by environmental variables, particularly those related to the microhabitat (microhabitat model: pseudo-$R^2 = 0.107$, cAIC = 1513.1, Table S2.7; macroenvironment model: pseudo-$R^2 = 0.043$, cAIC = 1531.8, Table S2.8; full model: pseudo-$R^2 = 0.136$, cAIC = 1501.1, Table S2.9). In the full model, phylogenetic diversity was negatively affected by northness, soil substrate, r-selected bryophyte strategy, pleurocarpous growth form and mat and turf life forms (Figure 4). In all the models, standardized phylogenetic diversity was negatively affected by species richness.

Functional underdispersion was significant in 224 samples (one-tailed Monte Carlo test, $p < 0.05$), while functional overdispersion was observed in four samples (0.8%). Standardized functional diversity was more strongly affected by microhabitat than by macroenvironmental variables (microhabitat model: pseudo-$R^2 = 0.111$, cAIC = 1344.5, Table S2.10; macroenvironment model: pseudo-$R^2 = 0.030$, cAIC = 1375.1, Table S2.11; full model: pseudo-$R^2 = 0.130$, cAIC = 1342.8, Table S2.12). In the full model, functional diversity was negatively affected by altitude, pleurocarpous growth form, shoot size, shaded microhabitats, and turf life form (Figure 4). In all the models, standardized

Patterns of beta diversity

The mean pairwise Jaccard turnover index was 0.862 (SD = 0.198), with complete turnover (i.e., no shared OTUs) observed between 49.94% of sample pairs. The overall variation in tardigrade communities was primarily driven by the turnover component of beta diversity rather than the nestedness component, with turnover accounting for 99.90% of total taxonomic beta diversity. Thus, we did not run separate analyses for the negligible nestedness component and performed only the analyses on the overall Jaccard beta diversity for taxonomic diversity. The univariate db-RDA models revealed a significant effect of altitude on all the three beta-diversity aspects: taxonomic ($R^2_{adj} = 0.035$), phylogenetic ($R^2_{adj} = 0.049$) and functional ($R^2_{adj} = 0.022$), which was confirmed by permutation tests (n=999), (p=0.001 in all three models).

In the full models, the analyzed predictors explained a small proportion of total beta diversity in db-RDA results: taxonomic ($R^2_{adj} = 0.107$), phylogenetic ($R^2_{adj} = 0.149$), and functional dissimilarity ($R^2_{adj} = 0.176$; Figure 4). Permutation tests using anova.cca (n = 999) showed that all terms in the db-RDA models were significant ($p < 0.05$), except for the effect of dbMEM1 (p=0.218), r/K strategy (p=0.218), and bryophyte size (p = 0.337) on functional dissimilarity (Tables S2.13-15).

In the taxonomic and phylogenetic datasets, the first db-RDA axes captured the altitudinal gradient and large-scale spatial structure, explaining 4.29% of the constrained variance of the taxonomic diversity and 5.82% of the constrained variance of phylogenetic diversity. For trait-based beta diversity, the first db-RDA axis explained 14.04% of the constrained variance and was associated with microhabitat light and moisture conditions. Positive axis values corresponded to shady microhabitats, turf life form, and north-facing slopes (Figure 4). Model comparison revealed that macroenvironmental variables were stronger predictors than microhabitat-related variables for explaining taxonomic and

phylogenetic beta diversity (Figure 4). In contrast, microhabitat variables explained more variance in functional dissimilarity (Figure 4). These findings suggest that large-scale variables, including the altitudinal gradient, primarily influence taxonomic community composition, while species traits are more closely linked to local-scale (microhabitat) conditions such as bryophyte life form, substrate type, and sample exposition.

An analysis of deviance from multivariate GLMs indicated that including bryophyte species identity did not significantly improve model performance ($p = 0.076$, Table S2.16). Consequently, we focused further analyses on models incorporating bryophyte trait predictors ($\sum$AIC = 22,754.9) and discarded the species-level model ($\sum$AIC = 49,845.42), as species-level patterns could be accounted for by general trait categories. The final analysis of deviance showed that all remaining predictors significantly explained variation in OTU distributions (Table S2.17).

Taxon-environment and trait-environment relationships

The genus-level multivariate model indicated that several environmental predictors significantly influenced the distribution of tardigrade taxa (Figure 5). The genera *Mesobiotus*, *Ramazzottius*, and *Minibiotus* were strongly associated with lower altitudes, whereas *Milnesium* exhibited a weak opposite trend. Different taxonomic groups also displayed preferences for distinct microhabitat conditions. Several genera were clearly linked to more humid or shaded microhabitats (characterized by tall/long shoots, pleurocarpous mosses, turf/weft/dendroid life forms, north-facing slopes, or overall shade), including *Degmion*, *Dianea*, *Diphascon*, and *Guidettion*. In contrast, genera such as *Echiniscus*, *Milnesium*, and *Ramazzottius* were associated with more xerothermic conditions, including cushion-forming mosses and exposed environments. Spatial variables (dbMEM1 and dbMEM2) revealed multiple significant associations, indicating variation in community composition across localities. Vegetation type and substrate-related variables also showed specific associations, for example, *Adropion* was linked to bryophytes growing on soil, while *Degmion* was associated with samples from coniferous forests.

The fourth-corner analysis further revealed numerous trait–environment relationships (Figure 6). Among the strongest associations were those between tardigrade body length and both pleurocarpous growth form and north-facing (negative northness) microhabitats. Oviposition in exuviae was positively associated with both altitude and pleurocarpous bryophytes. Buccal tube width showed negative associations with several variables, including pleurocarpous bryophytes, exposed samples, and meadow habitats. Pigmentation was negatively associated with microhabitats that received less light (e.g., pleurocarpous bryophytes, wefts or dendroid life forms, shaded environments, north-facing slopes, and soil substrates), and positively associated with sun-exposed microhabitats, including those with acrocarpous and cushion mosses, as well as anthropogenic or tree bark substrates.

Discussion

Overview and key findings

Our results indicate that multiple mechanisms influence the distribution and diversity of tardigrade communities across altitudinal gradients. However, macroenvironmental- and microhabitat-related variables explain only a small fraction of the observed patterns. This limited explanatory power seems to align with broader theories of community assembly, which emphasize the role of stochastic processes in shaping communities of small, highly dispersive taxa (Jenkins *et al.*, 2007; De Bie *et al.*, 2012; Farjalla *et al.*, 2012; Soininen *et al.*, 2013). Such processes likely result from frequent colonization-extinction cycles driven by the ephemeral nature of their microhabitats, disrupting the tight coupling between habitat conditions and community composition. In contrast, larger, more actively mobile taxa tend to exhibit more stable hierarchically structured communities shaped by competitive interactions and habitat selection (Van Allen & Rudolf, 2015; Boyce *et al.*, 2019; Martin & Ghalambor, 2023; Chen & Lewis, 2024), underlying difference in the mechanisms driving diversity across size scales. Tardigrade communities in bryophyte microhabitats are highly diverse, with rapid species turnover across landscapes, and tend to show high redundancy and low functional and phylogenetic diversity. While the altitude and geographical location are the primary variables shaping compositional turnover in tardigrade communities, microhabitat conditions primarily drive community trait patterns. This indicates that macroenvironmental variables shape species distributions, but trait-driven environmental filtering operates at the microhabitat scale. In the following sections, we discuss the effects of environmental variables and trait-environment associations in detail.

Altitudinal gradient of tardigrade diversity

Our study reveals a shift in tardigrade communities along the altitudinal gradient: from species-rich, functionally redundant, and phylogenetically underdispersed communities at lower altitudes to less diverse communities comprising phylogenetically and functionally more distant species at higher altitudes. The altitudinal decline in species richness observed in our study reflects a well-documented pattern across diverse taxa, including plants, vertebrates, and invertebrates (Rahbek, 1995; McCain & Grytnes, 2010). This pattern seems also to be the most prevalent in tardigrades, supported directly by several studies (e.g., Dastych, 1985; Collins & Bateman, 2001; Zawierucha *et al.*, 2015, 2019). Other studies suggest a unimodal distribution, with species richness peaking at mid-altitudes (Rodríguez-Roda, 1951; Beasley, 1988; Dastych, 1988; Guil *et al.*, 2009; Kaczmarek *et al.*, 2011). Among other meiofauna, such as rotifers and nematodes, similar taxonomic richness patterns in mountain environments have been reported. Specifically, Fontaneto & Ricci (2006) and Obertegger *et al.*, (2010) found that rotifer species richness decreases at higher altitudes, a pattern also observed in soil nematodes (e.g., Afzal *et al.*, 2021; Kashyap *et al.*, 2022; Li *et al.*, 2024).

While altitudinal patterns of tardigrade taxonomic diversity have been extensively studied, phylogenetic and functional diversity have received far less attention. Previous studies have not explicitly tested phylogenetic diversity in tardigrades along altitudinal gradients, and functional

565 diversity has generally shown weak or no altitudinal effect (Zawierucha *et al.*, 2019). Our findings,
566 however, suggest that standardized phylogenetic and functional diversity is higher at higher altitudes,
567 though this trend is largely driven by microhabitat variation (Figure 3) and potentially biased by reduced
568 species richness. This pattern aligns with observations that extreme environments tend to support
569 communities of fewer specialized species (e.g., Swenson *et al.*, 2012; Stuart-Smith *et al.*, 2013; Siefert
570 *et al.*, 2015). Tardigrades that are better competitors in extreme environments are typically those with
571 enhanced cryptobiotic capabilities, allowing them to survive prolonged unfavorable conditions while
572 other organisms perish (Jönsson, 2001, 2005). In the harsh localities at high altitude, we detected
573 specialized taxa representing the clades of Parachela, Apochela, and Echiniscoidea, which represent
574 divergent clusters in both phylogenetic and functional trait space (Figures 2 and 7a). This well
575 corresponds with previous studies which observed Echiniscoidea and Apochela more frequently at high
576 altitudes (e.g., Ramazzotti & Maucci, 1983; Dastych, 1985, 1988; Guil *et al.*, 2009; Zawierucha *et al.*,
577 2015, 2016, 2019). Notably, Heterotardigrada and Eutardigrada independently evolved cryptobiotic
578 adaptations to survive desiccation (Fleming *et al.*, 2024), but despite their long evolutionary history
579 which led to morphological, physiological, and phylogenetic distinctiveness, both lineages include
580 exceptionally resilient extremophiles capable of occupying similar niches. It suggests that phylogenetic
581 distance is not necessarily a proxy for the difference in habitat preferences among tardigrade taxa.
582 Despite the covarying effects of altitude and microhabitat features, our results reveal a
583 compositional shift along the altitudinal gradient, highlighting the predominant role of macro-
584 environmental variables, including altitude, in shaping taxonomic beta diversity. This is exemplified by
585 the genus *Macrobiotus* (Figure 6b), which includes multiple co-occurring species at lower altitudes (e.g.,
586 *M. sandrae*, *M. vladimiri*, *M. hufelandi*) that are morphologically and phylogenetically similar
587 (Bertolani *et al.*, 2011; Stec *et al.*, 2021). In contrast, at higher altitudes, only *Macrobiotus* aff. *hufelandi*
588 (OTU2) tends to dominate, possibly due to competitive exclusion. Notably, this altitude-related pattern
589 mirrors that observed in the original study describing these haplogroups: *M.* aff. *hufelandi* was
590 predominantly found at higher altitudes (Bertolani *et al.*, 2011). At lower altitudes, multiple closely
591 related species often coexist, leading to higher taxonomic richness but lower phylogenetic and functional
592 diversity, while higher altitudes typically support taxonomically poorer but more functionally and
593 phylogenetically dispersed communities (Figures 2 and 7a). This pattern likely reflects a shift in
594 microhabitat conditions along the altitudinal gradient, which, despite not being captured in full models
595 (Figure 4), contributes to the observed higher values of standardized phylogenetic and functional
596 diversity at higher altitudes. This divergence may result from stronger selection favoring stress-tolerant
597 species in extreme, high-altitude environments.
598
599 *Microhabitat effect on tardigrade diversity*
600 Our results highlight the importance of microhabitat variables in shaping tardigrade diversity,
601 particularly functional diversity (Figure 4). We found that large, pleurocarpous mosses and bryophytes

602 growing on rocks host species-rich communities, while short, acrocarpous mosses in exposed habitats
603 support fewer but more functionally and phylogenetically diverse species. This pattern is consistent with
604 island biogeography models, where larger, more structurally complex habitats support higher species
605 richness and stronger phylogenetic underdispersion (Zhang *et al.*, 2023). In our dataset, pleurocarpous
606 moss mats or carpets at lower altitudes supported diverse but functionally underdispersed communities
607 dominated by Parachela (Figures 3, 4, and 7a), while smaller patches of acrocarpous mosses in exposed
608 habitats hosted fewer, more specialized taxa. This finding aligns with previous studies, such as (Guil &
609 Sanchez-Moreno, 2013), which demonstrated that habitat structure has a stronger influence on
610 tardigrade diversity than altitude, with more complex substrates supporting more species. Similarly,
611 (Jönsson, 2003) found that pleurocarpous mosses (e.g., wefts) host more abundant and diverse tardigrade
612 assemblages, a pattern also observed in Norwegian forests (Guidetti *et al.*, 2024). More broadly, the
613 positive relationship between habitat complexity and species diversity is well established (MacArthur
614 & MacArthur, 1961) and well documented also for macro- and microinvertebrate taxa (e.g., Dean &
615 Connell, 1987; Rantalainen *et al.*, 2005; Nielsen *et al.*, 2010; Ossola *et al.*, 2015; Loke & Todd, 2016;
616 Ramsay *et al.*, 2021). Beyond habitat structure, the substrate on which a bryophyte grows can influence
617 community composition. We found a positive effect of rock substrate on taxonomic richness, with lower
618 standardized phylogenetic diversity in soil samples, consistent with previous studies reporting more
619 speciose communities in these substrates (Dastych, 1988; Guil & Sanchez-Moreno, 2013). However,
620 this effect is likely influenced by other microhabitat variables, such as substrate pH and nutrient content.
621 For example, species-rich tardigrade assemblages have been reported in more alkaline and nutrient-rich
622 substrates (Dastych, 1988; Guil *et al.*, 2009). While our study did not directly assess these factors, the
623 correlation analysis (Supplementary Figure S2.2) suggests that pleurocarpous mosses in our dataset are
624 associated with higher nutrient content and alkaline conditions, potentially supporting these patterns.
625 However, it remains unclear whether these effects reflect direct influences on tardigrades or simply
626 reflect the traits of their bryophyte hosts, which provide complex, moisture-retentive microhabitats.
627
628 *Community assembly and trait-environment interactions*
629 Our results show no significant association between specific bryophyte and tardigrade species,
630 consistent with previous studies on tardigrades (Nelson, 1975; Ramazzotti & Maucci, 1983; Kathman
631 & Cross, 1991; Meyer, 2006; Guidetti *et al.*, 2024) and other microinvertebrates (e.g., Burger, 1948;
632 Kaya *et al.*, 2010; Božanić *et al.*, 2013; Jattupan *et al.*, 2024). Instead, our findings suggest that broader
633 categories of moss structure shape tardigrade communities, reflecting a gradient of moisture and light
634 availability: from pleurocarpous mosses with long shoots in shaded environments, to short, acrocarpous
635 mosses in exposed habitats. These microhabitat effects likely interact with macroenvironmental factors,
636 including altitude and slope exposition, creating complex, spatially structured patterns of community
637 composition. The altitudinal gradient was the primary driver of taxonomic composition, while
638 phylogenetic and functional beta-diversity were more closely linked to microhabitat conditions. For

example, pigmentation was positively associated with short, acrocarpous mosses, cushion growth forms, anthropogenic substrates, and exposed environments, reflecting the strong cryptobiotic capabilities of pigmented taxa like *Milnesium*, *Echiniscus*, and *Ramazzottius* (Guidetti *et al.*, 2011a; Roszkowska *et al.*, 2021, 2023). In contrast, unpigmented tardigrades were more common in moist, shaded habitats, often associated with pleurocarpous mosses, weft or dendroid growth forms, and north-facing slopes, which retain more moisture and thus provide stable microhabitats (Stec *et al.*, 2025). Oviposition strategy was also related to altitude, with more species depositing eggs in exuviae at higher altitudes, potentially reflecting the dominance of high-altitude taxa belonging to Echiniscoidea and Apochela (Figure 7a). However, this strategy is likely a phylogenetically conserved trait rather than a direct response to environmental conditions, as some species that also lay eggs in exuviae (e.g., *Degmion*, *Dianea*) are found in shaded, while *Astatumen* is more common at low altitudes. Body length showed positive associations with mosses having long shoots and pleurocarpous growth forms, but negative associations with north-facing slopes and short-lived mosses (r-strategy). This pattern may reflect the influence of nutrient availability or links between body size and desiccation performance (Jönsson & Rebecchi, 2002; Zawierucha *et al.*, 2015, 2018; Nagwani *et al.*, 2024), though the large size variation among different clades (e.g., large-bodied Apochela vs. small-bodied Echiniscoidea) may obscure these relationships. A general pattern known from established ecological theory states that trait-based environmental filtering favors stress-tolerant species in harsh conditions, while more competitive species dominate in milder environments, reflecting trade-offs between stress tolerance and competitive ability (Belyea & Lancaster, 1999; Ackerly, 2003). Our results only partially align with these patterns, as we observed trait-based environmental filtering under harsh conditions, but in milder environments, we did not detect clear effects of competitive interactions (instead, we observed species-rich, functionally and phylogenetically underdispersed communities). This suggests that in milder microhabitats, multiple species with similar ecological preferences coexist without obvious signs of competitive exclusion. This pattern might be explained by the frequent colonization-extinction cycles driven by the environmental fluctuations typical of bryophyte microhabitats. Overall, our findings are consistent with previous studies on the role of moss moisture retention in shaping tardigrade diversity (Morgan, 1977; Horning *et al.*, 1978; Ramazzotti & Maucci, 1983; Dastych, 1988) and the role of desiccation dynamics in determining cryptobiotic efficiency (Vecchi *et al.*, 2024; Stec *et al.*, 2025). This suggests that desiccation tolerance, alongside habitat complexity and patch size, is a critical factor in structuring tardigrade communities in mountain ecosystems.

Conclusions

Our study provides one of the most extensive assessments of environmental effects on tardigrade communities and reveals several patterns that seem to reflect general rules of microscopic animal community assembly. Tardigrade communities are not strictly shaped by the taxonomic identity of their bryophyte hosts but rather by their functional traits, suggesting that microhabitat characteristics, rather

than host specificity, shape meiofaunal diversity. Apart from that, we documented the effect of macro-environmental factors on the communities, especially the altitude, which shapes the compositional turnover and general diversity patterns. We conclude that the patterns commonly observed in larger taxa, such as niche-based habitat sorting and interspecific competition, may be less relevant for bryophyte-dwelling meiofauna, which seem to be ruled by dynamic community assembly processes, where ecological drift is stronger than deterministic selection. This may explain why, despite the clear habitat preferences of tardigrade species, their distribution remains enigmatic. Nonetheless, understanding the relative roles of different factors shaping the distributions of microscopic taxa, together with transferable methods for monitoring their diversity, is a major contribution towards including the 'neglected' phyla into conservation and biomonitoring programs.

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Data availability statement: The raw sequence reads are deposited in NCBI SRA under accession number PRJNA1216760 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1216760). All data and code are provided as supplementary materials associated with this submission. The data is also deposited in FigShare under the reference number: https://doi.org/10.6084/m9.figshare.29153042.

Benefit-Sharing Statement: Benefits from this research accrue from the sharing of our data and results on public databases as described above.

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Authors contribution: DS conceived and designed the study; **DS** and **DF** design and participate in field works; **DS** and **DF** provided resources; **DS** performed wet-lab part of research; **GV** identified bryophytes; **BS, DS, DF** analyzed data; **DS** and **BS** wrote the paper with contributions from **DF** and **GV**;

Tables

Table 1. Summary of explanatory variables tested in our study. DEM - Digital Elevation Model (OpenTopography, 2013); BET - Bryophytes of Europe Traits (van Zuijlen *et al.*, 2023)

N	Variable	Source	Values	Description
Macroenvironmental predictors				
1	Altitude	field data	305 – 2486 [m asl]	Elevation at which the sample was collected
2	Vegetation type	field data	meadow/mixed_forest/deciduous_forest/coniferous_forest/shrubs	Vegetation-related environment in which the sample was collected
3	Northness	extracted from DEM	[-1 – 1]	Slope orientation at which the sample was collected; cosine-transformed aspect
4	Eastness	extracted from DEM	[-1 – 1]	Slope orientation at which the sample was collected; sine-transformed aspect
5	Geographical coordinates	field data		In the analyses of beta-diversity transformed to dbMEMs
Microhabitat predictors				
6	Sample exposition	field data	shady/exposed	samples were classified as shady if they were collected from beneath a tree, shrub, or rock overhang, where direct sunlight and exposure to open space were obstructed; otherwise scored as exposed
7	Substratum	field data	rock/soil/tree_bark/man_made	substrate on which the bryophyte sample was growing
8	Bryo_species	identification made in this study	103 binominal species names	bryophyte species constituting the sample; each name associated with respective higher taxonomic levels (genus, family, etc.)
9	Size	BET	10.00 – 166.67 mm	mean size of shoot/gametophyte as a proxy for microhabitat size

10	Gform	BET	acr (acrocarpous)/ple (pleurocarpous)	bryophyte growth form
11	Lform	BET	cushion/dendroid/mat/turf/weft	bryophyte life form
12	rK	BET	r or K strategy, derived from life strategy: r (lstrat = a/c/f/s), K (lstrat = l/p); a (annual shuttle), c (colonist), f (fugitive), l (long-lived shuttle), p (perennial), s (short-lived shuttle)	bryophyte life strategy – referring mostly to long- (K) and short-lived (r) life strategies
13	indL	BET	1 (deep shade) to 9 (full light), x (indifferent)	indicator value L (light)
14	indT	BET	1 (cold indicator, alpine-nival) to 9 (extreme warmth indicator), x (indifferent)	indicator value T (temperature)
15	indF	BET	1 (extreme dryness) to 9 (wet-site indicator), x (indifferent)	indicator value F (moisture)
16	indR	BET	1 (extreme acidity) to 9 (high pH soils), x (indifferent)	indicator value R (reaction/acidity)
17	indN	BET	1 (nutrient poorest) to 9 (nutrient richest), x (indifferent)	indicator value N (nutrients)

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Figure legends and embedded figures

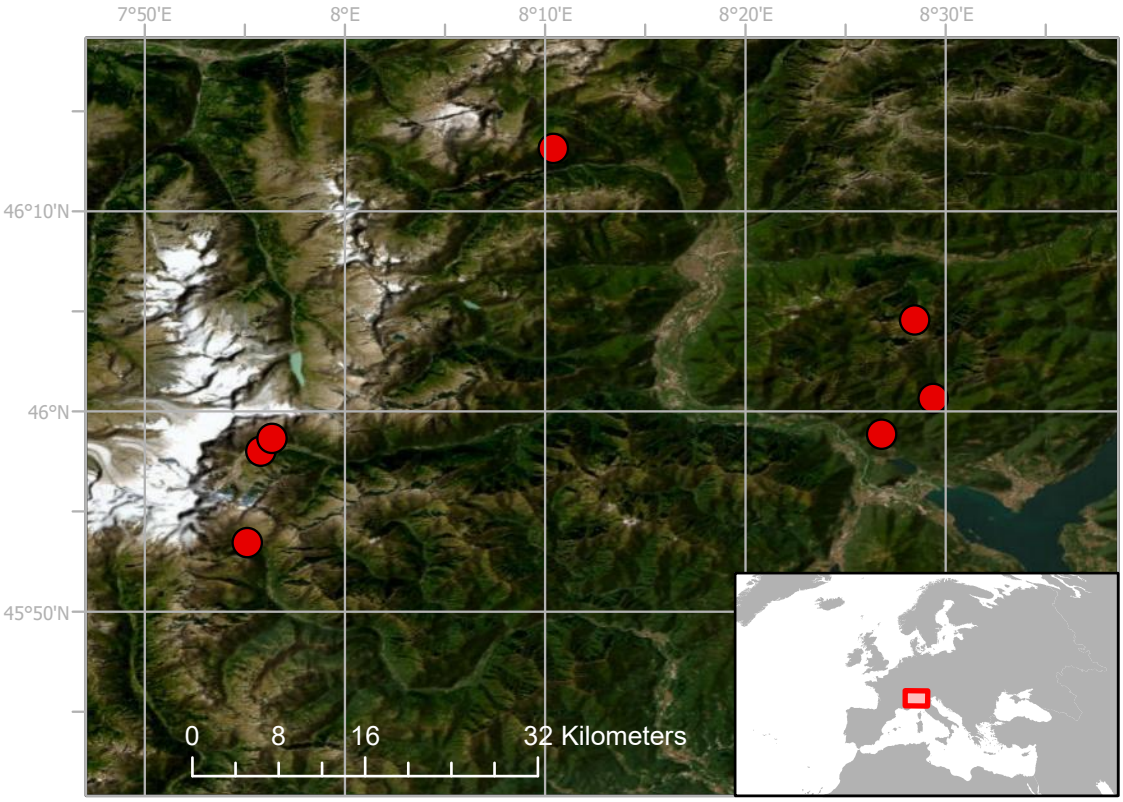


Figure 1. Study area. Red dots correspond to the locations of the seven transects. Source of background satellite imagery: Earthstar Geographics.

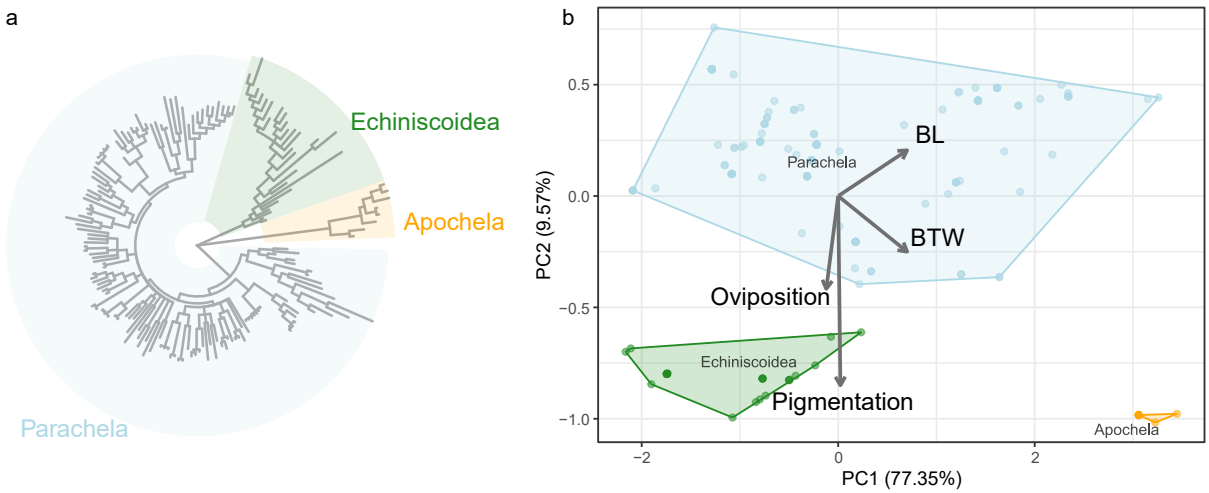


Figure 2. Overview of the datasets: (**a**) phylogenetic tree of OTU reference sequences classified as Tardigrada detected in the metabarcoding data; (**b**) PCA biplot of the trait dataset. Colors in both panels correspond to OTU taxonomic classification to the order level.

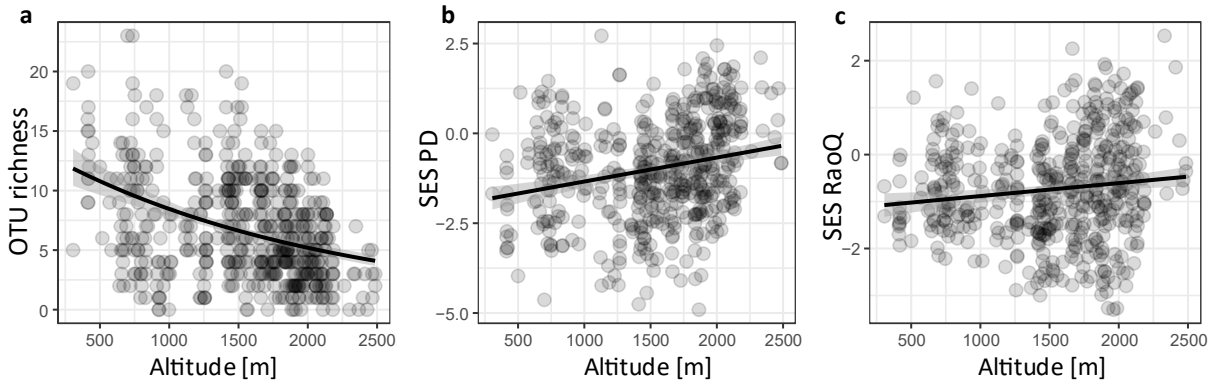


Figure 3. Patterns of three aspects of tardigrade diversity along the altitudinal gradient: (**a**) taxonomic diversity (OTU richness); (**b**) standardized phylogenetic diversity; and (**c**) standardized functional diversity. The regression lines and 95% confidence intervals represent the coefficients of univariate models with altitude as the sole predictor (a negative binomial GLM for taxonomic diversity and linear models for phylogenetic and functional diversity).

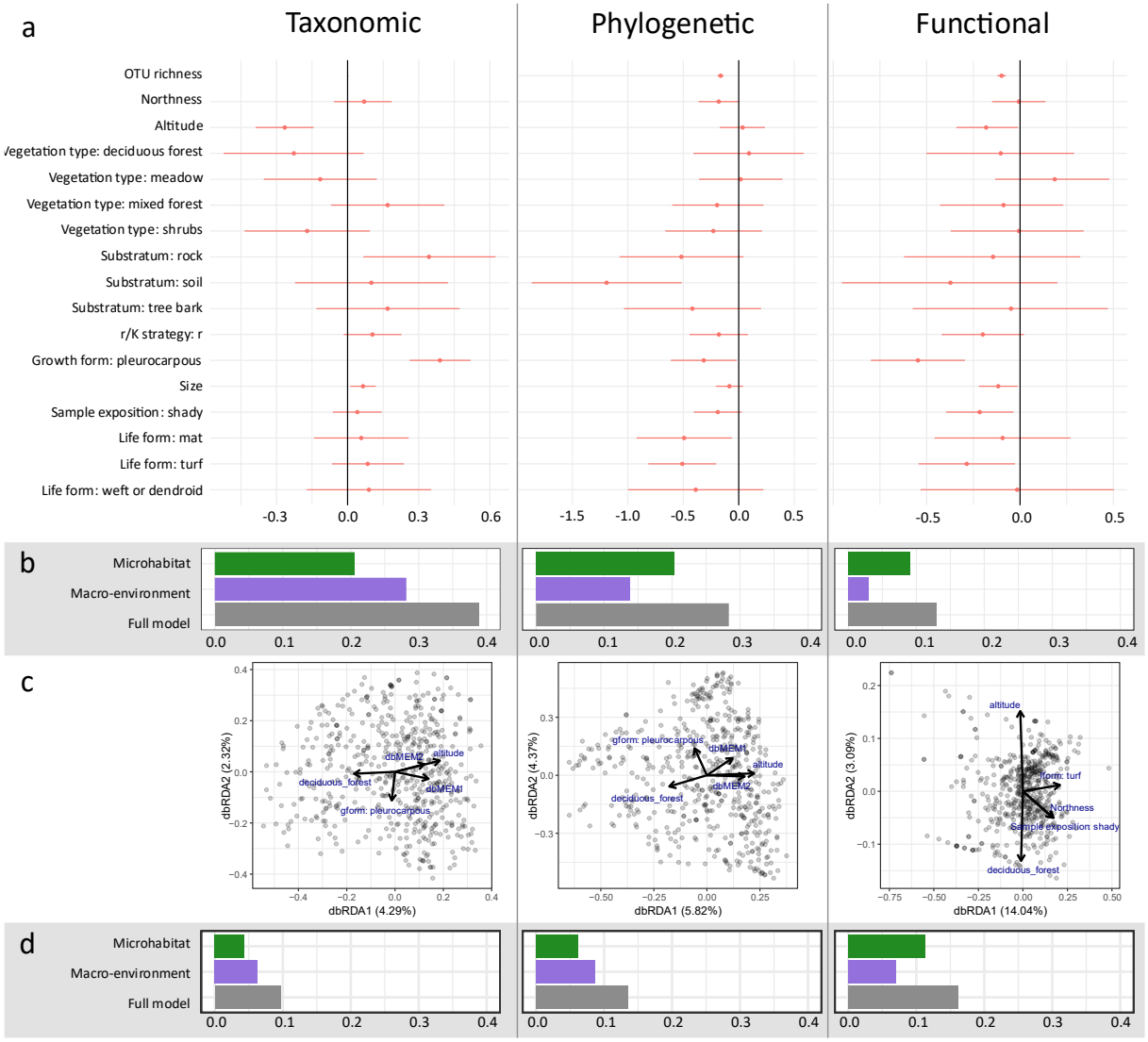


Figure 4. Summary of models linking alpha and beta diversity of tardigrade communities with macroenvironmental and microhabitat variables using taxonomic, phylogenetic, and functional diversity measures. Panel **a** shows the coefficients from the full models for alpha diversity. Points represent model estimates, with horizontal bars indicating 95% confidence intervals. Panel **b** compares pseudo-R^2 values for microhabitat, macroenvironment, and full models explaining alpha diversity patterns. Panel **c** presents dbRDA biplots illustrating beta diversity patterns (five variables with the highest vector length are displayed in each plot), and panel **d** compares adjusted R^2 values (R^2_{adj}) for microhabitat, macroenvironment, and full models explaining beta diversity patterns.

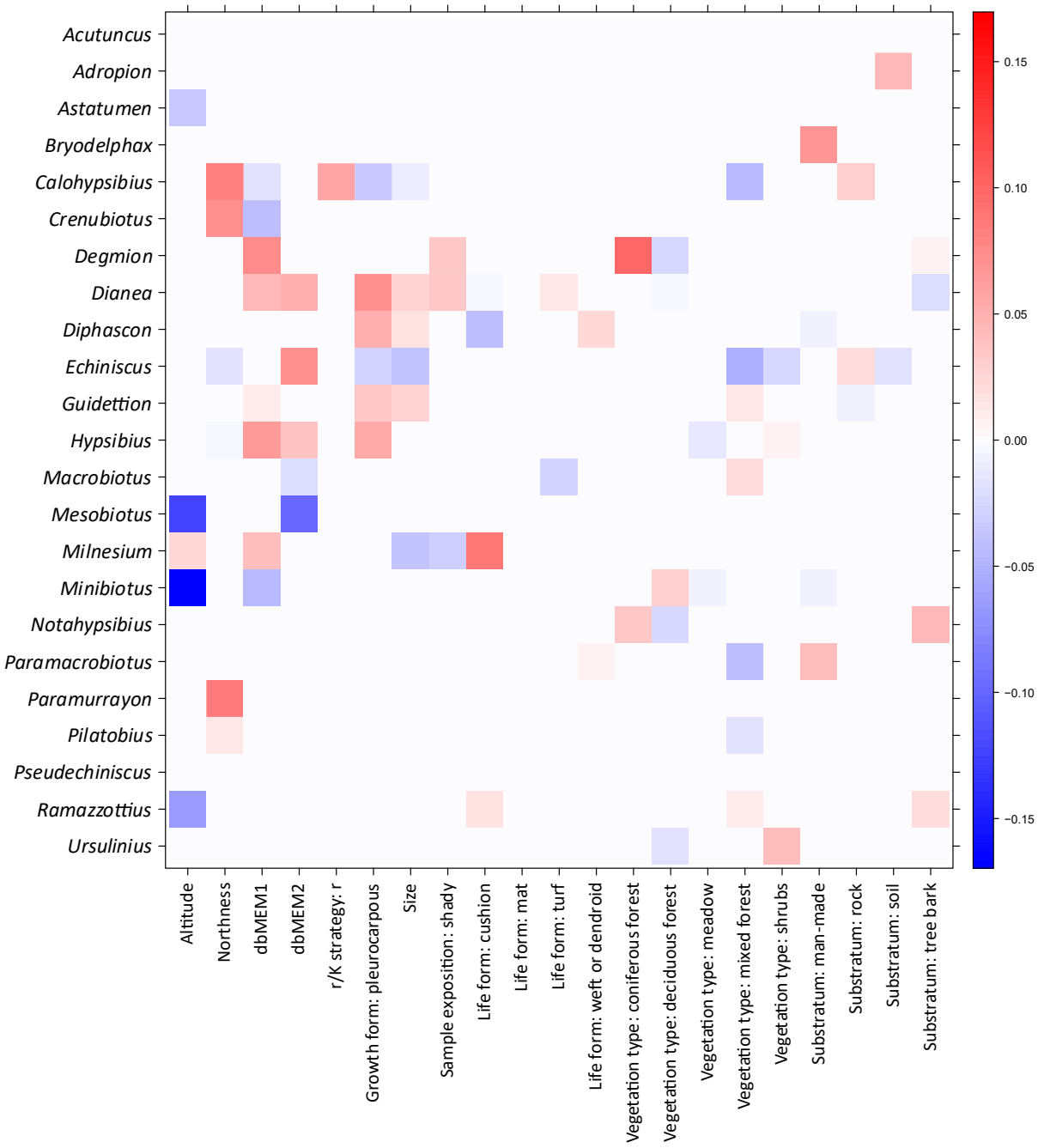


Figure 5. Responses of tardigrade genera (presence–absence data) to environmental covariates, as revealed by a multivariate species distribution model with LASSO penalty. Values represent model coefficients, with colors indicating the effect sizes on the occurrence probabilities of each genus. Shaded cells denote positive (red) or negative (blue) associations between taxa and environmental variables, with shading intensity corresponding to the strength of the association.

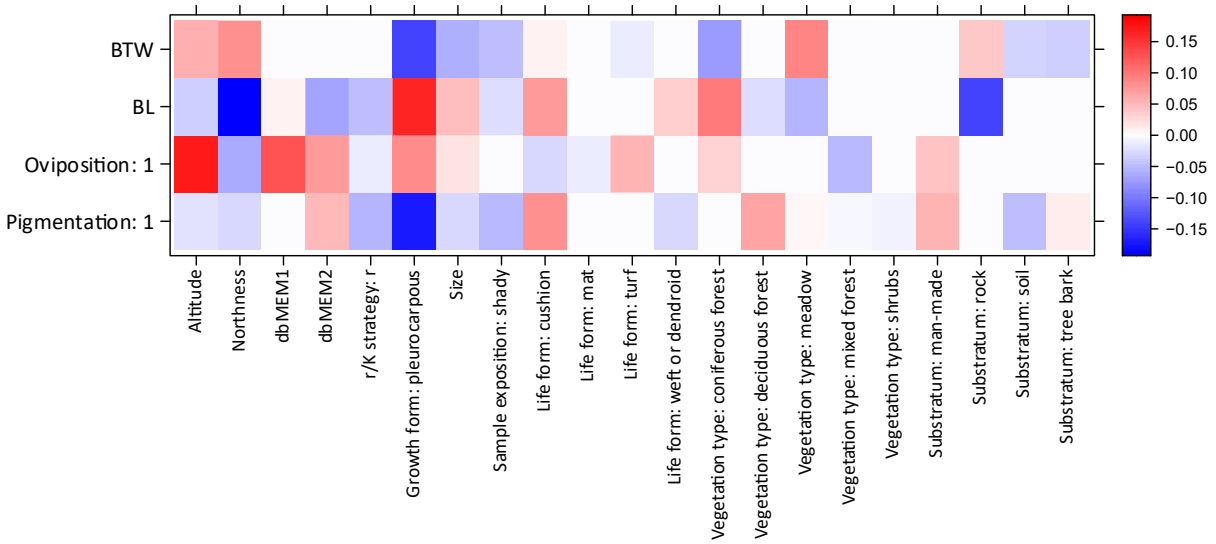
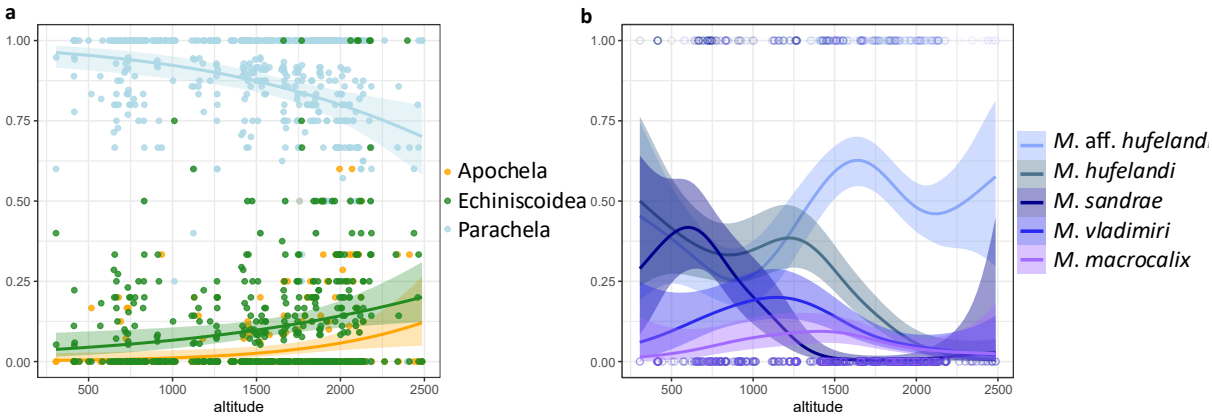


Figure 6. Results of the fourth-corner analysis of tardigrade traits, showing standardized coefficients for all environment–trait interaction terms from generalized linear models with LASSO regularization. Shaded cells represent positive (red) or negative (blue) associations between traits and environmental predictors, with shading intensity indicating the strength of the association.



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1139 **Figure 7**. Altitudinal patterns of selected tardigrade taxa, illustrating the overall taxonomic and phylogenetic
1140 diversity trends found in the study. (**a**) trends of the relative share of tardigrade orders within communities: logistic
1141 regression curves with 95% confidence bands; (**b**) altitudinal distribution of the five most common *Macrobiotus*
1142 OTUs: GAM splines with 95% confidence intervals.

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Supporting information

SM.1: Sample information: collection data and all tested variables

SM.2: Models' results and additional figures supporting the analyses:

Figure S2.1 – Species accumulation curve showing the total number of tardigrade OTUs in random subsets of samples of a given size from a study area, calculated using 999 permutations.

Figure S2.2 – Left panel - Correlation analysis of indicator values and moss growth forms, right panel - comparison of indicator values for reaction (indR) and moss growth forms.

Table S2.1 – Summary of the regression model of tardigrade taxonomic diversity (OTU richness) – negative binomial GLM.

Table S2 – Summary of the regression model of tardigrade phylogenetic diversity (standardized Faith's PD).

Table S2.3 – Summary of the regression model of tardigrade functional diversity (standardized Rao's Q).

Table S2.4 – Model results for taxonomic alpha diversity (microhabitat model, spaMM::fitme). Model formula: OTU_Richness ~ substratum + rK + gform + size + Sample_exposition + lform, family: negbin2(shape=1e+06)(link = log), N=546, Conditional AIC: 2955.434.

Table S2.5 – Model results for taxonomic alpha diversity (macroenvironment model - spatial, spaMM::fitme). Model formula: OTU_Richness ~ Northness + altitude + Vegetation type + Matern(1|latitude + longitude), family: negbin2(shape=1e+06)(link = log), N=546, Conditional AIC: 2839.242. Random effect correlation parameters: nu=0.373 rho = 375.64, random effect variance parameter 'lambda'=0.116.

Table S2.6 – Model results for taxonomic alpha diversity (full model - spatial, spaMM::fitme). Model formula: OTU_Richness ~ Northness + altitude + Vegetation type + substratum + rK + gform + size + Sample_exposition + lform + Matern(1|latitude + longitude), family: negbin2(shape=1e+06)(link = log), N=546, Conditional AIC: 2721.869. Random effect correlation parameters: nu=0.22 rho =563.6, random effect variance parameter 'lambda'=0.1384

Table S2.7 – Model results for the standardized effect size of Faith's Phylogenetic Diversity – SES_PD (microhabitat model, spaMM::fitme). Model formula: SES_PD ~ substratum + rK + gform + size + Sample_exposition + lform, family: gaussian, N=500, Conditional AIC: 1513.089.

Table S2.8 – Model results for the standardized effect size of Faith's Phylogenetic Diversity – SES_PD (macroenvironment model - spatial, spaMM::fitme). Model formula: SES_PD ~ Northness + altitude + Vegetation type + Matern(1|latitude + longitude), family: gaussian, N=500, Conditional AIC: 1531.841. Random effect correlation parameters: nu=16.66 rho =2818.9, random effect variance parameter 'lambda' = 0.066.

Table S2.9 – Model results for the standardized effect size of Faith's Phylogenetic Diversity – SES_PD (full model - spatial, spaMM::fitme). Model formula: SES_PD~ Northness + altitude + Vegetation type + substratum + rK + gform + size + Sample_exposition + lform + Matern(1|latitude + longitude), family: gaussian, N=500, Conditional AIC: 1501.146. Random effect correlation parameters: nu=16.66 rho =2955.3, random effect variance parameter 'lambda'=0.0514.

Table S2.10 – Model results for the standardized effect size of Rao's Quadratic Entropy – Rao_Q (microhabitat model, spaMM::fitme). Model formula: Rao_Q ~ substratum + rK + gform + size + Sample_exposition + lform, family: gaussian, N=500, Conditional AIC: 1344.479.

Table S2.11 – Model results for the standardized effect size of Rao's Quadratic Entropy – Rao_Q (macroenvironment model - spatial, spaMM::fitme). Model formula: Rao_Q ~ Northness + altitude + Vegetation type + Matern(1|latitude + longitude), family: gaussian, N=500, Conditional AIC: 1375.112. Random effect correlation parameters: nu=2.926 rho =17290.2, random effect variance parameter 'lambda'=0.070.

Table S2.12 – Model results for the standardized effect size of Rao's Quadratic Entropy – Rao_Q (full model - spatial, spaMM::fitme). Model formula: Rao_Q~ Northness + altitude + Vegetation type + substratum + rK + gform + size + Sample_exposition + lform + Matern(1|latitude + longitude), family: gaussian, N=500, Conditional AIC: 1342.810. Random effect correlation parameters: nu=16.66 rho =6370.2, random effect variance parameter 'lambda'=0.050

Table S2.13 – Results of the permutation test for the db-RDA model using 999 permutations for taxonomic beta diversity (Jaccard dissimilarities).

Table S2.14 – Results of the permutation test for the db-RDA model using 999 permutations for phylogenetic beta diversity (unweighted Unifrac distances).

Table S2.15 – Results of the permutation test for the db-RDA model using 999 permutations for functional beta diversity (Euclidean distances between community-weighted means).

Table S2.16 – Analysis of deviance table for multivariate generalized model fits comparing the model including bryophyte species (Y~altitude+Northness+dbMEM1+dbMEM2+Vegetation type+Sample exposition+substratum +Bryophyte species) to the model with bryophyte traits only (Y~altitude+Northness+ dbMEM1+dbMEM2+Vegetation type+Sample exposition+Growth form+Size+Life form+r/K strategy+substratum) calculated using 999 iterations of PIT-trap resampling.

Table S2.17 – Analysis of deviance for the multivariate generalized linear model (GLM) linking environmental variables to tardigrade OTU distributions. Test statistics were calculated using 999 iterations of PIT-trap resampling using likelihood ratio test with a multivariate GLM explaining the deviance as a measure of the quality-of-fit as well as the residual degree of freedom (Res.Df).

SM.3: Archive including pipelines and raw data used for the analysis (Metabarcoding_pipeline.html, Statistical_procedures.html, Phylogenetic_tree.tre, OTU_table.xlsx, OTU_x_trait_matrix.xlsx, Sample_data.xlsx, Metadata.xlsx).

SM.4: Tardigrade traits data.