**Functional convergence underground? The scale-dependency of community assembly processes in European cave spiders**

**Abstract**

*Aim.* An elusive question in community ecology is quantifying the relative contribution of environmental filtering versus limiting similarity. The issue remains unresolved because i) environmental filtering and limiting similarity often act simultaneously in shaping communities; and ii) their effect is scale-dependent. Focusing on caves, island-like natural laboratories characterized by largely constant environmental gradients and a limited diversity of species and interactions, we tested: i) the relative contribution of environmental filtering and limiting similarity in determining community assembly in caves; ii) how the relative contribution of these driving forces change along environmental gradients.

*Location.* Europe.

*Time period.* Present.

*Major taxa studied.* Subterranean spiders.

*Methods.* We leveraged uniquely available data on distribution and traits for European cave spiders (n = 475 cave communities). We estimated the trait space of each cave community using probabilistic hypervolumes, and obtained estimations of functional richness independent of the species richness of each community via null modelling. We model functional diversity change along environmental gradients using generalized dissimilarity modeling.

*Results.* Sixty-three percent of subterranean spider communities exhibited a prevalence of trait underdispersion rather than overdispersion. However, most subterranean spider communities display trait dispersion that do not depart significantly from random, indicating that environmental filtering and limiting similarity were both exerting equally weak or strong, yet opposing, influences. Communities with a prevalence of trait overdispersion were concentrated in southern latitudes, particularly in the Dinaric karst, where there is greater subterranean habitat availability and, consequently, more availability of niche space. Pairwise comparisons of functional richness across caves revealed these effects to be strongly scale-dependent, largely varying across gradients of cave development, elevation, precipitation, entrance size, and annual temperature range. Conversely, the impact of geographic distance on trait composition was weak, suggesting convergence in traits among communities that are far apart.

*Main conclusions.* Even systems with stringent environmental conditions, such as caves, maintain the potential for trait differentiation, especially in areas of greater habitat availability. Yet, the relative influence of environmental filtering and limiting similarity change with scale, along clear environmental gradients. The interplay of these processes may explain the assembly of species-poor subterranean communities displaying high functional specialization.

**Keywords:** Araneae;beta diversity; cave; functional diversity; functional guild; morphology; troglobiont, trait space

**INTRODUCTION**

An omnipresent scheme in introductory textbooks of ecology illustrates the numerous filters selecting which species end up assembling into local communities from a regional pool. An elusive problem concerning this ‘filtering’ metaphor is quantifying the relative contribution of abiotic and biotic factors in shaping communities (Chalmandrier *et al.* 2022; Kraft *et al.* 2015; Lamanna *et al.* 2014). In a nutshell, environmental filtering is the process whereby abiotic constraints prevent species from establishing in a community, selecting for a narrow set of traits suitable to cope with the local conditions, leading to lower differences in trait composition than expected by chance (“trait underdispersion”). Conversely, limiting similarity drives functionally similar species to diverge in key phenotypic traits to reduce niche overlap, leading to higher differences in trait composition than expected by chance (“trait overdispersion”). It follows that looking at biological communities through the lens of functional ecology (i.e., the traits expressed in each community) is one of the most effective ways we have to quantify the interplay between these two assembly processes (McGill *et al.* 2006). The use of traits in lieu of species identities allows an explicit focus on the mechanisms generating biodiversity patterns, often facilitating the conceptualization of general principles that are valid across species pools or distantly related taxa (Luza *et al.* 2023).

Even with trait-based approaches, however, it remains difficult to separate the main mechanisms filtering the species pool of potential resident species to the subset that occurs within a given community (α-diversity) and in driving variations across communities (β-diversity) (Vellend 2010). The distinction between environmental filtering and limiting similarity is too often conceptualized as a “black or white” dichotomy, whereby communities are described to be dominated by one or the other process. The ecological reality is instead more nuanced, with the two processes acting simultaneously in shaping communities, although with different intensities given the local conditions (Germain *et al.* 2018; Loughnan & Gilbert 2017). Furthermore, like any dimension of biodiversity, functional diversity change is scale-dependent (Graco-Roza *et al.* 2022; Jarzyna & Jetz 2018), forcing us to account for the pervasive effect that scale has on emerging patterns (McGill 2010). Since biotic interactions require spatial proximity, the effect of limiting similarity should often decrease with increasing scale and, vice versa, the filtering effect posed by the abiotic environment should increase with spatial scale—generally resulting in a predominance of trait overdispersion at local scales and trait underdispersion at broader scales (Belmaker *et al.* 2013; Lhotsky *et al.* 2016).

Mounting evidence demonstrates how the relative influence of environmental filtering and limiting similarity broadly changes along spatial and temporal gradients—e.g., for vertebrates (Belmaker *et al.* 2013; David *et al.* 2014; Jarzyna & Jetz 2018; McLean *et al.* 2021; Toussaint *et al.* 2021) and plants (Kraft *et al.* 2008; Lamanna *et al.* 2014; Lhotsky *et al.* 2016). However, there is still controversy on the direction of these changes and their causes (Germain *et al.* 2018; Kraft *et al.* 2015; Loughnan & Gilbert 2017). To minimize confounding factors and achieve a better understanding of community assembly rules, scientists are therefore increasingly turning their attention to island-like systems (e.g., oceanic islands, lakes, tank-bromeliads, floating plant-island, mountain summits; Itescu 2019; Srivastava et al. 2004) and specific biological communities within them [e.g., plants (Ottaviani *et al.* 2020; Schrader *et al.* 2021); birds (Ross *et al.* 2019; Sato *et al.* 2020; Triantis *et al.* 2022)], as models. The use of island-like systems, i.e., mostly closed, with known histories, and with a relatively low richness of species, allows ecologists to more easily disentangle community assembly processes while controlling for immigration, extinction, and dispersal dynamics (Itescu 2019; Mammola 2019; Whittaker *et al.* 2017).

Under this framework, caves and other subterranean ecosystems stand out as ideal model systems for the study of community assembly processes through a functional lens. Foremost, caves are semi-closed systems extensively replicated across the Earth (Culver & Pipan 2019), where stringent environmental conditions promote trait convergence among successful colonizers (Cardoso 2012; Trontelj *et al.* 2012). Second, subterranean communities generally exhibit lower species richness and functional diversity than neighboring surface communities (Gibert & Deharveng 2002; Cardoso 2012; Hose et al. 2022; but see Fernandes et al. 2016), making it easier to disentangle the relative effect of environmental conditions in selecting species possessing specialized traits within the community (Mammola *et al.* 2020b). Third, caves have clear environmental gradients (Kozel *et al.* 2019; Lunghi *et al.* 2015; Tobin *et al.* 2013) and display a reduced variability in their abiotic conditions (Badino 2010), two factors that avoid many of the confounding factors typical of other systems (Mammola 2019).

To study community assembly rules, we leveraged the unprecedented amount of data available for subterranean spiders in Europe (Mammola *et al.* 2018), namely community composition data for selected caves across the continent (Mammola *et al.* 2019a), and standardized traits for all species (Mammola *et al.* 2022). A previous analysis of the taxonomic component of this dataset demonstrated a quick turnover in the taxonomic diversity of subterranean spiders across Europe, mediated primarily by geographic distance among caves, and secondarily by the climatic conditions and availability of karst. Conversely, local-scale characteristics of caves exerted a negligible effect on species turnover (Mammola *et al.* 2019b). Here, we explore the functional dimension of these patterns, testing: i) the relative contribution of environmental filtering and limiting similarity in determining community assembly in caves; ii) how the contribution of these driving forces change along environmental gradients.

At the α-diversity level, we expect (**H1a**) communities to be predominantly functionally underdispersed because the stringent environmental conditions of caves should filter a narrow set of trait combinations, resulting in lower functional richness than what would be expected given species richness. Concurrently, we predict that (**H1b**) limiting similarity will play a stronger role in cases where there are more available niches and where local conditions allow for smaller niche overlap among species (e.g., larger caves, larger karst areas, regions with higher diversity of climates), leading to character displacement.

At the β-diversity level, we hypothesize that (**H2**) environmental factors will have a stronger effect than geographic distance on functional turnover. This is because we expect that functional composition will be strongly influenced by local environmental conditions, which modulate the availability of niches and the potential for species interactions

**MATERIAL & METHODS**

**Community-level data**

We obtained data for subterranean spider communities across Europe from Mammola et al. (2019a), which we refer to for a full account of the dataset and the methods used to assemble it. The dataset comprises data from 475 subterranean sites (limestone, volcanic, talus, and salt caves, as well as artificial sites including mines, blockhouses, and cellars; the general term ‘cave’ is used hereafter) across 27 European countries, covering a latitudinal range from 35° to 70°. The dataset only includes subterranean sites for which spider fauna is exhaustively known. For each site, the spider composition is represented as incidence data—presence/absence of each species. The database includes 326 species [average (± s.d.) number of species per cave of 4.3 (± 2.35); range: 0–15]. Note that we focused solely on “subterranean spiders” (Mammola *et al.* 2018, 2022), excluding “accidental” surface species (Trajano & de Carvalho 2017) occasionally found underground.

The functional composition of the species included in our dataset clustered in two major groups, followed by two other minor clusters. The species clustered mainly based on strategies such as cave adaptation, mainly hunting strategies and adaptation to darkness (Fig. S1). Most traits included in the analyses contributed to the three synthetic axis, except for XXX, XXX, XXX (Table S1)

**Environmental and geographic gradients**

We collated a site-by-environment matrix including local-scale environmental characteristics of each cave and broad-scale variables extracted from rasters using the coordinates of the cave entrance. Furthermore, from the coordinates of each cave we calculated pairwise geographic distances among caves (expressed in decimal degrees), useful for β-diversity analyses (see subsection “*Calculation of α- and β-diversity*”).

As local-scale predictors, we used the altitude of the cave entrance (in meters a.s.l.), the main entrance size (a numerical estimation of the dimension of the main entrance in square meters), cave development (total planimetric development of the cave in meters), and cave depth (total drop in meters). These are frequently used variables in macroecological analyses focused on caves (Jiménez-Valverde *et al.* 2017), which we here interpreted as proxies for local-scale conditions and niche space availability. For example, caves with a vertical drop and a large entrance tend to accumulate more external food resources (detritus) than horizontal caves with a very narrow entrance.

As broad-scale predictors, we included three climatic variables (mean annual temperature, annual temperature range, cumulative precipitation), one variable reflecting availability of carbonatic rocks (karst), and one biogeographical factor (the distance of each cave to the margin of the glacier in Last Glacial Maximum; ca. 21,000 years ago). We extracted climatic data from WordClim 2 rasters (Fick & Hijmans 2017) at a resolution of 2.5 minutes. Although broad-scale variables may fail-short to capture microclimatic variability within caves (Ficetola *et al.* 2020), they are good surrogates for general subterranean climatic conditions (Christman *et al.* 2016; Mammola & Leroy 2018; Sánchez-Fernández *et al.* 2018; Zagmajster *et al.* 2014). We extracted the size of the karst patch in which a cave occurs using the World Map of Carbonate Rock Outcrops (version 3.0). Given that most locations in our database were karst caves, we interpreted this variable as a *proxy* of habitat availability in the surrounding of each cave, and an indirect measure of habitat connectivity (Bregović & Zagmajster 2016; Curl 1986). Finally, we derived the distance of each cave from the Last Glacial Maximum glacier from reconstructions by Ehlers et al. (2011). We interpreted this as a *proxy* for the influence of past glacial cycles on the current distribution of subterranean species (Assmann *et al.* 2010; Mammola *et al.* 2019d).

**Functional traits**

For each spider species included in the database, we derived functional traits from Mammola et al. (2022), which we refer to for a full description of the trait data matrix and data collection methods. This trait matrix contains 64 traits, with some redundancy across traits, and many traits riddled with a high proportion of missing data (>50% observations). For the purposes of the analysis, we selected a subset of 20 traits (8 continuous, 9 binary, and 3 fuzzy coded) from the whole trait matrix. This subset of traits maximize the information contained in the trait database (Mammola *et al.* 2022), while minimizing the number of missing data and hence the need for trait imputation (the full list of trait and their distributions is available in Figure 1). The selected traits represent: i) general morphology and size of species (average body size, prosoma shape, sexual size dimorphism). Body size and shape are meant to be related to habitat (pore) size (Pipan & Culver 2017) and metabolic processes (Mammola & Isaia 2017). Sexual size dimorphism is meant to provide information on sexual selection mechanisms operating in subterranean environments (Mammola *et al.* 2022); ii) morphological adaptation to subterranean conditions [eye measures (AME, ALE, PME, PLE), eye reduction, degree of pigmentation, leg elongation]. This combination of traits captures the degree to which each species is morphologically adapted to the permanent darkness of subterranean ecosystems (Culver & Pipan 2015; Mammola & Isaia 2017); and iii) webs and hunting strategies, including numerous traits capturing important differences in the modes of life of spiders (Cardoso *et al.* 2011). To ensure exact matching between the spider species names in the community and trait matrices, we standardized and updated taxonomy using the function *checknames* in the R package ‘arakno’ version 1.1.1. (Cardoso & Pekar 2022).

**Data analysis**

We analyzed data in R version 4.1.2 (R Core Team 2021), using the suite ‘tidyverse’ (Wickham & et al. 2019) for data manipulation and visualization. In all functional diversity analyses, we followed the general analytical pipeline described in Mammola et al. (2021), and the protocol for transparent reporting by Palacio *et al.* (2022). A reproducibility checklist for the study is available in Table S1. Since functional analyses were computationally demanding, we ran all analyses in high-performance computing services (see “**Acknowledgments**”).

*Data exploration*

We carried out data exploration following Palacio *et al.* (2022), checking variable distribution, multicollinearity, and the presence of missing data (Figure 1). As a result of data exploration, we standardized all continuous traits (mean = 0 and standard deviation = 1) to ensure comparable ranges among different traits. In the environmental matrix, we checked variable distributions and log-transformed all numerical variables (except coordinates, annual temperature range, and mean temperature) to homogenize distribution and reduce the effect of outliers. None of the predictors showed correlation values higher than Pearson’s *r* > ±0.7 (Zuur *et al.* 2009).

*Functional space estimation*

We estimated the trait space of each cave using probabilistic hypervolumes (Blonder 2019; Blonder *et al.* 2014; Mammola & Cardoso 2020). Probabilistic hypervolumes have a key advantages over other commonly used trait-space characterizations [e.g., dendrograms (Petchey & Gaston 2002) or convex hulls (Cornwell *et al.* 2006)], in that they allow the detection of areas of higher or lower density in the trait space, thus representing uneven probabilities of finding a species with a given trait combination throughout the boundaries of the trait space (Blonder 2016; Mammola & Cardoso 2020).

Prior to analyses, we excluded caves with less than three species because these might lead to uninformative trait spaces, resulting in a total sample size of 367 caves. Since the trait matrix was a mixture of continuous, binary, and fuzzy-coded traits, and contained missing data for certain traits, we used a Gower distance to estimate trait dissimilarity among species (Gower 1971). In calculating Gower distance, we used the optimization method by de Bello *et al.* (2021) to attribute weight to traits within the three groups of variables (column “grouping” in Figure 1). This method addresses the issue of uneven contributions from different traits, especially fuzzy coded ones, in calculating multi-trait dissimilarities. The solution involves determining weights that minimize variations in the correlation between the dissimilarity of individual traits and the multi-trait dissimilarity. This ensures that each set of traits exerts a comparable influence on the overall multi-trait dissimilarity (de Bello *et al.* 2021).

We analyzed the resulting distance matrix through Principal Coordinate Analysis with the R package ‘ape’ version 5.5.0 (Paradis & Schliep 2019), extracting three orthogonal axes that we used to delineate the probabilistic hypervolumes for each cave. Using three trait axes ensures a good trade-off between accuracy and computation time (Graco-Roza *et al.* 2022; Mouillot *et al.* 2021). We constructed hypervolumes with a Gaussian kernel density estimator and a default bandwidth for each axis (Blonder *et al.* 2018), as implemented in the function *hypervolume\_gaussian* in the package ‘hypervolume’ version 3.0.1 (Blonder 2022).

*Calculation of α- and β-diversity*

We measured the properties of the estimated trait spaces using hypervolume-based functions (Mammola & Cardoso 2020) from the R package ‘BAT’ version 2.7.1 (Cardoso *et al.* 2015, 2021). We calculated the functional richness of each community (α-diversity) as the total volume of each hypervolume (*kernel.alpha* function). We estimated pairwise functional β-diversity among communities as a Sørensen dissimilarity index, calculated through a modified version of the *kernel.beta* function that enables parallel estimation of pairwise comparisons (Graco-Roza *et al.* 2022). This estimation of β-diversity further decomposes the two processes underlying overall dissimilarity (βtotal) among hypervolumes following Carvalho & Cardodo. (2020), namely: the replacement of trait space between communities (βreplacement), and the net differences between the amount of trait space enclosed by the two communities (βrichness). β-diversityranges from 0 (identical trait spaces) to 1 (fully dissimilar trait spaces).

*Null modeling*

Estimations of functional diversity are mathematically dependent on the taxonomic diversity, particularly the species richness feature for α-diversity and species composition for the β-diversity. Statistically controlling for this association may reveal the actual degree of importance of trait composition to community patterns (Götzenberger *et al.* 2016; Mammola *et al.* 2021). To this end, we randomly permuted without replication the rows of the trait matrix 999 times to generate a null distribution of each hypervolume-based trait space. For each random iteration, we calculated all α- and β-diversity measures. We estimated significant deviation of observed values from the null distribution as the proportion of instances where the estimated values were less than the observed values, plus half of the instances where the estimated values were equal to the observed values, divided by the total number of estimated values. Specifically, significance was determined by a threshold of Rank < 0.025 or > 0.975. Standard effect sizes (SES) were estimated using probit transformed p-values (Lhotsky *et al.* 2016). Probit transformation is used as an alternative to logit transformation in generalized linear models to transform probabilities into the minus-infinity-to-infinity range (Dobson 2002). We chose this approach because it is known to be less sensitive to skewed distribution of null values (Lhotsky *et al.* 2016), which was the case in our dataset. Notably, this approach is known to partially underestimate the effect size when the observed value is completely outside the null distribution; however, this problem was trivial in our case, as none of our observed values fell outside the null distribution (that is, p-value of 0 or 1).

*Hypothesis testing*

To test our first set of hypotheses on alpha diversity patterns (**H1**), we modeled the relationship between SES values for functional richness (α-diversity), and all local and broad-scale environmental characteristics of each cave using a generalized least squares fitted with the package “nlme” version 3.1-157 (Pinheiro J, Bates D, DebRoy S 2019). To account for spatial autocorrelation in the residuals, we introduced an exponential correlation structure on the longitude and latitude coordinates of each cave. Prior to model fitting, we standardized all predictors (mean = 0 and standard deviation = 1) to ease model convergence. We validated the model by inspecting the normality of residuals, heteroskedasticity, and degree of collinearity (Zuur *et al.* 2009).

To test our second hypothesis on beta diversity pattern (**H2**), we used a Bayesian bootstrap extension of generalized dissimilarity modeling (BBGDM), as implemented in the R package ‘bbgdm’ version 1.0.1 (Woolley *et al.* 2017). Generalized dissimilarity modeling is a matrix regression technique that incorporates variation in the rate of compositional turnover along an environmental or spatial gradient (non-stationarity) in a monotonic nonlinear fashion (Ferrier *et al.* 2007; Mokany *et al.* 2022). Because the elements of a dissimilarity matrix are not fully independent, BBGDM uses a Bayesian bootstrap procedure to correct the uncertainty of model parameters (Woolley *et al.* 2017). We used as input the predictors and the functional β-diversity matrices. We fitted individual BBGDMs for the three functional β-diversity matrices (βtotal, βreplacement, and βrichness) with default parameters of three I-splines for each predictor and default knot values.

In our study, we applied the BBGDM method to analyze both observed and null functional β-diversity matrices, the latter generated through null trait matrices (see section “*Null modeling*”). This allowed us to derive various metrics of SES to address different aspects of our research. Specifically, we aimed to assess whether specific variables promoted stronger or weaker changes in trait composition than expected given their changes in species composition. To achieve this, we extracted two key measures from the BBGDM runs: the sum of splines coefficients (i.e., the magnitude of relationship) for each variable and the fitted values (i.e., the shape of relationship). Both measures were extracted from the 999 BBGDM runs, creating a null distribution of magnitude and relationship shapes. Non-parametric SES was then employed to test and compare these distributions, and their significance were acquired through probit p-values.

The sum of splines provides a measure of the observed effect size, reflecting the total change in functional β-diversity associated with a single predictor while holding other predictors constant, indicating whether the overall effect is stronger or weaker than expected. On the other hand, the fitted values offer insight into the shape of the relationship along the gradient, allowing us to discern whether the effect in specific parts of the gradient is stronger or weaker than expected. This distinction is crucial, as it accounts for situations where the overall effect may appear random, but specific gradient values exhibit notable strength or weakness. For example, we can evaluate if changes in trait composition exhibit disproportionate strength (positive SES) when caves are in close proximity and disproportionate weakness (negative SES) when caves are farther apart.

**RESULTS**

**α-diversity**

SES values for the functional richness of each community were left-skewed, with 63% of caves displaying a prevalence of functional underdispersion over functional overdispersion (Figure 2a, 2b). Still, most of these caves clustered towards SES values close to zero (Figure 2b), with only seven communities completely underdispersed and one completely overdispersed (p < 0.05). In general, caves with a prevalence of overdispersion were concentrated at southern latitudes, especially in the Dinaric karst (western Balkans) (Figure 2a). A generalized least squares model fitted through the data suggested that communities in caves with a greater depth (negative drop), occurring within larger karst areas, and in areas with a broader annual temperature range were more likely to be functionally overdispersed (Figure 2c). The addition of spatial component did not improve our model significantly, suggesting the absence of spatial correlation in our data.

**β-diversity**

Patterns of functional β-diversity were primarily driven, based on effect sizes, by cave development, elevation, precipitation, entrance size, and annual range of temperature. The contribution of additional predictors was negligible (Figure 3). The rate of turnover along the cave development gradient was monotonically asymptotic, with rates of turnover steeply increasing in the first portion of the gradient before reaching a plateau (Figure 3a). This effect was significant along the whole gradient (Figure 3c). We also observed some degree of turnover along the gradients of elevation, precipitation, and temperature range. That is, communities in caves with different elevations, temperatures, and precipitation regimes tend to express different functions. For precipitation, SES values indicated that there is underdispersion along the first half of the gradient and an increasing predominance of functional overdispersion in the second half of the gradient. The pattern was reversed for the annual range of temperature.

The effect of geographic distance on functional β-diversity followed a power-law curve (linearly asymptotic), and was rather weak—that is to say, at increasing distance between two caves, there was only a limited turnover in functional richness (Figure 3a). Interestingly, when looking at variation in the effect over the geographic gradient (Figure 3c), we observed a prevalence of trait overdispersion at a smaller spatial scale which progressively decreased toward zero when caves were >2000 km apart.

Most of the variation in β-diversity was due to replacement of trait space among communities (βreplacement; Figure S1), with patterns largely mimicking the variation in total β-diversity (βtotal). Conversely, the contribution of βrichness was negligible in all cases except for cave drop (Figure S2).

**DISCUSSION**

Focusing on the underexploited natural laboratory offered by caves, we studied functional diversity patterns in subterranean spider communities across Europe, testing general hypotheses ruling community assembly. Two important points, largely generalizable across systems and species pools, emerge from our analysis.

The first point is that environmental filtering and limiting similarity are not mutually exclusive processes (Pillar *et al.* 2009). Even in caves, ecological systems where environmental filtering is meant to be particularly strong (Gibert & Deharveng 2002), the relative influence of these two processes varied substantially given the local habitat conditions. Whereas the direction of SES for functional richness was predominantly towards underdispersion (Figure 2b), the majority of values were close to zero, meaning environmental filtering and limiting similarity were both acting in equally weak or strong, but opposing, directions. Environmental filtering is indeed a demonstrably strong factor in caves, with many traits and parts of the potential functional space being absent. Yet, our results add quantitative evidence to a growing body of literature (Culver & Pipan 2015; Fernandes *et al.* 2016; Mammola *et al.* 2016; Trontelj *et al.* 2012) emphasizing the importance of reconsidering the role of niche-based processes as an important force driving the evolution of cave communities.

Subterranean communities with local trait overdispersion were more frequently associated with large karst patches, areas with broader temperature ranges, and deeper caves (Figure 2c), all conditions that provide more niche space to be exploited. This was particularly evident in the Dinaric karst (western Balkans), the most important global hotspot of subterranean biodiversity (Culver *et al.* 2006; Sket 2011), where virtually all cave trait spaces were predominantly overdispersed. Large patches of karst, such as in the Dinarides, implies greater habitat availability (Bregović & Zagmajster 2016) and possibly connectivity (Curl 1986), hence a higher niche space. We hypothesize that species were often able to move within this karst region in geological time and, when in sympatry, character displacement with speciation would occur. With time, this allowed the trait space to expand and intermediate forms to lose the competitive edge over species that minimized competition. In contrast, in smaller regions, there were no opportunities for expansion of the trait space, as very few forms could co-exist and survive for long enough for speciation to occur, keeping the trait space limited and hence no overdispersion. However, we outline that our data is not enough to confirm the relevance of limiting similarity to our patterns and the patterns can also emerge from weaker environmental filtering or stronger niche complementarity. Likewise, the positive association between trait overdispersion and temperature range can be interpreted in the light of the influence of temperature variability on species range size and dispersal (Ghalambor *et al.* 2006; Janzen 1967; Polato *et al.* 2018), including subterranean spiders(Mammola *et al.* 2019c). Finally, communities in caves with a greater drop tend to be, on average, predominantly overdispersed. Deeper caves tend to express more areas with differing availability of resources, offering more possibility for different communities with contrasting traits.

The second key point emerging from our study is about the importance of scale in the perception of community assembly patterns. Accounts for scale in trait analyses have been achieved, for example, by looking at variations in individual trait values along ecological gradients (e.g., elevation; Swenson et al. 2011), or by contrasting taxonomic and functional diversity change in highly dispersive organisms (e.g., birds; Jarzyna & Jetz 2018). Here, we devised a novel approach to account for the magnitude of trait dispersion change along the studied ecological gradients, combining gradients and traits all together in a single model. We observed how functional β-diversity patterns varied along multiple ecological gradients. The most important one was the difference in the development among the studied caves, whereby the largest replacement of functions occurred between pairs of caves with divergent development (i.e., large versus small caves; with an inflection point with cave >5 meters in development). A plausible explanation is that cave development is a *proxy* for the availability of spatial niches. In particular, small caves will be primarily colonized by spiders adapted to the cave entrance conditions, and large caves will often sustain a greater number of specialized species, accounting, overall, for drastically different functions. Other important gradients of variations were elevation and precipitation, reflecting the influence of climatic conditions and habitat heterogeneity in the local structuring of functions.

In terms of distance decay in functional diversity, trait composition changed in a random way with respect to species composition, with a slightly higher likelihood of a more pronounced replacement of traits that decreased along the gradient (Figure 3c). This means that, although some replacement of traits do occur, overall turnover happens by substitution of species pursuing similar functions. Still, when decoupling functional patterns from taxonomic diversity, the functional responses varied along the geographic gradient according to theoretical expectations, showing stronger overdispersion at smaller distances, and progressive moving toward SES values of zero at larger distances. This highlights the scale dependency of regional trait dispersion, with nearby caves more likely to have interacting communities and such effect becoming weaker with increasing spatial distance.

**CONCLUSIONS**

The use of caves as model systems for investigating (macro-)ecological patterns in space and time is still underexploited (Mammola *et al.* 2020a). This is partially a problem related to the objective difficulties of working in caves (resulting in a general lack of data at the right resolution) and partly a methodological problem. Nonetheless, thanks to the recent development in databases of species distributions and traits, and the emergence of novel analytical tools, there is a vast potential to leverage these systems as ideal settings in which to model across space. Using an explicit functional diversity approach, we showed that i) even systems with stringent environmental conditions maintain the potential for trait differentiation, especially in areas of greater habitat connectivity; and ii) the relative influence of environmental filtering and limiting similarity change with scale, along clear ecological gradients. Overall, our findings reconcile contrasted views about the relative importance of the two main mechanisms shaping patterns of biodiversity, and provide a conceptual foundation to account for scaling effects in the study of community assembly. This information is key amidst escalating global anthropogenic threats affecting surface (Bowler *et al.* 2020) and subterranean ecosystems (Nanni *et al.* 2023; Vaccarelli *et al.* 2023), insofar as realistic predictions of biodiversity change require explicitly accounting for community assembly processes (Ovaskainen *et al.* 2019).

**SUPPLEMENTARY MATERIALS**

Functional diversity protocol checklist

Figure S1

Figure S2

Supporting literature

**LITERATURE CITED**

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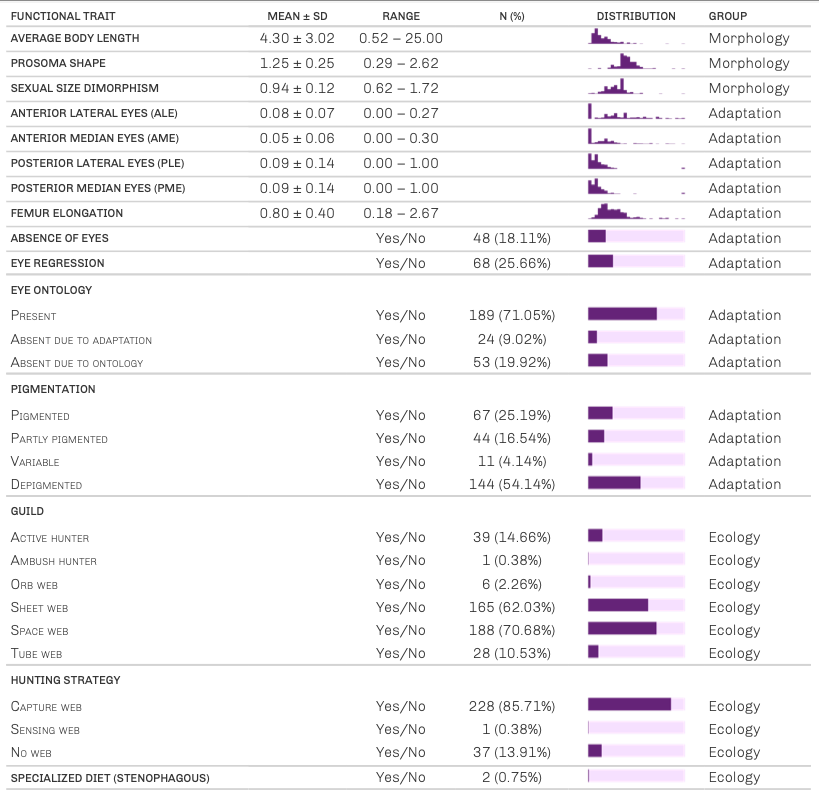
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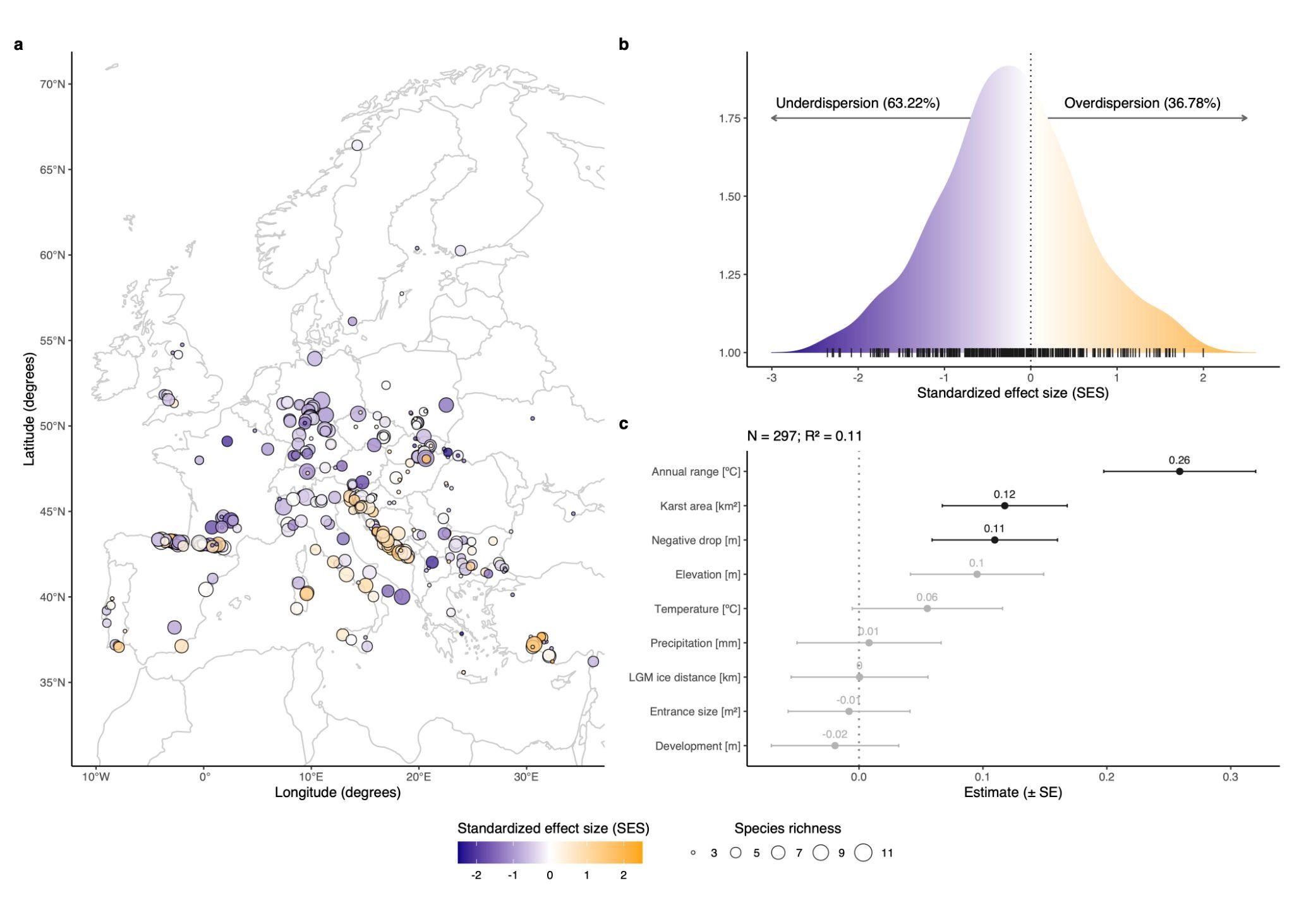
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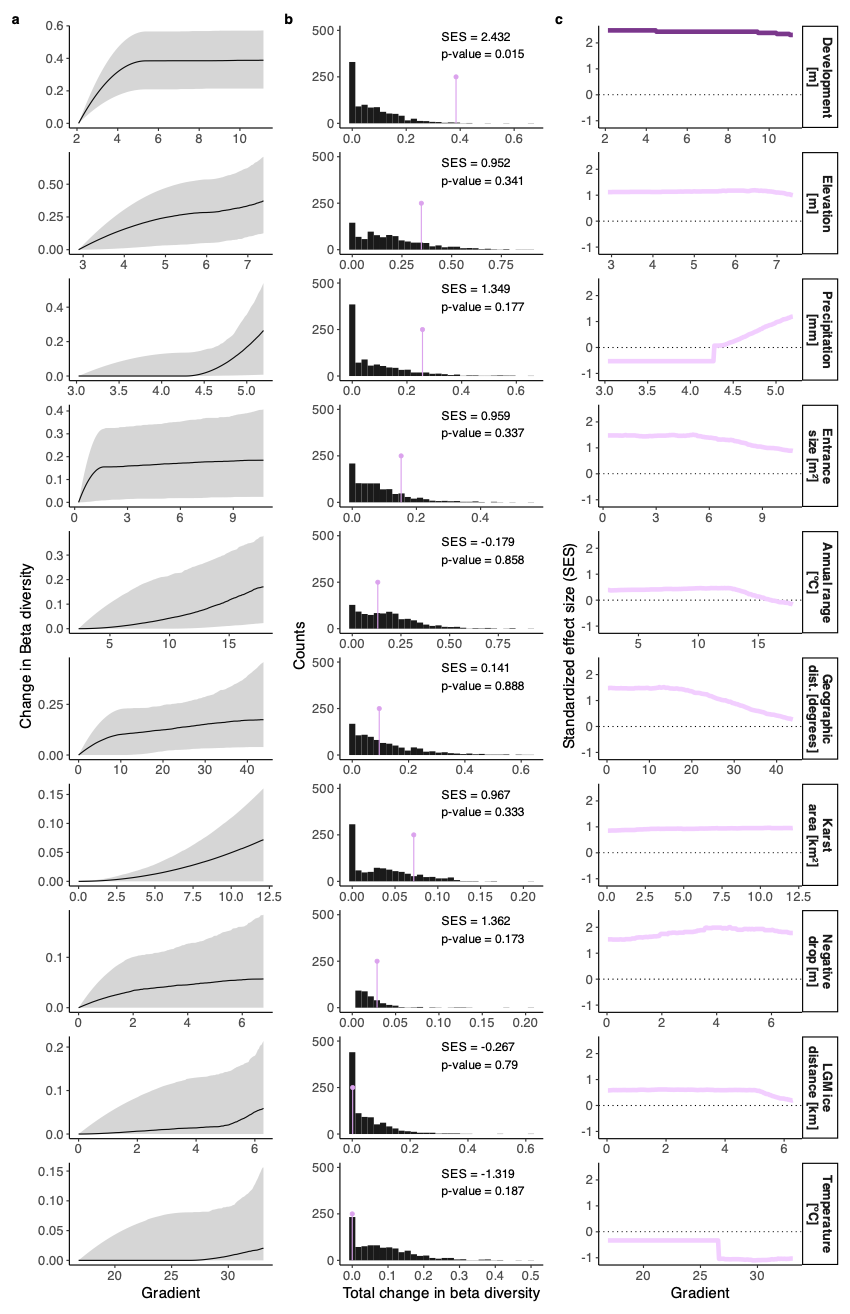
**Figure 1.** Summary of the traits used in the analysis. We refer to Mammola et al. (2022) for a full description of traits and their hypothesized functional meaning. Column “Group” refers to the grouping used in the estimation of weights for the Gower distance *sensu* de Bello et al. (2021), whereby: “Adaptation” are traits related to morphological adaptation to subterranean conditions (especially darkness); “Morphology” are traits describing general morphology of species; and “Ecology” refers to traits describing webs and hunting strategies.



**Figure 2.** Distribution of European subterranean spiders along the first two axes of a principal coordinate analysis describing the trait similarity among species. Gradient of colour denote density of species—higher density in darker areas. The small dots in the figure represent the position of single species across the trait space while the larger circles indicate the centroid position of each trait value.



**Figure 2.** Functional diversity at the α-diversity level for subterranean spider communities in Europe. **a**) Distribution of the studied caves (N = 367 caves). The size of each dot represents species richness. Dots are colored according to their standard effect size (SES) value for functional richness, where functional richness is estimated as the volume of the hypervolume representing each cave’s trait space. **b**) Density of SES for functional richness across the studied caves. Percentage of caves with negative or positive SES values are indicated. Dark lines at the bottom of the density curve show the frequency of observed values. **c**) Environmental factors driving variation of SES values for functional richness. Estimated parameters are based on a generalized least square model (significant effect in a darker color). Error bars indicate standard errors. The exact estimated regression parameters and p-values for the model are in Table 1. Note that the sample size of this model is 297 (not 367) because of missing data in the environmental data for some caves. LGM = Last Glacial Maximum.



**Figure 3.** Results of Bayesian bootstrap generalized dissimilarity modeling for change in total functional β-diversity (βtotal) of subterranean spider communities across Europe (i.e., unit increase in mean β along a given gradient). Variables are sorted by their contribution (on top: highest contribution). **a**) Fitted I-splines (partial regression fits) for the considered environmental and geographic gradients. The maximum height reached by each curve indicates the total amount of compositional turnover explained by that variable (holding all other variables constant), whereas the shape of each spline indicates how the rate of compositional turnover varies along the gradient. **b**) Distribution of expected values (histogram) versus the observed value (colored line) of each gradient, based on null modeling (999 iterations). In other words, these panels provide information as to whether the effect of a given variable is higher or smaller than expected given species composition. **c**) Variation in the magnitude of the standard effect size (SES) values along the observed gradient. In other words, these panels provide information as to whether the effect of a given variable in determining trait dispersion changes along the gradient. In **b** and **c**, significant effects (Rank < 0.025 | > 0.975) are highlighted with a darker purple.

**Table 1.** Estimated regression coefficients for the generalized least square model. Significant values are highlighted in bold. LGM = Last Glacial Maximum.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| TERM | ESTIMATE | STANDARD ERROR | T-STATISTIC | P-VALUE | VIF*1* |
| **Intercept** | −0.23 | 0.05 | −5.02 | **> 0.001** |  |
| **Entrance size**  **[m²]** | −0.01 | 0.05 | −0.16 | 0.870 | 1.17 |
| **Development**  **[m]** | −0.02 | 0.05 | −0.38 | 0.707 | 1.27 |
| **Negative drop**  **[m]** | 0.11 | 0.05 | 2.16 | **0.031** | 1.21 |
| **Elevation**  **[m]** | 0.1 | 0.05 | 1.77 | 0.077 | 1.35 |
| **LGM ice distance**  **[km]** | 0 | 0.06 | 0.01 | 0.994 | 1.3 |
| **Karst area**  **[km²]** | 0.12 | 0.05 | 2.33 | **0.020** | 1.22 |
| **Temperature**  **[ºC]** | 0.06 | 0.06 | 0.91 | 0.364 | 1.86 |
| **Annual range**  **[ºC]** | 0.26 | 0.06 | 4.22 | **> 0.001** | 1.66 |
| **Precipitation**  **[mm]** | 0.01 | 0.06 | 0.14 | 0.889 | 1.7 |

*1* Variance Inflation Factor.

Table SXX. Trait Analysis of European Cave Spiders. The table illustrates the contribution of individual traits to the functional space through mapping on PCoA 1, PCoA 2, and PCoA 3 synthetic axes. All traits contributed significantly to the axes except for spiders with sensing web, ambush hunters and food specialists. Significance values were obtained trhough ENVFIT analysis on the first three PCoA axes scores.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **PcoA 1** | **PCoA 2** | **PCoA 3** | **r2** | **Pr(>r)** | **Signif. Codes** |
| **VECTORS** |  |  |  |  |  |  |
| **AME** | -0,47507 | 0,69214 | -0,54337 | 0,5613 | 0,001 | \*\*\* |
| **ALE** | -0,35357 | 0,74591 | -0,56445 | 0,836 | 0,001 | \*\*\* |
| **PLE** | -0,45528 | 0,86957 | -0,19122 | 0,461 | 0,001 | \*\*\* |
| **PME** | -0,46345 | 0,86505 | -0,19211 | 0,4383 | 0,001 | \*\*\* |
| **Femur elongation** | 0,54638 | 0,81337 | 0,19972 | 0,4295 | 0,001 | \*\*\* |
| **Sexual size dimorphism** | 0,84637 | -0,10171 | -0,5228 | 0,0959 | 0,021 | \* |
| **Body length** | -0,98363 | 0,06607 | -0,16766 | 0,6031 | 0,001 | \*\*\* |
| **Prosoma shape** | -0,61681 | -0,6306 | -0,47104 | 0,293 | 0,001 | \*\*\* |
| **FACTORS** |  |  |  |  |  |  |
| **Pigment** |  |  |  | 0,3503 | 0,001 | \*\*\* |
| *Fully* | -0,0909 | **0,095** | -0,0235 |  |  |  |
| *Variable* | 0,0142 | **0,0527** | -0,0215 |  |  |  |
| *Partly* | -0,0004 | **0,0175** | -0,0173 |  |  |  |
| *Depigmented* | 0,0017 | **-0,0891** | 0,0196 |  |  |  |
| **Eyeless** |  |  |  | 0,2801 | 0,001 | \*\*\* |
| *No* | -0,0178 | **0,0328** | -0,0298 |  |  |  |
| *Yes* | -0,0622 | **-0,1245** | 0,0842 |  |  |  |
| **Eyes regression** |  |  |  | 0,1624 | 0,001 | \*\*\* |
| *No* | **-0,0485** | 0,0074 | 0,0228 |  |  |  |
| *Yes* | 0,0422 | -0,0487 | **-0,0913** |  |  |  |
| **Capture web** |  |  |  | 0,3224 | 0,001 | \*\*\* |
| *No* | -0,1437 | **-0,1545** | 0,0628 |  |  |  |
| *Yes* | -0,0043 | **0,0263** | -0,0161 |  |  |  |
| **Sensing web** |  |  |  | 0 | 1 |  |
| *No* | **-0,0285** | -0,005 | -0,0024 |  |  |  |
| *Yes* | -- | -- | -- |  |  |  |
| **No web** |  |  |  | 0,3224 | 0,001 | \*\*\* |
| *No* | -0,0043 | **0,0263** | -0,0161 |  |  |  |
| *Yes* | -0,1437 | **-0,1545** | 0,0628 |  |  |  |
| **Tube web** |  |  |  | 0,1769 | 0,001 | \*\*\* |
| *No* | -0,0068 | **-0,0086** | 0,0052 |  |  |  |
| *Yes* | **-0,2119** | 0,0255 | -0,0667 |  |  |  |
| **Sheet web** |  |  |  | 0,1137 | 0,001 | \*\*\* |
| *No* | -0,0386 | **-0,0549** | 0,0242 |  |  |  |
| *Yes* | -0,0195 | **0,0395** | -0,0262 |  |  |  |
| **Space web** |  |  |  | 0,4567 | 0,001 | \*\*\* |
| *No* | **-0,173** | -0,053 | 0,0081 |  |  |  |
| *Yes* | **0,0449** | 0,0193 | -0,0078 |  |  |  |
| **Orb web** |  |  |  | 0,0363 | 0,017 | \* |
| *No* | -0,0233 | -0,0062 | -0,0021 |  |  |  |
| *Yes* | -0,2009 | 0,0366 | -0,0142 |  |  |  |
| **Ambush hunter** |  |  |  | 0 | 1 |  |
| *No* | -0,0285 | -0,005 | -0,0024 |  |  |  |
| *Yes* | -- | -- | -- |  |  |  |
| **Active hunter** |  |  |  | 0,3214 | 0,001 | \*\*\* |
| *No* | -0,0013 | 0,0256 | -0,016 |  |  |  |
| *Yes* | -0,15 | -0,142 | 0,0582 |  |  |  |
| **Food specialist** |  |  |  | 0,0176 | 0,189 |  |
| *No* | -0,0274 | -0,0034 | -0,0034 |  |  |  |
| *Yes* | -0,1353 | -0,1675 | 0,0932 |  |  |  |
| **AME type** |  |  |  | 0,3352 | 0,001 | \*\*\* |
| *Absent due to adaptation* | 0,0586 | -0,0578 | 0,0611 |  |  |  |
| *Absent due to Ontology* | -0,1135 | -0,1352 | 0,0686 |  |  |  |
| *Present* | -0,0196 | 0,0363 | -0,0301 |  |  |  |
| Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1 | | | |  |  |  |