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ECOLOGY
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Climatic stability, not average habitat temperature, determines thermal tolerance of subterranean beetles

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Handling Editor: Derek M. Johnson

Abstract

The climatic variability hypothesis predicts the evolution of species with wide thermal tolerance ranges in environments with variable temperatures, and the evolution of thermal specialists in thermally stable environments. In caves, the extent of spatial and temporal thermal variability experienced by taxa decreases with their degree of specialization to deep subterranean habitats. We use phylogenetic generalized least squares to model the relationship among thermal tolerance (upper lethal limits), subterranean specialization (estimated using ecomorphological traits), and habitat temperature in 16 beetle species of the tribe Leptodirini (Leiodidae). We found a significant, negative relationship between thermal tolerance and the degree of subterranean specialization. Conversely, habitat temperature had only a marginal effect on lethal limits. In agreement with the climatic variability hypothesis and under a climate change context, we show that the specialization process to live in deep subterranean habitats involves a reduction of upper lethal limits, but not an adjustment to habitat temperature. Thermal variability seems to exert a higher evolutionary pressure than mean habitat temperature to configure the thermal niche of subterranean species. Our results provide novel insights on thermal physiology of species with poor dispersal capabilities and on the evolutionary process of adaptation to subterranean environments. We further emphasize that the pathways determining vulnerability of subterranean species to climate change greatly depend on the degree of specialization to deep subterranean environments.

KEYWORDS
cave fauna, climate change, climatic variability hypothesis, deep subterranean environment, habitat temperature, Leiodidae, mountain passes hypothesis, thermal tolerance

INTRODUCTION

Amid an ongoing climate emergency (Ripple et al., 2019, 2021), scientists are renewing their efforts to anticipate the impact of climate change on biodiversity (Beaumont et al., 2011; Román-Palacios & Wiens, 2020; Sala et al., 2000). To assess the species capability to cope with changing temperatures, it is crucial to understand which eco-evolutionary factors affect their thermal tolerance (Calosi et al., 2008; Huey et al., 2012). In this sense, one

of the most important factors typically linked to intra- and interspecific variation of thermal tolerance is the environmental conditions that a species experiences in its habitat. The mountain passes hypothesis (Janzen, 1967) and the climatic variability hypothesis (CVH; Stevens, 1989) represent a cornerstone of thermal ecology. Both hypotheses predict the evolution of species with wide thermal tolerance ranges in environments with variable temperatures, and vice versa, the evolution of thermal specialists in thermally stable environments (Janzen, 1967; Stevens, 1989). Macroecological tests of these hypotheses have focused on latitudinal variations in thermal physiology, demonstrating general decreases in species' thermal tolerance breadth toward the equator (Bozinovic et al., 2011; Deutsch et al., 2008; Mermillod-Blondin et al., 2013; Mittelbach et al., 2007; Muñoz & Bodensteiner, 2019; Shah et al., 2017; Sunday et al., 2011). However, Payne and Smith (2017) suggested that temperature variability may not be the ultimate mechanism underlying latitudinal and elevational trends in thermal tolerance, providing an alternative explanation for global diversity patterns in thermal tolerance. They showed that latitude gradients could vary not only in temperature variability but also in mean temperature, and therefore thermal adaptations could be selected for by both types of pressures.

Due to their climatic stability, subterranean environments constitute ideal experimental settings for studying thermal niche evolution under a context of increasing temperatures and climate change, and to extend general thermal tolerance hypotheses beyond the understanding of patterns across environmental gradients at broad spatial scales (Mammola et al., 2019b; Sánchez-Fernández et al., 2018). In the subterranean environment, the extent of spatial and temporal thermal variability experienced by taxa is largely set by their degree of specialization to deep subterranean habitats. In temperate caves, thermal variability increases from deeper or inner sectors (with annual variations usually narrower than 1°C; Badino, 2004, 2010; Cigna, 2002) to shallow or outer sectors (in which annual temperature variations can be more similar to those in the outside environment; Pipan et al., 2010; Mammola et al., 2019b). In the so-called 'superficial subterranean habitats' (Culver & Pipan, 2008) such as small cavities in the uppermost karst layers, deep soil and litter strata, and surface cracks and fissures, thermal variation can even be comparable with that on surface habitats (Mammola et al., 2016; Pipan et al., 2010).

In agreement with Steven's and Janzen's hypotheses, recent studies in subterranean fauna have demonstrated that, in the process of specialization to the subterranean environment, thermal tolerance breadth (Mammola et al., 2019a; Raschmanová et al., 2018) and thermal plasticity via acclimation (Pallarés et al., 2019) have been reduced. However, given that most subterranean species show a low capacity for either microhabitat selection or dispersal, it could be expected that a thermal specialization would involve not only the reduction of thermal tolerance limits but also a fine-tune adjustment of the thermal tolerance range to the specific, highly constant temperature of the local habitat (Huey & Kingsolver, 1989; Peck et al., 2004). Therefore, even among species with similar degrees of subterranean specialization, thermal tolerance ranges might differ depending on the temperature at which they are exposed. Surprisingly, a recent study investigating thermal tolerance in some highly specialized beetles suggested that upper thermal limits (UTL) of subterranean species are uncoupled to current habitat temperature (Rizzo et al., 2015). Therefore, a more nuanced consideration of the interaction between the degree of subterranean specialization (which, in turn, is linked with the thermal stability of the habitat) and average habitat temperature, is needed (Mammola et al., 2019a).

Addressing the relationship among thermal tolerance, habitat temperature, and subterranean specialization remains challenging, for at least three reasons. First, establishing the degree of subterranean specialization requires the collection of detailed traits of subterranean taxa to determine morphological modifications such as eye loss or reduction, depigmentation, increase of adult size, and elongation of appendages (Christiansen, 2005), or even more subtle modifications of life history traits and behavior (Cieslak et al., 2014a; Parzefall, 1982). Second, to accurately address species' thermal tolerance, experimental measurements of physiological thermal limits are needed. However, obtaining such data for subterranean species is not trivial, given the logistic difficulties for sampling in caves and conducting experiments with subterranean animals (Castaño-Sánchez et al., 2020; Culver et al., 2006; Mammola et al., 2021; Schneider & Culver, 2004; Wynne et al., 2018). Moreover, the rarity of subterranean species limits the number of specimens available for robust quantitative analyses (Mammola et al., 2021). Finally, interspecific comparisons of thermal tolerance need to be made with an appropriate phylogenetic control to ensure species comparisons are independent, but phylogenetic data are rarely available and therefore relationships remain unresolved for most subterranean groups (Juan et al., 2010).

Here, we overcome these three limitations to explore the relationship among thermal tolerance, subterranean specialization, and habitat temperature in a lineage of subterranean beetles. As climate change in deep subterranean environments will operate by warming the systems, we experimentally measured UTLs and used ecomorphological traits to characterize the degree of subterranean specialization in a set of closely related species exposed to differing

habitat temperatures and degrees of thermal stability. We expect habitat temperature and the degree of subterranean specialization to be related to thermal tolerance, so that highly specialized species living in colder environments will show the lowest thermal tolerances.

METHODS

Study group and sampling

The study species belong to the *Speonomus* and *Bathysciola* clades of a lineage of the beetle tribe Leptodirini (Coleoptera, Leiodidae, Cholevinae) distributed in the Pyrenees, Basque Country, and coastal mountain systems in Catalonia (Cieslak et al., 2014a; Fresneda et al., 2007; Fresneda & Costas, 2016; Ribera et al., 2010) (Appendix S1: Table S1; Figures S1, S2). We used 16 species of such lineage that show different specificity for the subterranean environment (from facultative subterranean species that live under forest litter or upper layers of fractured soil to obligate subterranean species living in deep subterranean habitats) and different ecomorphological traits related to the degree of specialization to the subterranean environment.

We collected living adult specimens by hand in their natural habitat (Table 1) using baits and food traps. Once collected, we transported specimens to the laboratory in a portable refrigerator with moss to maintain cool and humid (>90% relative humidity) conditions. In the laboratory, we placed specimens inside plastic containers with a white plaster layer (~1 cm diameter), moss, small stones, and tissue papers that we wetted daily. We closed the plastic containers with plastic film with small holes for aeration.

We fed specimens ad libitum with freshly frozen *Drosophila melanogaster* and acclimated them for 2 days in an incubator (Radiber ERF-360; Radiber, Barcelona, Spain) at the average temperature of the locality where each species was collected (Table 1). Such temperatures were obtained from the mean annual temperature available in the WorldClim database v. 1.4 (https://www.worldclim.org/) at 0.08° spatial resolution. It is well established that cave temperatures can be estimated from the mean temperature at the surface, because both are highly correlated (Badino, 2004; Culver & Pipan, 2009; Jeannel, 1926; Juberthie & Decu, 1994; Mammola et al., 2017; Poulson & White, 1969; Sánchez-Fernández et al., 2018; Smithson, 1991). We kept humidity close to saturation (>90% RH) by placing trays with water in the incubators. Temperature and humidity were monitored every 15 min with HOBO MX2301 (Onset Computer Corporation, Bourne, United States) and TFA 30.3039 (KlimaLogg Pro; TFA, Wertheim-Reicholzheim, Germany) dataloggers and

remained quite constant ($\pm 0.5°C$ and $\pm 10\%$ RH variation) along the experiments.

Characterization of the degree of specialization to the subterranean environment

To obtain quantitative estimates of the degree of subterranean specialization of the study species, we measured several biological and ecological traits commonly associated with adaptation to the deep subterranean environment in arthropods (Ribera et al., 2018; Trajano & Cobolli, 2012). We measured the total body length (LT), the length of the antennae (LA) and the tibias (LL) relative to the total body length (LA/LT and LL/LT) (Appendix S1: Table S2; Figure S3), using a stereoscopic microscope (Olympus SZX16; Olympus corporation, Tokyo, Japan) equipped with a camera Lucida (Olympus C5060WZ camera; Olympus corporation). Larger body size and elongated body appendages are typically found in highly specialized subterranean species (Barr, 1968; Galán, 1993; Schneider & Culver, 2004). We also used the number of larval instars of the life cycle. In Leptodirini, a reduction in the number of larval instars from three (in facultative subterranean species) to two or one (in shallow or deep subterranean specialists, respectively) is one of the life cycle modifications associated with subterranean colonization (Cieslak et al., 2014a, 2014b). We assigned a continuous value of each of these traits to each species, except from the number of instars, which was included only for those species whose life cycle has been experimentally studied (i.e., *Bathysciola mystica*, *Machaeroscelis infernus*, *Speonomus curvipes*, and *Speonomus longicornis*) (Appendix S1: Table S2). We performed a principal component analysis (PCA) with the R package *FactoMineR* v.2.3 to summarize the degree of subterranean specialization of the study species according to the morphological parameters and life cycle data. Additionally, we performed another PCA including also habitat specificity (i.e., the assignment of each species as obligate or facultative subterranean; as shown in Appendix S1: Table S2) among the traits, obtaining qualitatively similar results (as shown in the Results section).

Thermal tolerance experiments

We measured the UTLs of the study species by a static method (Jørgensen et al., 2019; Lutterschmidt & Hutchison, 1997), assessing survival under exposure at constant temperatures for 7 days. For this, we placed

TABLE 1 Information on the collection sites

Species	Locality	Mean annual temperature (°C)[a]	Collection habitat
Bathysciola mystica	Grotte d'Escarchein (Escarchein, Ariège, France)	7.6	• Forest litter; shallow/outer cave sector
Bathysciola rugosa	Dolina de Orobe (Alsasua, Navarra, Spain)	12	• Forest litter; shallow/outer cave sector
Nafarroa sorogainensis	Cueva del Carlista (Sorogain, Navarra, Spain)	10	• Forest litter; shallow/outer cave sector
Euryspeonomus breuili	Cueva de Akelar (Alli, Navarra, Spain)	10.6	• Shallow/outer and deep/inner cave sectors
Speocharidius breuili	Cueva de Mendikute (Albiztur, Guipúzcoa, Spain)	10	• Deep/inner cave sector
Parvospeonomus canyellesi	Forat no. 2 de les Pedreres (Gualba de Dalt, Barcelona, Spain)	13.7	• Deep/inner cave sector
Stygiophyes puncticollis	Cova Negra de Tragó (Ós de Balaguer, Lleida, Spain)	12.6	• Deep/inner cave sector
Machaeroscelis infernus	Grotte d'Escarchein (Escarchein, Ariège, France)	7.6	• Deep/inner cave sector
Pseudospeonomus raholai	Cau del Lliri (Cap de Creus, Girona, Spain)	15	• Deep/inner cave sector
Speonomites crypticola	Forat Negre (Serradell, Lleida, Spain)	10.4	• Deep/inner cave sector
Speonomites velox	Querant del Riu Merlé (Vilanova de Meià, Lleida, Spain)	12	• Deep/inner cave sector
Speonomus curvipes	Grotte de Ludax (Belesta, Ariège, France)	10.7	• Deep/inner cave sector
Speonomus longicornis	Grotte Pigaï (Aigües Juntes, Ariège, France)	11.8	• Deep/inner cave sector
Trapezodirus arcticollis	Cova de Sant Salvador (Bonansa, Huesca, Spain)	6.9	• Deep/inner cave sector
Troglocharinus ferreri	Cova de Coll Verdaguer (Vallirana, Barcelona, Spain)	14.9	• Deep/inner cave sector
Troglocharinus fonti	Cova d'Ormini (Montanisell, Lleida, Spain)	10.3	• Deep/inner cave sector

[a]Obtained from the WorldClim database v.1.4 (https://www.worldclim.org/) at 30 arc-sec resolution (~1 km² at the equator).

specimens (6–12 replicates per treatment) in plastic containers in Radiber ERF-360 incubators at different test temperatures, with constant RH (>90%), permanent darkness, and food provided ad libitum. The test temperatures included a control (the same temperature as the collection cave, specific for each species and ranging from 6.9 to 14.9°C; as listed in Table 1) and three higher temperature treatments at 20, 23, and 25°C. Previous studies on cave beetles have reported lethal temperatures between 20 and 23°C (Pallarés et al., 2021; Rizzo et al., 2015). We checked survival every 24 h during the 7-day trial by carefully touching specimens with a brush. The plastic containers, stones, and the tissues were wetted daily and food was provided ad libitum to avoid any potential stress by desiccation or starvation.

We compared the survival of each species at different temperatures using Kaplan–Meier survivorship curves (Altman, 1990). We specified right censored data for those individuals that were alive at the end of the 7 days of exposure (Therneau, 2015). We assessed the overall effect of temperature treatment on survival time using a log-rank test (Harrington & Fleming, 1982), and the R package *survival* v.3.1-12. As a measure of the upper lethal temperature, we calculated the LT_{50} (median lethal temperature, i.e., the temperature at which 50% of the exposed individuals had died) at 7 days' exposure. To obtain LT_{50}, we fitted survival data to a logistic regression model using the R package *brglm* v.0.6.2, and estimated LT_{50} values with the "dose.p" function in the R package *MASS* v.7.3-51.5.

Relationship among thermal tolerance, habitat temperature, and the degree of specialization to the subterranean environment

We explored the relationship among UTLs (using LT_{50} as the response variable), subterranean specialization (approximated as the first PCA component because it explained more than 75% of variance in our data set; see results), and the temperature of each species' locality (i.e., mean annual temperature obtained from WordClim, Table 1), with phylogenetic generalized least squares (PGLS) in the R package *caper* v.1.0.1. The choice of PGLS allowed us to account for the non-phylogenetic independence among species. We incorporated species relationships in the models using the most recent species-level phylogeny of the *Speonomus* group (Cieslak et al., 2014a, 'unpublished data'), updated by including new sequenced specimens that became available after 2014. The phylogenetic tree was pruned to keep only the tips that correspond to the species used in the thermal tolerance experiments, while preserving the overall backbone tree structure. As some of the nodes were not supported (posterior probability <0.95), we accounted for the topological uncertainty using six alternative tree topologies from the postburn-in Bayesian samples as inputs in the PGLS. We used a model weighting procedure to estimate the most important drivers for thermal tolerance (e.g., Gutiérrez-Cánovas et al., 2021). First, we fitted four PGLS models that included all the possible combinations of our predictors: (i) an interactive model with subterranean specialization, habitat temperature, and their interaction, (ii) an additive model with both predictors but without the interaction term, and (iii, iv) two single-predictor models with each variable. Predictors were standardized (mean = 0, SD = 1) to facilitate model coefficient comparison (standardized effect sizes). We then ranked these models using the Akaike

information criterion corrected for small sample size (AIC_c) and evidence weights, using the *MuMIn* package (Bartoń, 2016). All statistical analyses were conducted in R v.3.6.3 (R Core Team, 2020).

RESULTS

Temperature had a significant effect on survival time in most species (see log-rank test in Appendix S1: Table S3), which showed a high survival (100% or nearly) both in the control and 20°C treatment for the entire duration of the experiments (Appendix S1: Table S3). Survival at 23°C was variable among the species, whereas at 25°C no species survived longer than 24 h, except for *Pseudospeonomus raholai*. LT_{50} ranged from 14.90 ± 1.70 (*Euryspeonomu breuili*) to 28.59 ± 3.97 (*Pseudospeonomus raholai*) (Appendix S1: Table S3).

The first principal component used to characterize the degree of subterranean specialization explained 88.7% of variance (number of instars 28.1%; LL/LT: 25.3%; LA/LT: 25.9). In general, species with one larval instar and elongated appendages had a higher degree of specialization (Appendix S1: Figure S4).

The best performing model for thermal tolerance was the one including only subterranean specialization as a predictor (lowest AIC_c value and highest weight). Although the additive model including also habitat temperature was ranked close to the specialization model ($\Delta AIC_c = 1.97$), the effect of temperature on UTL was marginal (see standardized coefficients and R^2 in Table 2). PGLS showed a significant negative relationship between UTLs and the specialization to the subterranean environment (Table 2; Figures 1, 2), which was consistent for the six alternative phylogenetic trees (Appendix S1: Table S4). However, no significant relationship was found between UTLs and the temperature of the cave,

TABLE 2 Comparison of the different phylogenetic generalized least squares models fitted to test the relationship between thermal tolerance, the specialization to the subterranean environment (spec), and mean habitat temperature (temp)

Predictors	Coefficients (mean ± SD)	AICc	ΔAIC	R^2	Weight
Specialization	Spec: −1.975 ± 0.757	82.6	0.00	0.278	0.637
Additive model	Spec: −2.092 ± 0.768 Temp: 0.742 ± 0.768	84.6	1.97	0.275	0.238
Temperature	Temp: −0.309 ± 0.686	86.7	4.11	−0.056	0.081
Interactive model	Spec: −2.087 ± 0.794 Temp: 0.720 ± 0.796 Spec × Temp: -0.273 ± 0.660	88.0	5.38	0.226	0.043

Note: Models are ranked following decreasing statistical support based on AIC_c values. Standardized regression coefficients (standardized effect sizes), ΔAIC_c values (difference with the model with the lowest AIC value), model weights and goodness-of-fit (R^2) are also shown.
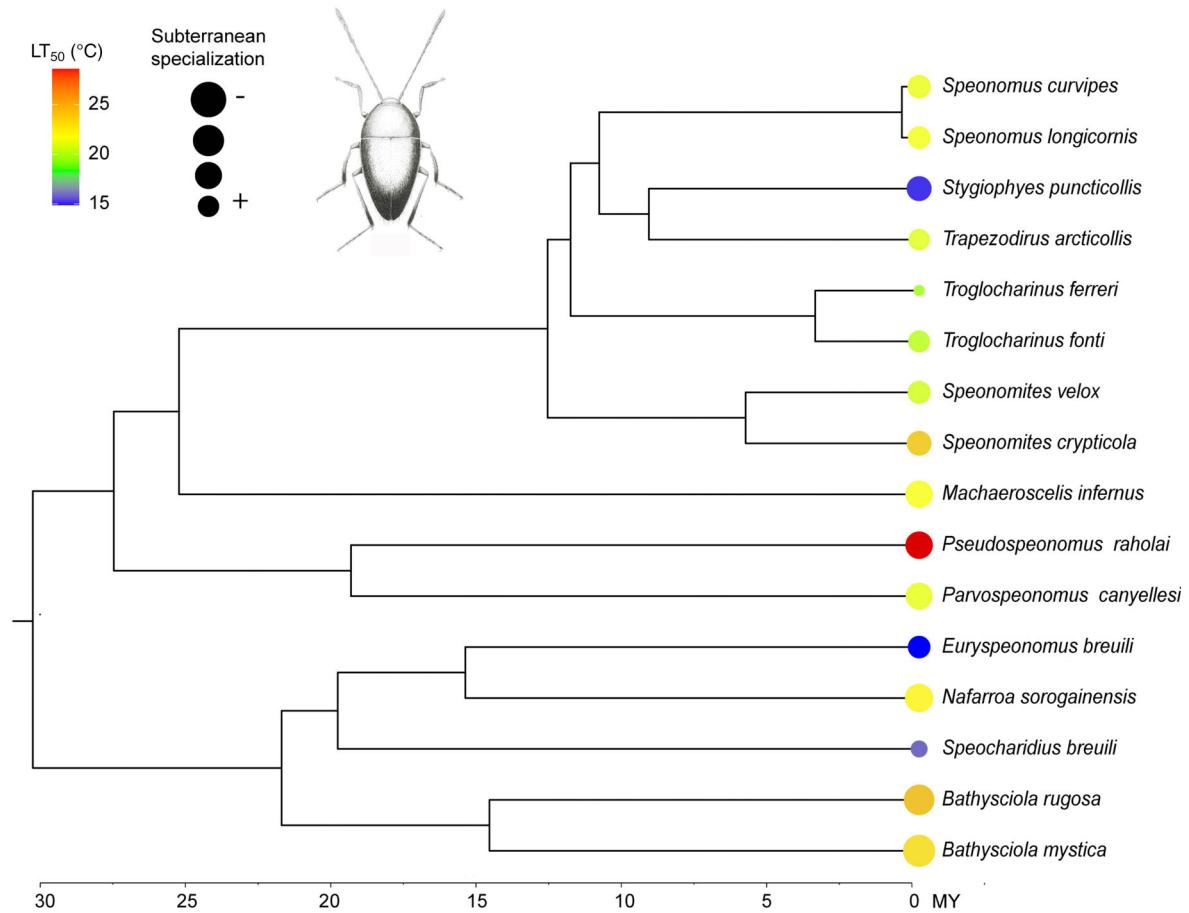
FIGURE 1 Example of one of the calibrated ultrametric phylogenetic trees of the studied clade showing median lethal temperature (LT$_{50}$) values and the degree of specialization to the subterranean environment. Abbreviation: MY, millons of years
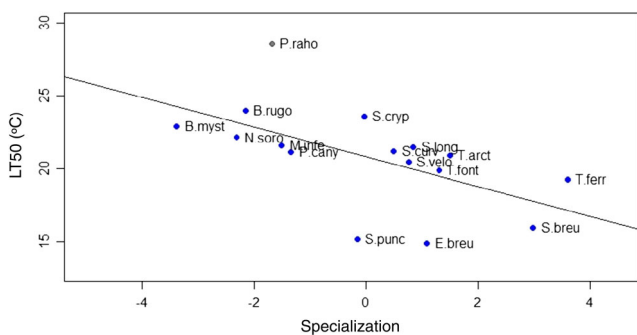
FIGURE 2 Relationship between median lethal temperature (LT$_{50}$) and the degree of specialization to the subterranean environment of the study species (see full species names in Table 1)

neither independently nor in combination with the degree of specialization. No phylogenetic signal in the residuals was found ($\lambda = 0.00$ for the six phylogenetic trees). Analyses made including habitat specificity for the quantification of subterranean specialization provided consistent and similar results (Appendix S1: Table S5).

DISCUSSION

If subterranean organisms have lost the ability to adapt to a changing environment has been a fundamental question in subterranean biology (Mammola et al., 2020), because the degree of specialization to deep subterranean environments is a key driver for the vulnerability to climate change (Pallarés et al., 2021). Therefore, shedding light in the relationship among thermal tolerance, subterranean specialization, and current habitat temperature is pivotal for the conservation of subterranean fauna from a climate change perspective.

Our results support the CVH and Janzen's hypotheses, extending the relationship between physiological thermal tolerance and environmental stability to small spatial scales within subterranean environments, in agreement with previous studies in independent groups of subterranean invertebrates (Raschmanova et al. 2018; Mammola et al., 2019a; Pallarés et al., 2021; Jones et al., 2021). Therefore, the loss of thermal tolerance in the process of specialization to the deep subterranean environment emerges as an intriguing, non-

morphological example of convergent evolution in caves, as the same pattern has been found in disparate groups. Historically, the most well studied subterranean adaptations were those involving morphological changes or behavioral modifications (e.g., feeding, reproduction, life cycle, and sociality) related to permanent darkness or low food availability as the main selective pressures (Culver & Pipan, 2015). Although there are some examples showing pronounced modifications of thermal physiology in organisms inhabiting other highly stable habitats, such as the absence of a heat shock response in Antarctic nothothenioid fish (Somero, 2005), the role of large spatial and temporal thermal stability as a potential selection pressure shaping thermal physiology features remains largely unexplored.

Our results show that the specialization process to live in deep subterranean habitats involves a loss of heat tolerance and this is irrespective of the temperature at which species are exposed, because habitat temperature only had a marginal effect on UTLs. Then, there is not a fine-tune specialization of the thermal limit. Therefore, we confirm the lack of adjustment of UTLs to habitat temperature previously suggested by Rizzo et al. (2015) for deep subterranean species, and extend it to the whole lineage studied here irrespective of the degree of subterranean specialization. Therefore, thermal variability seems to exert a higher evolutionary pressure than mean habitat temperature to configure the thermal niche of species. For the less specialized species, subjected to greater daily and seasonal fluctuations, such decoupling between mean temperature and the UTL could be somehow more predictable, but it is surprising that the most specialized species are able to survive at temperatures well above those experienced for millions of years with an extremely low temporal and spatial variation. It is known that UTL has little evolutionary potential to increase in ectotherms (Kellermann et al., 2012; Araújo et al., 2013, Hoffmann et al., 2013). Our results further suggest that it has little evolutionary potential also to decrease, and apparently the maintenance of physiological tolerance to high temperature up to some point (~20°C in the studied clade) might not pose an excessive energetic cost for these species.

It is worth noting some conspicuous differences in thermal tolerance between species with similar degrees of subterranean specialization (e.g., *Pseudospeonomus raholai* or *Stygiophyes puncticollis* among the species with an intermediate degree of specialization, *Euryspeonomus breuili* and *Speocharidius breuili* among specialists). These differences cannot be explained by the different temperatures of their localities. Further work must be directed to explore other factors that could explain the particularly high or low tolerances of these species (e.g., other natural or anthropic stressors that could affect these populations). In addition, further experimental work to complete the information on the number of larval instars for all considered species could be useful, as it is likely to be the most reliable trait to act as surrogate of the degree of specialization to deep subterranean environment in beetles (Cieslak et al., 2014a). Therefore, including this new information for some species in the model could refine the residuals for these species and the general relationship with thermal tolerance.

Adaptation to stable environments implies the reduction of thermal tolerances and therefore, the ability to cope with climate change. Our results agree with those of Johansson et al. (2020) that recently demonstrated that temperate insects with narrow seasonal activity periods can be as vulnerable to climate change as tropical insect species. However, the overall vulnerability of a species will depend on both its intrinsic thermal sensitivity and the magnitude and rate of climate change at which they are exposed in their habitats (Foden et al., 2019). Among subterranean species, those living in the deepest part of caves are the less thermally tolerant according to our results, but they will be more buffered from thermal changes than species living in the outer sections of the caves or forest litter. However, some of these deep subterranean specialists currently inhabiting warm caves, would have a narrower thermal safety margin in a climate change scenario than more superficial species (Arribas et al., 2012; Colado et al., 2021; Deutsch et al., 2008). For example, *Troglocharinus ferreri* has UTL values only ~4°C above its habitat temperature. Contrarily, less specialized species inhabiting more superficial habitats (e.g., *Bathysciola mystica* and *B. rugosa*), could be less stressed under climate change as they have much broader tolerance ranges, ~12–13°C above the temperature under which they are currently living, showing therefore, broader thermal safety margins. However, contrary to deep and climatically stable habitats, superficial subterranean habitats are subjected to higher variations in their maximum temperatures (Mammola et al., 2016; Pipan et al., 2010). Maximum temperature, as well as the frequency of temperature extremes, has been shown to be more meaningful than average temperature in explaining species extinction risk due to climate change (Román-Palacios & Wiens, 2020). Also, it must be considered that some metabolic functions can be compromised in the long term at temperatures much below the thermal tolerances found here. Therefore, it would be interesting to complement survival experiments with studies of biochemical biomarkers that reflect physiological changes triggered by thermal stress (e.g., oxidative stress) for a number of species with different degrees of subterranean specialization, to obtain more

accurate estimates of the species capacity to face climate change (Pallarés et al., 2020).

Exploring in detail the relationship between thermal tolerance and specialization in ecomorphological traits could be helpful to predict the relative sensitivity to climate change of subterranean communities without needing to measure thermal limits. We show that the pathways that determine the vulnerability of subterranean species to climate change greatly depend on their degree of specialization to deep subterranean environments. This information, combined with evaluation of exposure to climatic changes at their present locations, is needed to identify species or populations at greatest risk and to preserve this fragile and valuable component of global biodiversity, especially when it is systematically neglected by global climate change conservation strategies and agendas (Arneth et al., 2020; Sánchez-Fernández et al., 2021). In the era of climate change, when insect populations are going extinct at an unprecedented peace (Cardoso et al., 2020; Eisenhauer et al., 2019), it is important not to forget the biodiversity that dwells below our feet (Mammola et al., 2019c; Sánchez-Fernández et al., 2021; Wynne et al., 2021). It will be affected the same, if not more so, by a global temperature increase.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Raquel Colado, Susana Pallarés, and David Sanchez-Fernandez conceived the ideas and designed the methodology; Raquel Colado, Susana Pallarés, Javier Fresneda, Valeria Rizzo, and David Sanchez-Fernandez collected the data; RC and SP analyzed the data; Raquel Colado, Susana Pallarés, Stefano Mammola, and David Sanchez-Fernandez directed the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

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