# Climate change may drive cave spiders to extinction

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**Running title:** Predicting cave spiders' extinction

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**Authors contribution statement**

MI and SM conceived the study, collected and analyzed the data. SLG provided fundamental arguments for the discussion. All authors contributed to the writing of the manuscript.

## Abstract

Subterranean ecosystems present ideal opportunities to study mechanisms underlying responses to changes in climate because species within them are often adapted to a largely constant temperature. We have characterized the thermal conditions of caves in the Western Alps, and relate these hypogean climate data to the occurrence of *Troglohyphantes* spiders (Araneae, Linyphiidae). Our data indicate that present distributions reflect Pleistocene glaciation events and also point to specific responses as a consequence of changes in temperature. Constant temperatures recorded inside caves provide an approximation of the mean annual temperature outside, thus we extend the results to a regional scale. We used ecological niche modeling to predict habitat suitability both in the Pleistocene and under future global warming scenarios. These analyses point toward a future decline in habitat suitability for subterranean spiders and the potential extinction of the most restricted endemic species. When compared with other species that live in confined habitats such as islands and mountains, we expect cave species to be as much, if not more, vulnerable to climate change.

## Introduction

The potential impact of global climate change on a broad range of organisms occurring in different ecosystems and showing a very diverse pattern of distributions is well documented (Walther et al. 2002, Parmesan and Yohe 2003, Parmesan 2006, Walther 2010, Chen et al. 2011, Dawson et al. 2011, Bellard et al. 2012). However, the effects on biological communities in hypogean ecosystems have not been well explored (but see Brandmayr et al. 2013, Wynne et al. 2014, Sánchez-Fernández et al. 2016).

From an ecological standpoint, hypogean ecosystems are considered extreme, lacking solar radiation, receiving poor energy inputs, and through being characterized by low abundance and diversity of organisms (Poulson and White 1969, Culver and Pipan 2009, Romero 2009). Most caves have almost constant temperature over the year, with an annual variability decreasing progressively with increasing distance from the entrance until a remarkable temperature steadiness is reached (Moore and Sullivan 1964, Smithson 1991), usually varying by only a few tenths of a degree over the year (Badino 2010).

According to direct field observations (Fejér and Moldovan 2013, Domìnguez-Villar et al. 2015) and theoretical models (Badino 2004, Covington and Perne 2015), anthropogenic global warming may significantly influence and modify the underground climate in different ways to that which happens outside. The most important difference refers to the thermal inertia of caves, which explains time lags of up to several decades in the warming of the system (Badino 2004). This increased flux of energy from the atmosphere to the subterranean environment is expected to be deposited primarily in the superficial sections of caves (Badino 2004) and in other superficial subterranean habitats (Mammola et al. 2016). Energy fluxes accumulating underground may have dramatic cascade effects on both physical and biological components of hypogean ecosystems. By affecting air density gradients, for instance, small changes in cave temperature modify air circulation, with potential fallouts on speleothem growth, seasonal ventilation rates, and processes of condensation and corrosion (Domìnguez-Villar et al. 2015).

Given the reduced physiological tolerance to temperature fluctuations in subterranean species (e.g. Bernabò et al. 2011, Mermillod-Blondin et al. 2013, Novak et al. 2014, Rizzo et al. 2015) as well as their low dispersal capacity (Sánchez-Fernández et al. 2016), ongoing human-driven climatic shifts are also expected to affect subterranean biocoenosis and ecosystems (Wynne et al. 2014). From a conservation perspective, the ecological and biogeographical peculiarity of the subterranean fauna enhances the risk of local extinction (Cardoso 2011a, b, 2012) and strengthens the importance of considering subterranean organisms as model species for ecological studies dealing with climatic changes (Chevaldonné and Lejeune 2003, Wynne et al.

63    2014, Mammola et al. 2015b).

64    We investigated the response of hypogean specialized spiders to increases in temperature in the Western

65    sector of the Alps. Among the range of subterranean taxa occurring in the Western Alps, we chose the cave-

66    dwelling spiders *Troglohyphantes* (Araneae, Linyphiidae) as model organisms. The reliability of this model

67    comes from empirical observations pointing out their preference for narrow, stable and cool microclimatic

68    conditions (Deeleman-Reinhold 1978, Isaia and Pantini 2010, Isaia et al. 2011, 2016, Mammola and Isaia

69    2016). Accordingly, *Troglohyphantes* spiders are characterized by moderate to high levels of

70    *troglobiomorphy* (*sensu* Juberthie and Decu 1994) and show restricted or point-like ranges of distribution

71    (e.g. Brignoli 1971, Deeleman-Reinhold 1978, Isaia and Pantini 2010, Isaia et al. 2011). On top of that,

72    populations of *Troglohyphantes* are usually extremely structured from a genetic point of view, lacking shared

73    haplotypes between caves and showing highly reduced dispersal ability (Mammola et al. 2015a).

74    We modelled presence/absence of *Troglohyphantes* spiders in relation to cave microclimate, past glacial

75    dynamics and other cave features in order to assess their sensitivity to potential subterranean climatic

76    variation induced by climate change.

77    Because constant temperatures recorded inside caves provide an approximation of the mean annual

78    temperature outside (Moore and Sullivan 1964, Smithson 1991, Badino 2004, 2010), in a second step we

79    extend the results to a bigger dataset. We used Ecological Niche Modeling (ENM) to extend to a wider scale

80    the response of our model organism to climatic changes, estimating the impact in terms of loss of habitat

81    suitability. In particular, we hypothesized i) a significant relationship between the occurrence of

82    *Troglohyphantes* spiders and different climatic conditions as a function of their adaptation to narrow and

83    specific ranges of temperature; and ii) that climate affected the distribution of our model species in the past

84    and will affect it in the future, through a decline in habitat suitability.

85

## Materials and methods

### Study area

We studied caves in the administrative regions of Piemonte and Valle d'Aosta (Western Alps, Italy), a coherent biogeographic area of the Alps in which the distribution of *Troglohyphantes* spiders has been well documented (e.g. Isaia and Pantini 2010, Isaia et al. 2011, 2016). We used a regional scale for analyses instead of smaller or larger scales, as the former has been shown to be appropriate for species-level responses to global warming (Walther et al. 2002). The Western Alps contain more than 2,500 caves developed in carbonate and non-carbonate substrates (AGSP, 2016), plus a variety of artificial hypogean habitats such as bunkers and abandoned mines.

We selected 33 hypogean localities over a north-south range in the Western Alps (Fig. 1), from the Tanaro Valley (South; Province of Cuneo) to the Strona Valley (North; Province of Verbania). These included karst and tectonic caves as well as artificial subterranean sites, with an altitudinal range of 415–2,357 m a.s.l, a planimetric development range of 7– 3,440 m and an ascending/descending structure (i.e. drop) range from –143 to +184 m. We deliberately choose localities with an established, recently and well-documented spider fauna (see Isaia et al. 2011). A description of each site is provided in Supplementary Material Appendix 1.

### Sampling design

Temperature (T) was monitored from 2012 to 2014 using Hygrochron™ devices (accuracy: ±0.5 °C) placed inside the cave ($T_{int}$) and at cave entrances ($T_{ext}$). Measurements were taken every 3 hours and the devices were replaced after 12 months to allow download of data part way through the study.

The presence of *Troglohyphantes* at each site was verified during all surveys. The final presence/absence dataset was verified and integrated with available literature data (Isaia and Pantini 2010, Isaia et al. 2011, 2016), wherever possible.

### Fine-scale modeling

Data from the Hygrochron™ devices were used to generate values of mean annual temperature, daily and annual range at the cave entrance ($T_{ext}$) and at the deeper zone ($T_{int}$). Sites were sorted according to previous glacial history (ICE), as identified through an in-field geological survey by Motta (2014) or by using paleo-glacial reconstruction for sites not included in the Motta study (Ehlers et al. 2011)—see Supplementary

115     Material Appendix 1. The relationship between temperature variables was explored using linear regression

116     models—*lm* command in the "stats" R package (R Development Team 2015). Presence/absence of spiders

117     was modelled as a function of temperature and the glacial history (ICE) via generalized linear models (*glm*

118     command in the "stats" R package) assuming a Bernoulli error structure (0–1 discrete). Prior to model fitting,

119     we tested for multi-collinearity among covariates and excluded collinear variables (Zuur et al. 2010). We

120     tested for spatial autocorrelation among environmental variables and in species occurrence via Moran's I test

121     in the "ape" R package (Paradis et al. 2004), using the Gittleman and Kot (1990) method. After fitting the

122     initial model, we carried out model reduction by sequentially deleting terms—backward elimination—

123     according to AICc values (Hurvich and Tsai 1989). The process was repeated until a minimum adequate

124     model of fixed effects remained (Zuur et al. 2009).

125

126     **Wide scale modeling, past and future predictions**

127     Once the model had been fitted using the fine-scale dataset, we extended the detected trends to a wider

128     scale (regional) using ecological niche modeling. We modelled the current distribution of *Troglohyphantes* in

129     the study area, transferring the results into ancestral (Last Glacial Maximum; ~22,000 years ago; hereinafter

130     LGM) and future (2050 and 2070) climatic conditions in order to estimate variations in the distribution ranges

131     relative to past and future climate changes.

132

133     *Occurrence points*

134     Geo-referenced occurrence localities of *Troglohyphantes* spp*.* were taken from Isaia et al. (2011). This

135     dataset covers 361 caves and virtually all possible thermal ranges experienced by *Troglohyphantes* spiders

136     in the Western Alps. *T. lucifuga* and *T. lucifer*, which are not exclusively restricted to subterranean habitats

137     (Isaia and Pantini 2010, Isaia et al. 2016), were excluded from our analysis. Prior to model fitting, we

138     designated a sampling bias grid (Phillips et al. 2009, Syfert et al. 2013). Within each cell in the grid—which

139     had a width equal to the grain of the bioclimatic rasters—all the points apart from one were randomly

140     removed (see Newbold 2010). In this way, we minimised spatial autocorrelation, given that proximate

141     localities likely comprise similar environmental variables.

142

143     *Explanatory variables*

144     We represented present-day climate with 19 bioclimatic variables (1950–2000; Hijmans et al. 2005;

145  resolution: 30''; available at: www.worldclim.org; Table 1), and analogous data layers for past and future

146  climate. For both past and future climate, multiple GMCs were used to take into account variation and

147  uncertainty among simulations (Kageyama et al. 2001, Beaumont et al. 2008, Diniz-Filho et al. 2009). Paleo-

148  bioclimatic variables for the LGM were derived from simulations available from three General Circulation

149  Models (GCMs) from the Coupled Model Intercomparison Project Phase 5 (CMIP5; Taylor et al. 2012).

150  These were CCSM4, MIROC-ESM and MPI-ESM-P (resolution of 2.5'). Future data represent downscaled

151  and calibrated climate projections from three GCMs (CCSM4, BCC-CSM1-1, MIROC5) under two

152  Representative Concentration Pathway scenarios (rcp 2.6 and rcp 8.5 respectively) for both 2050 and 2070,

153  at a resolution of 30''. Ice cover during Pleistocene was derived from paleo-reconstruction, by converting the

154  shape of glacial masses provided by Ehlers et al. (2011) into a binary raster. This categorical variable was

155  further included in the models. Prior to model computing, we investigated multi-collinearity among covariates

156  via Pearson *r* correlation (Zuur et al. 2009, 2010), selecting a final set of uncorrelated bioclimatic variables

157  ($r < \pm 0.7$) (Table 1).

158

159  *Algorithm choice and projections*

160  A wide suite of algorithms exists in the realm of bioclimatic envelope modeling (Peterson et al. 2011).

161  Considering the conceptual ground of the "no free lunch" theorem (Ho and Pepyne 2002), Qiao et al. (2015)

162  recently suggested that no single best algorithm should be chosen *a priori* for modeling the ecological

163  niche/species distribution of a certain organism. We thus began the analysis of this particular dataset with an

164  assessment of performance of potential competing algorithms (see also Elith et al. 2006). In particular, we

165  tested the performance of three well-acknowledged statistical techniques, belonging to the three existing

166  categories of algorithms for computing ENM/SDM: regression [Generalized linear models (GLM); McCullagh

167  and Nelder 1989], regression trees [Boosted Regression Trees (BRT); Friedman, 2001] and machine-

168  learning [Maximum entropy model (MaxEnt); Philips et al. 2006]. Details of the modeling procedure and

169  settings for each technique are provided in Supplementary Material Appendix 2.

170  As is widely recommended in the literature (e.g. Peterson et al. 2011, Saupe et al. 2012, Merow et al. 2013),

171  models were calibrated within the accessible area—often referred to as area M (Barve et al. 2011; details in

172  Supplementary Material Appendix 2). **M** is the geographic extent hypothesized to fall within the long-term

173  dispersal and colonization potential for a particular species over its evolutionary history. Considering the

174  reduced dispersal potential of *Troglohyphantes* spiders, in our case area **M** was approximated by masking

175  the bioclimatic layers with a rectangle delimiting the Western alpine chain.

176 We computed the models using the three modelling techniques in order to relate the occurrence points to the

177 set of non-collinear variables. For each technique, we ran twenty replicate of models via a loop in R, keeping

178 a random partition of 20% of the points for each run to assess the predictive ability of each algorithm.

179 Predictive abilities of the three models were obtained by calculating two different metrics (average of the

180 twenty runs): the area under the Receiver Operating Characteristics (ROC) curve (AUC; Fielding and Bell

181 1997) and the True Skill Statistics (TSS; Allouche et al. 2006), whereby an AUC < 0.60 and/or a TSS < 0.1

182 denotes a predictive ability no better than random. We employed two metrics because it is not recommended

183 to rely on a single measure of model fit for comparing different techniques (Elith and Graham 2009).

184 We finally retained the modeling technique showing the best performance. A final model was generated

185 using the same parameters and calibrated with the complete occurrence dataset.

186 Subsequently, we transferred the model into each LGM and future GCMs, and we calculated the median

187 value across all projection for each combination of GCM. We estimated environmental suitability for each

188 projection by rescaling raster cell values above the threshold of 0.4 (see discussion in Liu et al. 2005)

189 between 0 and 1. Areas below a threshold value of 0.4 were set to 0.

190

191

192

## Results

194 **Fine-scale modelling**

195 As expected, we observed a strong linear relationship between mean annual $T_{int}$ and mean annual $T_{ext}$

196 (Estimated β±se: 0.901±0.041, p<0.001), with only 3 out of 33 caves showing $T_{int}$ values 1 °C higher (Grotta

197 di Bergovei and Fessura di Verrogne) or lower (Grotta Testa di Napoleone) than $T_{ext}$ values (Supplementary

198 material Appendix 2, Fig. A1).

199 No variables considered in the GLM analysis were found to be spatially autocorrelated (Moran's I test, all

200 *p*>0.05) apart from the mean annual $T_{int}$ range (p<0.01) which therefore was not considered further. Moran's I

201 test results are reported in the Supplementary Material Appendix 2, Table A1. Daily range of $T_{int}$ was highly

202 collinear with past ice cover (ICE) and thus excluded from the analysis (Supplementary material Appendix 2,

203 Fig. A2). All variables related to $T_{ext}$ (mean and range) were highly collinear with $T_{int}$ (Pearson *r*>±0.7), and

204 thus not included in the model (Supplementary material Appendix 2, Table A2). Backward elimination

205 revealed that variables related to cave features—type of cave, total development, drop—were not influential

206 in determining the presence/absence of *Troglohyphantes* and these were therefore not considered in further

207 analytical steps. Accordingly, the structure of the minimum adequate model included mean annual $T_{int}$ and

208 the past glacial cover (ICE) as covariates. Outcomes of the GLM pointed to a significant decrease in the

209 probability of occurrence of *Troglohyphantes* with increasing mean annual $T_{int}$ values (Estimated β±se: -

210 0.543±0.249, p=0.02). We also found a significant negative effect of glacial history, namely a lower

211 probability of occurrence of the model organisms in caves covered by the ice during past glaciations (ICE,

212 test relative to the level "uncovered"; Estimated β±se: -2.584±1.052, p=0.01; Fig. 2).

213

214 **Model on the regional dataset, past and future predictions**

215 MaxEnt performed better than other algorithms tested in terms of predictive ability (MaxEnt: mean AUC±sd =

216 0.873±0.09; mean TSS±sd = 0.51±0.08; GLM: AUC±sd = 0.676±0.378; mean TSS±sd = 0.32±0.221; BRT:

217 AUC±sd = 0.78±0.02; TSS±sd = 0.43±0.12). We therefore retained the former technique for further analysis

218 and projections. Uncorrelated variables introduced in the final model are reported in Table 1. The final model

219 included mean annual temperature (Bio01), mean diurnal range (Bio02), and ice cover (ICE)—response

220 plots are reported in Supplementary Material Appendix 2, Fig. A3. Suitable areas predicted by the model for

221 the current climate (Fig. 3) roughly overlay the known distribution of *Troglohyphantes* in the study area, with

222 higher suitability predicted in the low-medium mountain belt, ranging from the districts of Maritime to Graian

223 Alps. The most suitable and unfragmented habitat is found at the southern border of the Alps. Based on the

224 model projections, the average probability of occurrence is 0.68 across all known populations of

225 *Troglohyphantes*.

226 With regard to LGM projections (Fig. 3), the district of Maritime Alps (South-western Alps) and the

227 surrounding plains appear to contain the most suitable areas of habitat. Areas covered by ice were predicted

228 to be largely unsuitable, with areas bordering the glacial masses possibly acting as microrefugia (*sensu* Rull

229 2009).

230 Future forecasts based on different emission scenarios indicate a general decline of suitability throughout the

231 distribution range (Fig. 4), with major effects at higher emissions. For 2050 we predict a general decrease in

232 suitable areas (threshold 0.4) of ~ 45% for the low emission (rcp 2.6) and of ~ 55% for the high emission

233 scenario (rcp 8.5) in respect to the present-day projection. Habitat loss is further intensified in the 2070 high-

234 emission scenario, with an overall reduction of ~ 70%. The predicted loss of suitable habitat appears to be

235  more intense at lower altitudes. In particular, for the high-emission scenario in 2070 approximately half of the

236  current localities are predicted to be unsuitable (Fig. 5).

237  Across all known populations, the average decrease in the probability of occurrence of *Troglohyphantes*

238  drops from 0.68 to 0.51 in the low emission scenario (both 2050 and 2070). For the high emission scenario,

239  the probability of occurrence drops to 0.40 for 2050 and 0.17 for 2070.

# DISCUSSION

**Caves as laboratories for the study of climate change**

In contrast with fluctuating surface temperatures, temperature stability in the subterranean domain allows the detection of temperature changes over longer time periods. Indeed, this working hypothesis provides a stimulating starting point for the ecological modeling of hypogean ecosystems. In spite of that, correlative distribution modelling techniques have been rarely—and only recently—applied to these particular biological systems (e.g., Bryson et al. 2014, Naranjo et al. 2014, Fišer et al. 2015, Mammola et al. 2015a, Soto-Centeno et al. 2015, Christman et al. 2016, Copper-Bohannon et al. 2016, Ferreira et al. 2016, Sánchez-Fernández et al. 2016).

However, the link between the external climate and the subterranean habitat has been well documented in speleological literature (Moore and Sullivan 1964, Smithson 1991, Badino 2004, 2010, Covington and Perne 2015). The general explanation for this relationship is found in the physics of the infiltrating meteoric fluids. Over a geological timeframe, a given rock layer will be forced to the thermal equilibrium of the water flowing through it. As a consequence, the temperature of the rock surrounding the cave equilibrates to the mean temperature of the infiltrating waters, which, in turn, is equal to the mean temperature outside (Badino 2010). Depending on the thickness of the rock layer (i.e. the cave depth), the amplitude of the sinusoidal input signal (i.e. the external temperature range) can be shifted in time—i.e. there is a time lag between input and output maxima (Badino 2004), but this lag is likely to have a duration of decades. As an example, for the Postojna cave (Slovenia) Domìnguez-Villar et al. (2015) estimated a warming time lag of ~ 20 years, which is orders of magnitude less time than the glacial time frames that are the focus of the study. In practical terms, this implies that our future projections are mostly relevant for the superficial subterranean habitats—including the outermost sectors of caves—whereas they should be shifted in time of at least 20 years to obtain a more precise time scale for future habitat modifications in deep hypogean layers.

In an evolutionary sense, such a time lag is not large, but in an anthropogenic sense it might be significant. As a consequence, spiders that we see today may still be in the lag phase i.e. not yet fully adapted to the current temperature.

We note that, in general, correlative distribution models prioritize the use of extreme values—maxima and minima—instead of average values. Whilst extremes are often believed to reflect a realistic relationship with species-specific physiological constraints (Hijmans et al., 2005), in the case of caves with constant temperatures and reduced temperature excursions, average values are likely to be more appropriate. Our

270  use of average values thus likely increases the robustness of our results because GCMs models are better

271  at predicting average than extreme values (but see Zimmerman et al. 2009).

272

**Interpreting the effects of past climate change**

274  Two contrasting theories attempt to explain the origin and the distribution of the subterranean fauna (Culver

275  and Pipan 2009, 2010). The theory of the active colonization (Rouch and Danielopol 1987) or adaptive shift

276  hypothesis (Howarth, 1980) puts great emphasis on the process of active colonization of the hypogean

277  domain, with species being driven by the opportunity to occupy new, unexploited ecological niches. On the

278  other hand, the theory of relicts and refuges (Botosaneanu and Holsinger 1991) invokes long-term climatic

279  changes, such as glaciation cycles and other large-scale climate upheavals, as the main factors that

280  prompted the colonization of the subterranean habitat and causing the obliteration of surface-dwelling

281  populations (Holsinger 1988, Botosaneanu and Holsinger 1991).

282  Although this dichotomous vision exists, the case of our model species possibly supports the latter theory of

283  relict and refuges, given that the occurrence of *Troglohyphantes* appears to be significantly linked to the

284  glacial history. This pattern can be explained by taking into account the self-evident fact that, most likely,

285  natural populations do not survive in caves filled with ice (Culver and Pipan 2010). Remarkably, in our case

286  only two sister species (*T. lucifuga* and *T. lucifer*) were found to occur in caves that were covered by ice

287  during the Pleistocene. *T. lucifuga* is a common and widespread species in the North Western Alps showing

288  a low degree of troglobiomorphism, whereas the recently described *T. lucifer* represents its ecological

289  vicariant in the nearby alpine districts of Cottian and Southern Graian Alps (Isaia et al. 2016). Both species

290  occur in epigean as well as hypogean habitats (Isaia and Pantini 2010, Isaia et al. 2016), an occurrence that

291  could be explained by *T. lucifuga* and *T. lucifer* having colonized the area after the glaciers retreated.

292

**Global change in hypogean ecosystems**

294  Little attention has been given to the effects of global climate change on hypogean organisms and

295  ecosystems relative to their epigean counterparts. In this study, we show the extent to which cave-dwelling

296  biological communities are likely to be affected by changes in temperature. Specialized hypogean arthropods

297  are known to have a fine-tuned thermal tolerance to the constant and narrow temperature ranges of the

298  subterranean habitat (Delay 1978, Huevy and Kingsolver 1989, Rizzo et al. 2015). This extends even to a

299  greater reduction in thermal tolerance observed in species living in the buffered portion of caves compared

300 with those living in the vicinity of the cave entrance (Lencioni et al. 2010, Bernabò et al. 2011, Novak et al.

301 2014).

302 However, from a global change perspective, such an adaptation turns out to be a strong limitation. Indeed,

303 while most invertebrates living close to the surface—troglophiles—have retained their ability to withstand

304 temperature variations, many specialized hypogean organisms—troglobionts—have lost such

305 thermoregulatory mechanisms (Novak et al. 2014).

306 Modelling at both local and regional spatial scales indicates that subterranean *Troglohyphantes* spiders are

307 restricted to particular climatic environments, occurring only in caves with annual mean temperatures below

308 10–11°C. This finding implies that in most mid-altitude areas of the W-Alps, where annual mean

309 temperatures are around 10–12°C, populations of *Troglohyphantes* are close to their physiological limit. Due

310 to predicted temperature rises (IPCC 2014), it is therefore expected that large areas of currently suitable

311 habitat will become progressively unsuitable, therefore falling outside the climatic niche of our model

312 species.

313 Our results, indeed, point to a likely dramatic decline in habitat suitability (Fig. 5) for most of the species

314 included in the analysis—*T. bornensis, T. iulianae, T. konradi, T. lanai, T. nigraerosae, T. pedemontanus*,

315 and *T. pluto*. On the other hand, a higher chance of survival is expected in *T. vignai* because it shows a

316 wider distribution and is also recorded at higher altitudes (above 2,000 m; Isaia et al. 2011, Mammola et al.

317 2015a). In addition to troglobiomorphic species being restricted to areas of temperature stability, caves are

318 often isolated from each other and species within them, including spiders, often exhibit reduced vagility (e.g.,

319 Hedin 1997, Snowman et al. 2010, Yao et al. 2016). In turn, this latter factor significantly increases the threat

320 of global extinction because the rate of recolonisation is low (Cardoso et al. 2011a, b). This is particularly

321 evident for *Troglohyphantes* spiders, for which we previously demonstrated a very low dispersal ability within

322 caves (Mammola and Isaia 2016) and extreme levels of population differentiation (Mammola et al. 2015a).

323 It is interesting to note that in the case of other troglobiomorphic species, the assessment of global warming

324 impacts on subterranean species based exclusively on correlative niche models is suggested to be

325 unreliable if thermal tolerance is wider than that reflected by current distributions (Sánchez-Fernández et al.

326 2016). Physiological mechanisms that might account for such an observation include the ability to synthesize

327 stress proteins (e.g. heat shock proteins; Bernabò et al. 2011). Whilst the data for these particular species

328 (Leiodidae beetles) are compelling, the finding seems unlikely to apply more generally to the glacial relict,

329 cold-adapted spiders in our study. These have a rather different evolutionary history to that of the beetles

330 and are not shown to be able to over-express similar, protective proteins. It is clear, however, that an intrinsic

degree of uncertainty remains associated with interpreting the results of any statistical model of this kind. Accordingly, as a follow up of this study, we aim to combine multiple lines of evidence (see Peterson 2009)—such as thermal tolerance experiments and population genetic studies—in order to explore our predicted patterns further (see Krehenwinkel et al. 2015 for an example on spiders). Testing the physiological response of subterranean model species to altered climatic condition could help to identify their specific upper thermal limits, and thus provide experimental evidence for our findings. In parallel, molecular analysis could establish contemporary population structure and dispersal modes, thereby increasing our understanding of the ability of subterranean species to escape and survive climate changes.

**Conclusions**

Together with other species living in confined habitats such as islands (e.g., Bellard et al. 2014, Yalor and Kumar 2016) and mountain summits (e.g., Beniston 2003, Xu et al. 2009, Yoccoz et al. 2010), we expect cave adapted species to be as vulnerable, if not more so, to climate change. However, the principal mechanisms underlying the response of cave-dwelling species to global climate change are as yet poorly described. Caves are excellent natural laboratories for the study of global changes in environment because of their remarkable ecological and thermal stability. Our findings emphasise the importance of considering subterranean organisms as model species for ecological studies dealing with climatic changes, and to extend such investigations to other subterranean systems worldwide.

**Supplementary materials**

362    **Appendix 1 -** Raw data of the fine-scale dataset, with details about sampling sites.

363    **Appendix 2 -** Details on modeling procedure. Additional figures and tables.

364

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**Tables and Figures**

**Table 1**. List of the variables selected to construct the regional scale model. The uncorrelated variables (Pearson *r* <±0.7) used in the model are highlighted. Percent contribution and permutation importance (*sensu* Phillips 2011) of each variable for building the final MaxEnt model is reported.

| Variable | Description | Unit | Percent contribution | Permutation importance |
|---|---|---|---|---|
| **Bio01** | **Annual Mean Temperature** | **°C** | **29.6 %** | **27.4** |
| **Bio02** | **Mean Diurnal Range** | **°C** | **32.7 %** | **32.5** |
| Bio03 | Isothermality (Bio02/Bio07) (*100) | °C | - | |
| Bio04 | Temperature Seasonality (standard deviation*100) | °C | - | |
| Bio05 | Max Temperature of Warmest Month | °C | - | |
| Bio06 | Min Temperature of Coldest Month | °C | - | |
| Bio07 | Temperature Annual Range (Bio05–Bio06) | °C | | |
| Bio08 | Mean Temperature of Wettest Quarter | °C | - | |
| **Bio09** | **Mean Temperature of Driest Quarter** | **°C** | **Discarded (<5%)** | **Discarded** |
| Bio10 | Mean Temperature of Warmest | °C | - | |

| Variable | Description | Unit | Percent contribution | Permutation importance |
|---|---|---|---|---|
| | Quarter | | | |
| Bio11 | Mean Temperature of Coldest Quarter | °C | - | |
| Bio12 | Annual Precipitation | mm | - | |
| Bio13 | Precipitation of Wettest Month | mm | - | |
| Bio14 | Precipitation of Driest Month | mm | - | |
| Bio15 | Precipitation Seasonality (Coefficient of Variation) | mm | - | |
| Bio16 | Precipitation of Wettest Quarter | mm | - | |
| Bio17 | Precipitation of Driest Quarter | mm | - | |
| **Bio18** | **Precipitation of Warmest Quarter** | **mm** | **Discarded (<5%)** | **Discarded** |
| Bio19 | Precipitation of Coldest Quarter | mm | - | |
| Alt | Altitude a.s.l | m | - | |
| **Ice** | **Ice cover during Last Glacial Maximum** | **Categorical** | **37.7 %** | **40.1** |

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**Figure legends**

**Figure 1**. Hypogean localities selected in the present work. Ice cover during Last Glacial Maximum is reported in the top-right inset map. A code in brackets referring to the Italian speleological cadaster is provided for each locality—more details in Supplementary Material Appendix 1.

673

**Figure 2.** Predicted values (filled lines) and 95% confidence intervals (dashed lines) of the effect of mean annual cave temperature ($T_{int}$) on the probability of presence of *Troglohyphantes* spiders according to glacial history, derived from GLM analysis.

677

678 **Figure 3**. Projected environmental suitability of Western Alps for troglobiomorphic species of

679 *Troglohyphantes* spiders according to current and Last Glacial Maximum (LGM) climatic conditions. LGM

680 projection represents the average of three General Circulation Models (GCMs). Only suitable areas over the

681 threshold of 0.4 are shown.

682

683 **Figure 4**. Projected future environmental suitability of the Western Alps for troglobiomorphic species of

684 *Troglohyphantes* spiders in 2050 and 2070 according to the two Representative Concentration Pathways

685 (rcp) considered in the work. Each map represents the average of the projection into three General

686 Circulation Models (GCMs). Only suitable areas over the threshold of 0.4 are shown.

687

688 **Figure 5**. Current and projected future environmental suitability (high emissions scenario for 2070) of the

689 Western Alps for *Troglohyphantes* spiders. Orange dots represent current localities of *Troglohyphantes.*

690 Localities of *T. lucifuga* and *T. lucifer* are not shown in the map. Only suitable areas over the threshold of 0.4

691 are shown.

692