






# Vulnerability to climate change for two endemic high-elevation, low-dispersive *Annitella* species (Trichoptera) in Sierra Nevada, the southernmost high mountain in Europe

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**Abstract.** 1. Climate change is predicted to progressively shift habitat characteristics that will alter the distribution and eco-physiological responses of organisms. High-elevation stream insects without extensive dispersal are expected to be highly vulnerable if they are unable to track predicted shifts. Understanding dispersal is therefore crucial to implement practical solutions in conservation.

2. The evolutionary history of the Palaearctic genus *Annitella* (Trichoptera), the population dynamics and habitat distribution (present and future) of two endemic species (*A. esparraguera* and *A. iglesiasi*) confined to Baetic Mountains (SE-Iberian Peninsula) were assessed to gain fundamental insights into species responses to climate warming and to identify populations at risk.

3. Diversification of *Annitella* was dated to the Pleistocene and was likely associated with southern and extra-Mediterranean refugia located across Europe. The two endemic species belong to distant lineages that preferred similar cold headwater pristine habitats. The range of *A. esparraguera* was larger than that of *A. iglesiasi*. Both species showed low genetic diversity in *cox1*, but only *A. esparraguera* exhibit locally unique haplotypes, indicating limited gene flow. For *A. esparraguera*, modelled future habitat suitability showed 88.4% range contraction by 2050 (RCP scenario 8.5) and a displacement of 41.5% of the current potential distribution to higher elevations.

4. Populations of *A. esparraguera* are predicted to be lost because of the reduction of optimal habitat and limited propensity for tracking future suitable conditions. Beyond the preservation of their current habitat, their conservation might require proactive measures (translocations). Similar predictions may apply to other Mediterranean endemic headwater specialist restricted to isolated high-elevation streams.

**Key words.** Caddisfly, global warming, Iberian Peninsula, intraspecific diversity, Limnephilidae, phylogeography, SDM, stream ecology.

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## Introduction

Geographical ranges of species and the composition of local species assemblages are determined by multiple ecological factors, including dispersal limitation, environmental filtering, and species interactions (Ricklefs, 2004). Given that patterns of diversity have arisen over extended time scales, present-day distributions preserve signatures of evolutionary history at inter- and intraspecific levels (Marske *et al.*, 2013; Múrria *et al.*, 2017). Therefore, understanding mechanisms of species origins and community assembly requires integration of the evolutionary processes operating at large temporal scales with ecological processes effective at short temporal scales (Múrria *et al.*, 2017). These mechanisms can also reveal the adaptability and migratory potential of each species that can be used to predict how biodiversity will respond to ongoing climate change and the factors that will impact the distribution of species and communities (Parmesan, 2006).

Global climate change will result in a general increase of temperature, but also in greater variability of precipitation patterns and drought frequencies locally (Milly *et al.*, 2005). Varying climate will cause progressive shifts in habitat characteristics and the severity of changes will be critical for the survival at all levels of diversity from individuals to communities. In stream ecosystems, and especially in Mediterranean regions, climate change and the expected increase in frequency and magnitude of extreme events such as floods, droughts and fires (Ulbrich *et al.*, 2006) will result in local population extinctions and regional deterioration of freshwater diversity, leading to changes in ecosystems functioning of rivers (Palmer *et al.*, 2009; Woodward *et al.*, 2010). The warming and precipitation rates will determine the degree of vulnerability, but the effect may vary among species depending on the magnitude of climatic variation throughout its range, species tolerance to climatic shifts, the propensity for migration to localities where environmental conditions fit the eco-physiological requirements of the species, and adaptive plasticity to adjust to those changes (Bellard *et al.*, 2012; Garcia-Raventós *et al.*, 2017).

Commonly used bioclimatic envelope models predict future species distributions under varying scenarios of habitat suitability based on current geographical ranges and corresponding environmental variables (Araújo & Peterson, 2012). However, these methods do not generally take into account biotic factors such as dispersal abilities, adaptive capacity and species interactions that determine the responses of individual species, which are presumably not random across taxa and regions (Dawson *et al.*, 2011). Instead, inferences about demographic and evolutionary histories, life-history traits and population genetics can add the required information to estimate species-specific responses based on their adaptability and migratory potential. However, these aspects have been poorly explored to determine species vulnerability and to identify priority conservation areas (Dawson *et al.*, 2011; Bellard *et al.*, 2012; Paz-Vinas *et al.*, 2018; Ruegg *et al.*, 2018). At evolutionary scales, dispersal abilities and connectivity determine the intraspecific genetic structure (Papadopoulou *et al.*, 2009) and the rate of cladogenesis (Barraclough *et al.*, 1999; Múrria *et al.*, 2013). For instance, the persistence and isolation of resident populations due to low

dispersion may correlate with geographically highly diversified genealogies, which can be evident both at the population and deeper phylogenetic levels, as seen in high elevation tropical streams (Múrria *et al.*, 2015). Hence, intraspecific genetic structure and the rate of cladogenesis may be useful as an indicator of vulnerability, and, for instance, species that evolved in high elevation isolated habitats are expected to be vulnerable to extinction if climate change shifts habitat conditions because traits related to dispersion have not been favoured.

Modelled distribution of high-elevation aquatic insect species in Europe revealed how future range contractions under climate warming will imply losses of genetic diversity (Bálint *et al.*, 2011). However, not all species and European ecoregions are equally vulnerable to climate change (Hering *et al.*, 2009). Endemism, feeding type specialisation, short emergence period, and preference for cold water temperature, springs and streams at high altitudes explain the species' sensitivity to climate change impacts (Hering *et al.*, 2009; Tierno de Figueroa *et al.*, 2010). With respect to caddisflies (Trichoptera), which are the focus of the current study, according to such parameters, the highest fraction of potentially endangered species is found in the Iberic-Macaronesian region, mainly in the Iberian Peninsula (Hering *et al.*, 2009). A caddisfly lineage that may be negatively affected by climate change is the Palaearctic genus *Annitella* Klapálek, 1907 (Trichoptera: Limnephilidae: Chaetopterygini) because *Annitella* species (i) are apparently restricted to cold headwater streams, (ii) have a short emergence period (Graf *et al.*, 2008), and (iii) most of the described 17 morpho-species are endemic to single high-elevation European mountain ranges, being considered micro-endemics (Schmid, 1952; Oláh & Kovács, 2012; 2014; Malicky, 2013; Neu *et al.*, 2018) (Table 1, Fig. 1a). The only exception is *A. obscurata* (McLachlan, 1876), which is widely distributed in headwater streams from Scandinavia to the Pyrenees (González & Martínez-Menéndez, 2011; Malicky, 2013; Neu *et al.*, 2018). However, due to their low abundance and narrow distribution, the knowledge of the ecology and evolution of *Annitella* species is sparse and mainly based on a small number of collection specimens (Sáinz-Bariáin *et al.*, 2013). In the Iberian Peninsula, six endemic *Annitella* species are distributed in mountains of the Baetic System, which includes the Sierra Nevada, Ancares, Picos de Europa and Pyrenees (1–3 in Fig. 1a). Two out of these six endemic species, *A. iglesiasi* González & Malicky, 1988 and *A. esparraguera* Schmid, 1952, are found only in mountain ranges of the Southeast (Sipahiler, 1998; Sáinz-Bariáin *et al.*, 2013; Martín *et al.*, 2015), and historical records indicate that both species coexist in the Sierra Nevada (González & Malicky, 1988; Sáinz-Bariáin *et al.*, 2013).

There is currently a major gap between predictions of species vulnerability and management strategies, despite the fact that conservationists need to identify those species that are likely to be the most vulnerable to the impacts of climate change (Arribas *et al.*, 2012; Pacifici *et al.*, 2015). The two southernmost *Annitella* species are good model organisms to predict effects of climate change on Mediterranean cold stenotherm species that inhabit springs and high-altitude streams due to their narrow potential distribution and the predicted habitat shifts for these freshwater ecosystems (Hering *et al.*, 2009; Tierno de Figueroa *et al.*, 2010; Conti *et al.*, 2014). Otherwise, male genitalia of *A. iglesiasi* are morphologically similar to the widely

**Table 1.** Site location and *cox1* haplotype composition and diversity of the sampled populations for *Annitella esparaguera* and *A. iglesiasi*.

Species	Sites	Catchment	Code	X_UTM	Y_UTM	Alt.	Mountain Range	N	h (Id)	$\pi$	Snn (P-value)
<i>A. iglesiasi</i>	Río Alhama	Guadiana Menor	AL	477554	4112204	2150	Sierra Nevada	4	1 (1),2 (1),6 (1),8 (1)	0.00555	
	Barranco de los Pasillos	Guadiana Menor	PA	493948	4107674	1646	Sierra Nevada	2	6 (1),7 (1)	0.00185	
	Río Laroles	Adra	LA	495909	4102162	1753	Sierra Nevada	6	5 (1), 6 (1), 7 (2), 10 (2)	0.0037	
	Barranco de Manuel Casas	Genil	MA	460987	4105369	1704	Sierra Nevada	19	2 (4), 3 (6), 4 (4), 6 (4), 9 (1)	0.00481	
	Barranco de las moscas	Andarax	TE	504076	4107093	1500	Sierra Nevada	1	7 (1)	-	
Total								32	10	0.00444	0.13 (0.775)
<i>A. esparaguera</i>	Río Alhama	Guadiana Menor	AL	477554	4112204	2150	Sierra Nevada	2	5 (1),11 (1)	0.0037	
	Borreguiles del Monachil	Genil	MO	465816	4103202	2679	Sierra Nevada	14	2 (1), 3 (7), 4 (6)	0.00246	
	Borreguiles del Dífar	Genil	DI	466148	4104564	2855	Sierra Nevada	2	7 (1),8 (1)	0.00185	
	Río Gor	Guadalquivir	GO	513147	4133118	1773	Sierra de Baza	16	1 (1),6 (4),10 (9),12 (1),13 (1)	0.0037	
	Río Guadalquivir	Guadalquivir	GA	505475	4194974	1378	Sierra de Cazorla	8	9 (1),10 (7)	0.00185	
Total								42	13	0.00481	0.38 (0.036*)

N, number of individuals sequenced, h (Id), haplotype composition, nucleotide diversity ( $\pi$ ) and Snn values.

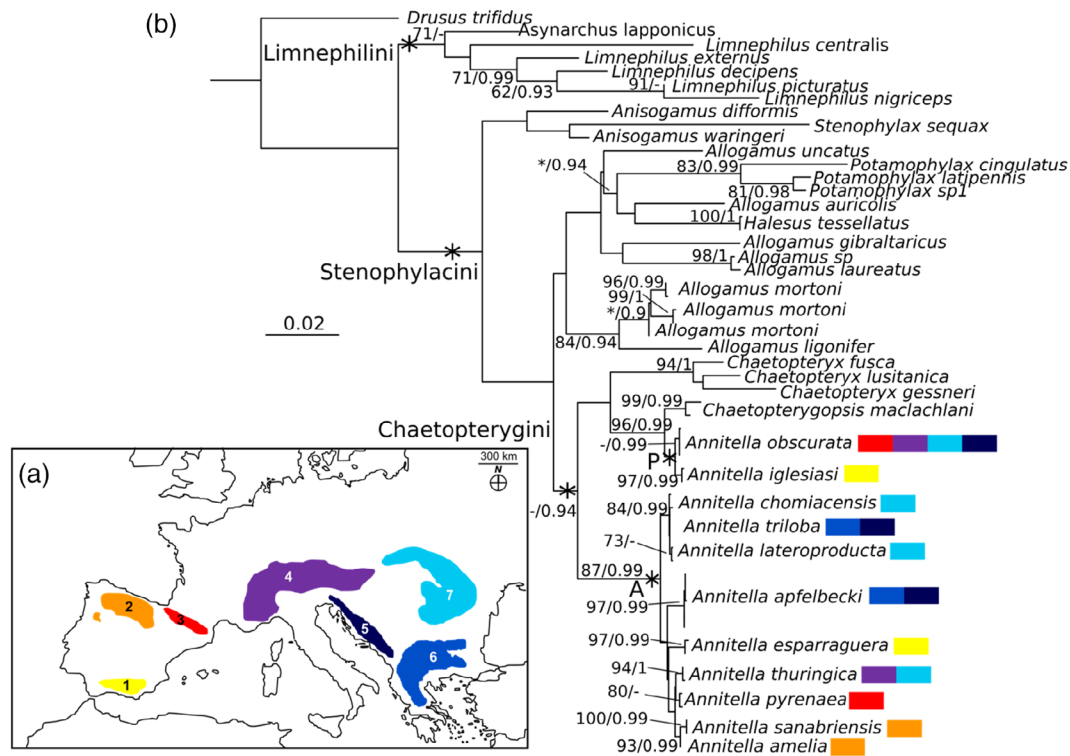
distributed species *A. obscurata*, and these two species have been included in the subgenus *Praeannitella* Schmid, 1952. On the other hand, the male genitalia of *A. esparaguera* are morphologically similar to the other European species included traditionally in the subgenus *Annitella* Klapálek, 1907 (Schmid, 1952; González & Malicky, 1988; Sipahiler, 1998). Because of their fairly distant relationships, these two species may differ in functional traits such as dispersal abilities that could result in different habitat preferences and/or vulnerability. Both species may be indicative of the effects of climate change on the biota of the Sierra Nevada, which is the second highest mountain range in Europe and has been selected in the Global Change in Mountain Regions initiative (GLOCHAMORE) by the UNESCO as a model system because of its unique climate conditions and high endemism (Sáinz-Bariáin *et al.*, 2013; Zamora *et al.*, 2016).

Here, an integrative approach for assessing the effects of climatic change on the extinction risk of *A. iglesiasi* and *A. esparaguera* confined to the Sierra Nevada and nearby Baetic System mountains is used. First, a phylogenetic tree of the genus *Annitella* was built to elucidate its evolutionary history. Geographical distributions and current habitat preferences were assessed for modelling future habitat availability, and intraspecific genetic diversity and structure was estimated as a measure of potential dispersal. Given high and steeply sloped topographic barriers among catchments and internal physical habitat heterogeneity across headwater river sections (Clarke *et al.*, 2008), the reduced connectivity among rivers is predicted to result in a structured distribution at species (high endemism) and populations (intraspecific genetic structure) levels (Finn *et al.*, 2011; Múrria *et al.*, 2013). Moreover, both *Annitella* species, as short-winged autumn-emerging caddisfly species (Schmid, 1952), are expected to have narrow geographical ranges and isolated populations, but it is unclear to what degree these ranges are determined by high habitat specificity or limited gene flow due to their low dispersal propensity. Finally, a proposal for conservation management of these two species is provided.

## Materials and methods

### DNA sequencing and DNA sequences compilation

To elucidate the evolutionary history of *Annitella*, 40 individuals belonging to the tribes Stenophylacini and Chaetopterygini within the subfamily Limnephilinae Kolenati, 1848 were sequenced (GenBank accession numbers MN606328-MN606406 and MN606430-MN606453; Supporting Information Appendix S1). Within the tribe Chaetopterygini, two out of five European genera were collected or provided to authors by colleagues, including 11 species of the subgenera *Annitella* and *Praeannitella* from several mountain ranges across Europe along different sampling campaigns in 2008, 2009 and 2010 (Fig. 1a), in addition to *Chaetopteryx lusitanica* Malicky, 1974. The rare Iberian species *Annitella cabeza* Sipahiler, 1998 and *Annitella lalomba* Sipahiler, 1998 were not available for DNA analysis, and the new species *Annitella ostrovicensis* Oláh & Kovács, 2012 and *Annitella jablaniensis* Oláh, 2014 from the Balkans were described after these analyses were performed (Oláh & Kovács, 2012, 2014). Within the tribe Stenophylacini, 4 out of 16 European genera were sequenced:



**Fig. 1.** (a) Locations of *Annitella* species across central and southern Europe: 1. Sierra Nevada; 2. Ancares and Picos de Europa; 3. Pyrenees; 4. Alps; 5. Dinarides; 6. Balkans; 7. Carpathians. (b) Phylogenetic relationship among species of tribes Limnephilini, Stenophylacini and Chaetopterygini included in this study and the outgroup *Drusus trifidus* based on combined Maximum Likelihood analysis of three genes *cox1*, *ef-1 $\alpha$*  and 28S. Branch support indicates Maximum Likelihood bootstrap (>60) and Bayesian inference posterior probability (>0.95). Distribution range of each species is indicated using the colours in (a). “P” indicates the clade that comprises the *Annitella* subgenus *Praeannitella*, whereas “A” indicated the subgenus *Annitella*. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

eight species of the genus *Allogamus* Schmid, 1955 together with *Halesus tessellatus* Rambur, 1842, two species of the genus *Potamophylax* Wallengren, 1891, and *Stenophylax sequax* (McLachlan, 1875). However, diagnoses of genera *Halesus*, *Allogamus* and *Stenophylax* within Stenophylacini are conflicting because genitalia are often highly variable and also phylogenetic analyses revealed a paraphyletic grade of this tribe with respect to Limnephilini (Vshivkova *et al.*, 2006). To increase the robustness and accuracy of the phylogenetic analyses, all relevant sequences of European species of the subfamily Limnephilinae (Malicky, 2013; Morse, 2017) were compiled from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>, accessed on October 2017). Finally, the phylogenetic analysis of Limnephilinae included 56 specimens belonging to 36 morphological species of tribes Limnephilini, Stenophylacini and Chaetopterygini and the out-group *Drusus trifidus* McLachlan, 1868 (Limnephilidae: Drusinae) (Supporting Information Appendix S1). For the population genetic analyses, all individual larvae captured during the intensive sampling (74 specimens) of *A. iglesiasi* and *A. esparaguera* from five populations of each species were analysed (Table 1) (GenBank accession numbers MN606407–MN606429).

DNA was extracted from the abdominal segments I–V using WizardSV 96 extraction plates (Promega, Southampton, UK). A total of 614 bp of the mitochondrial Cytochrome Oxidase I (*cox1*) gene was amplified using primer sets C1–J–2183 (Simon

*et al.*, 1994) and S20 (Pauls *et al.*, 2003) and PCR thermocycle programs described in the study by Pauls *et al.* (2006). The nuclear elongation factor 1- $\alpha$  (*ef1 $\alpha$* ; 484 bp) and 28S ribosomal RNA subunit domain D3 (28S; 703 bp) partial gene sequences were amplified using the primer sets described in the study by Múrria *et al.* (2012). Amplification products were purified using Millipore Multiscreen 96-well plates (Millipore, Billerica, MA) and sequenced in both directions using BigDye 3.1 and an ABI PRISM3700 DNA Analyser (Applied Biosystems). Sequence chromatograms were assembled and edited using the Sequencher 4.6 software (Gene Codes Corp, Ann Arbor, MI) and aligned using the online version of MAFFT 5.8 (Katoh *et al.*, 2005).

#### Phylogenetic and diverged time analysis

The best partition scheme and models for each of the two partial *cox1* and *ef1 $\alpha$*  genes were selected using Partition Finder (Lanfear *et al.*, 2012) based on the Akaike information criterion (AIC), which for each gene produced two partitions composed of the first plus second and the third codon positions. Maximum likelihood searches were conducted with RAxML 7.0.4 (Stamatakis, 2006) under the GTR +  $\Gamma$  model with default number of  $\Gamma$ -categories implemented independently for each partition. Clade support for

the best tree was assessed by means of 1000 nonparametric bootstrap resampling replicates of the original matrix. Bayesian analysis was conducted using MrBayes 3.2.5 (Ronquist & Huelsenbeck, 2003). Two independent runs with four simultaneous Markov chain Monte Carlo (MCMC) chains (one cold and three heated), each with random starting trees, were accomplished simultaneously, sampling 1000 generations until the standard deviation of the split frequencies of these two runs dropped below 0.01 (10 million generations). Tracer 1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>) was used to ensure that the MCMC chains had reached stationarity by examining the effective sample size (ESS) values and to determine the correct number of generations to discard as burn-in. The two phylogenetic analyses were run remotely at the CIPRES Science Gateway (Miller *et al.*, 2010).

To estimate divergence dates among species, Bayesian methods implemented in Beast 1.8 (Drummond *et al.*, 2012) were used. Based on the results of the phylogenetic tree, the dated *cox1* gene tree was rooted with *S. sequax*, the presumed sister group to Chaetopterygini. For this analysis, only one *cox1* sequence for each differentiated molecular species within the tribe Chaetopterygini was used, which removed *A. triloba* Marinkovic-Gospodnetic, 1957 and *A. lateroproducta* (Botosaneanu, 1952) because of the high similarity with *A. chomiensis* (Dziedzielewicz, 1908). In addition, the two *cox1* sequences of *Chaetopteryx moretti* Lodovici, & Valle, 2007 and *C. villosa* Fabricius, 1798, were downloaded from GenBank. Due to the absence of dated fossils or an unambiguous biogeographic event that could be used for calibration, a high (0.0168 substitutions/site/MY; Papadopoulou *et al.*, 2010) and low (0.0115 substitutions/site/MY; Brower, 1994) published estimate of substitution rates for *cox1* in insects was applied. Uniform evolutionary models were applied on the Yule speciation model with a lognormal relaxed molecular clock. Two independent analyses with the same settings were conducted running 20 million generations (saving trees every 1000) or until analyses converged and the number of trees was sufficient based on ESS values, as measured with Tracer 1.6. The majority rule consensus tree of the two runs was combined using the Beast 1.8 accompanying programs Logcombiner and TreeAnnotator (Drummond *et al.*, 2012), following burn-in to estimate the consensus chronogram. For selection of the best molecular clock, the modified Akaike information criterion (AICM) with the moments estimator (Baele *et al.*, 2012), as implemented in Tracer 1.6, with 1000 bootstrap replicates was used.

#### *Distribution of Annitella species in the Baetic system*

Across the Sierra Nevada, 47 pristine first- and second-order stream reaches were sampled in 2008 and 2009, for assessing the geographical range of *A. esparaguera* and *A. iglesiasi*. In addition, to cover the entire range of species (Bonada *et al.*, 2008; Ruiz-García *et al.*, 2016; Sipahiler, 1998; Sáinz-Bariáin & Zamora-Muñoz, 2012; Sáinz-Bariáin *et al.*, 2013; 2015; Martín *et al.*, 2015) sites at the adjacent Sierra de Baza and Sierra de Cazorla were sampled in 2009 and 2010. At all sites, larvae were captured once each season (spring, summer, and autumn) to assess voltinism by kick sampling using a 30 cm diameter hand net (mesh size = 500 µm) near banks at a water depth less than 50 cm of slow running creeks on different types of substrate:

muddy, moss, macrophytes or gravel. Adults were captured by sweeping the riparian vegetation with an entomological net and light-traps at night. All individuals were preserved in absolute ethanol for molecular analysis.

In order to determine the environmental conditions of each sampled stream reach, the water temperature and pH (Waterproof pHTestr 10, 20 and 30, Eutech Instruments, Oakton), and conductivity (Eutech Conductivimeter Eco-Scan Con6 Agua) were measured, and water samples were taken. In the laboratory, nutrients and cations were determined according to the methodology described in the study by Rodier (1998): ammonium, phosphate, nitrites, nitrates, sulphates, calcium, magnesium, and chloride (see details for Methods in the study by Sáinz-Bariáin *et al.*, 2016). To eliminate the redundancy and collinearity among variables, only one of the highly correlated variables (Pearson >0.9) was retained yielding a total of 10 variables. In order to distinguish whether isolated populations were determined by habitat specificity or limited gene flow due to low dispersal propensity, environmental differences between all sampled stream reaches, independently for each *Annitella* species were recorded and assessed by principal components analysis (PCA) using *ade4* (Dray & Dufour, 2007) of the R software (R Development Core Team). The ecological space generated on the PCA primarily indicated the ecological factors that delimit the distribution of the larvae.

#### *Intraspecific genetic structure*

Intraspecific genetic diversity of *A. esparaguera* and *A. iglesiasi* was measured by counting polymorphic sites and nucleotide diversity  $\pi$  (i.e. the average number of nucleotide differences per site between two sequences; Nei, 1987). The level of gene flow was inferred by estimating the genetic structure among populations using the Snn statistics (Hudson, 2000) in DnaSP (Librado & Rozas, 2009) considering the populations with at least two individuals sequenced. To visualise relationships among haplotypes, a statistical parsimony haplotype network was constructed with a 95% connection limit using TCS 1.21 (Clement *et al.*, 2000).

#### *Species distribution models*

To assess how the potential distribution area would change under ongoing climate change, species distribution models (SDM) were run separately for the occurrences of the two *Annitella* species at current and future (in 2050 and 2080) climatic conditions. Because of the very low occurrence of *A. iglesiasi* and the associated low statistical power, the SDMs were only significant for *A. esparaguera*, which had been historically recorded in 10 localities (Sipahiler, 1998; Sáinz-Bariáin *et al.*, 2016). For the 47 sites in Sierra Nevada sampled at multiple time points, the failure to detect a species was considered as an empirical absence in the models. SDMs were generated on the current occurrence data; 19 bioclimatic variables, which were based on temperature and rainfall values (available at Worldclim.org in ASCII format and 30-s resolution), and elevation (available at

centrodedescargas.cnig.es in ASCII format and 200 m grid spacing resolution). Bioclimatic and elevation variables are commonly used in SDM because they cover a large spatial scale and are of biological importance to distribution ranges (O'Donnel & Ignizio, 2012).

The final number of bioclimatic and elevation variables to be used in SDMs was reduced to eliminate the redundancy and collinearity among them but capturing the climatic variation without overfitting the SDMs (Feld *et al.*, 2016). First, to detect pairs of highly correlated variables and collinearity between predictor variables (threshold = 0.40), the Spearman correlation and VIF collinearity analyses were performed using the *usdm* package (Naimi, 2015) of the R software. Second, a random forest method was implemented using *randomForestSRC* (Ishwaran *et al.*, 2014) to identify complex interactions between variables and to arrange them by their relative contribution to the model ( $40 < \text{AIC} < 50$ ). Finally, only 3 out of 20 variables were uncorrelated and did not suffer collinearity problems: isothermality (the variance of day-to-night temperatures oscillation relative to the summer-to-winter oscillations, Bio3), Mean Temperature of Wettest Quarter (mean of the temperatures that prevail during the wettest season, Bio8) and Precipitation seasonality (coefficient of variation in monthly precipitation totals over the course of the year, Bio15). To account for uncertainties, different modelling techniques (GLM, GAM, and BRT) with the four selected variables were tested and an automatised stepwise model selection by AIC was used to classify the best modelling technique and variables. Given the low occurrence of *A. esparaguera* (10 sites), the occurrence data set was not split in two sets (training and test data sets), and the LOO-method (Leave-One-Out, a cross validation method) was used instead to validate the model. Finally, a generalised linear model (GLM) using the Precipitation Seasonality (Bio15) was statistically chosen as the best predictor to SDM. The classification threshold was obtained using the *SDMTools* package (VanDerWal *et al.*, 2014) of the R software, which also provided the confusion matrix and the sensitivity and specificity values. All distribution models were performed using the *stats* and *gbm* (Ridgeway, 2013) packages of the R software.

For future prediction for 2050 and 2080, four climate models with high resolution were selected to account for different sources of uncertainty (Thuiller, 2004): EC-Earth (European Centre of Medium Range Weather Forecast, ECMWF), HadGEM2 ES (Met Office-Hadley Centre, MOHC, and Instituto Nacional de Pesquisas Espaciais), MRI-CGCM3 (Meteorological Research Institute CGCM version 3, Japan) and CESM1(BGC) (National Science Foundation, Department of Energy, National Centre for Atmospheric Research). For HadGEM2 ES and MRI-CGCM3, climatic scenarios selected were RCP 2.6, 4.5 and 8.5 (from the lowest to highest emissions); for CESM1(BGC), only RCP 4.5 and 8.5 were available, while for EC-Earth, only RCP 8.5 was available. All future climatic conditions of bioclimatic variables were downloaded from ccafs-climate.org in the same format as current conditions.

To compare current and future predictions, a threshold classification ( $=0.5$ ) was applied to convert predictions into a binary response. This threshold was obtained as the result of the mean prediction for the occurrences (presences) that were tested using a confusion matrix (Nenzén & Araújo, 2011). This step was conducted using the *SDMTools* R Package (VanDerWal *et al.*, 2014). Then, the difference in pixels covered between current

and future predictions indicated the loss or gain of future total species range, whereas the overlap between future prediction and current distribution indicated the current areas that will be available in the future.

## Results

### *Evolutionary history of Annitella*

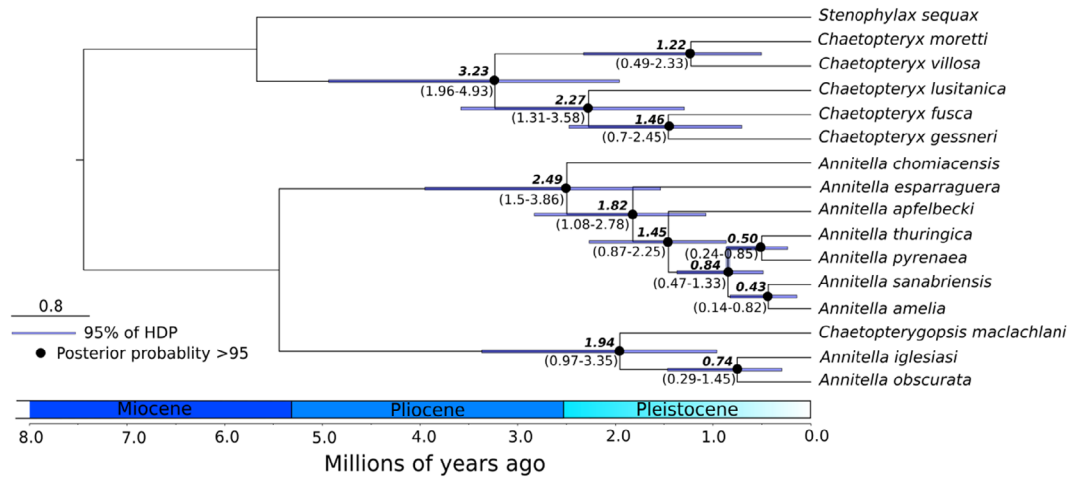
The maximum likelihood searches recovered the representatives of the tribe Limnephilini as sister to all other species in the tribes Chaetopterygini and Stenophylacini, whereby the latter was a paraphyletic grade with respect to Chaetopterygini (Fig. 1b). In the tribe Stenophylacini, the genera *Anisogamus* and *Stenophylax* were clustered together, whereas the genera *Potamophylax* and *Halesus* were placed within the paraphyletic genus *Allogamus*, although with weak support. Within the tribe Chaetopterygini, the genus *Annitella* was found in the two main clades, one formed exclusively of the subgenus *Annitella*, including nine endemic species from high mountain ranges across Europe, whereas the remaining two species of *Annitella* (the member of the subgenus *Praeannitella*) were sister to the two supported genera *Chaetopteryx* and *Chaetopterygopsis*. In the subgenus *Praeannitella*, the widespread *A. obscurata* and *A. iglesiasi* were clearly differentiated sister taxa. In contrast, in subgenus *Annitella*, the members of three eastern species, *A. chomiensis*, *A. triloba* and *A. lateroproducta* distributed at the Dinarides, Balkans and Carpathians, formed a cluster of closely similar haplotypes. The other six species belonging to the subgenus *Annitella* were phylogenetically differentiated. The Iberian *A. esparaguera* was related to *A. apfelbecki* (Klapálek, 1899) located in the Balkans and Dinarides, and *A. thuringinca* (Ulmer, 1909) from Alps and Carpathians. The other three Iberian species *A. amelia* Sipahiler, 1998, *A. sanabriensis* (González & Otero, 1985) and *A. pyrenaea* (Navás, 1930) were phylogenetically closely related. The two species present in the Sierra Nevada therefore are members of two different lineages.

The dated *cox1* gene tree topology was divided in two clades (Fig. 2). In one clade, the time span to the most recent common ancestor of the genus *Chaetopteryx* was 3.23 Mya (95% highest probability densities, 1.96–4.93 Mya), and the five included species diverged between 2.27 Mya (95% HPD 1.35–3.58) and 1.22 Mya (95% HPD 0.49–2.33). Within the subgenus *Annitella*, the species diverged between 2.49 Mya (95% HPD 1.5–3.86) and 0.43 Mya (95% HPD 0.14–0.82) when the two Iberian species, *A. sanabriensis* and *A. amelia*, diverged. The split between the genus *Chaetopterygopsis* and subgenus *Praeannitella* was 1.94 Mya (95% HPD 0.97–3.35), and *A. obscurata* and *A. iglesiasi* split 0.74 Mya (95% HPD 0.29–1.45). The temporal origin of *A. esparaguera* (1.82 Mya, 95% HPD 1.08–2.78) and *A. iglesiasi* (0.75 Mya, 95% HPD 0.29–1.45) was relatively recent in the Pleistocene.

### *Distribution of Annitella species in the Baetic system mountains*

Geographical ranges differed between the two species; *A. iglesiasi* had a narrower geographical distribution than *A. esparaguera*, which was located in the Sierra Nevada but also in the nearby

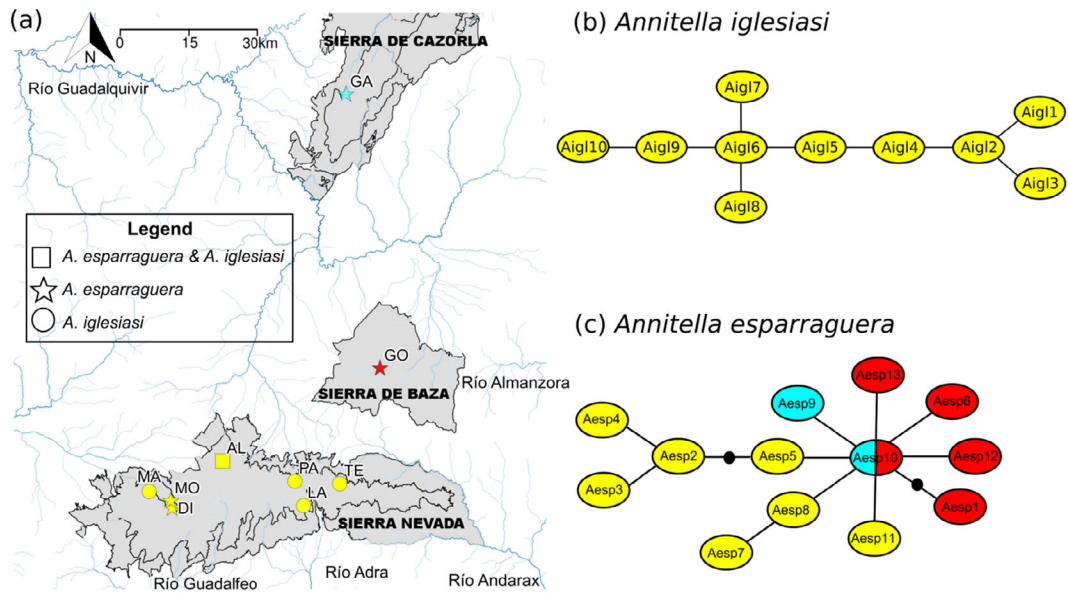




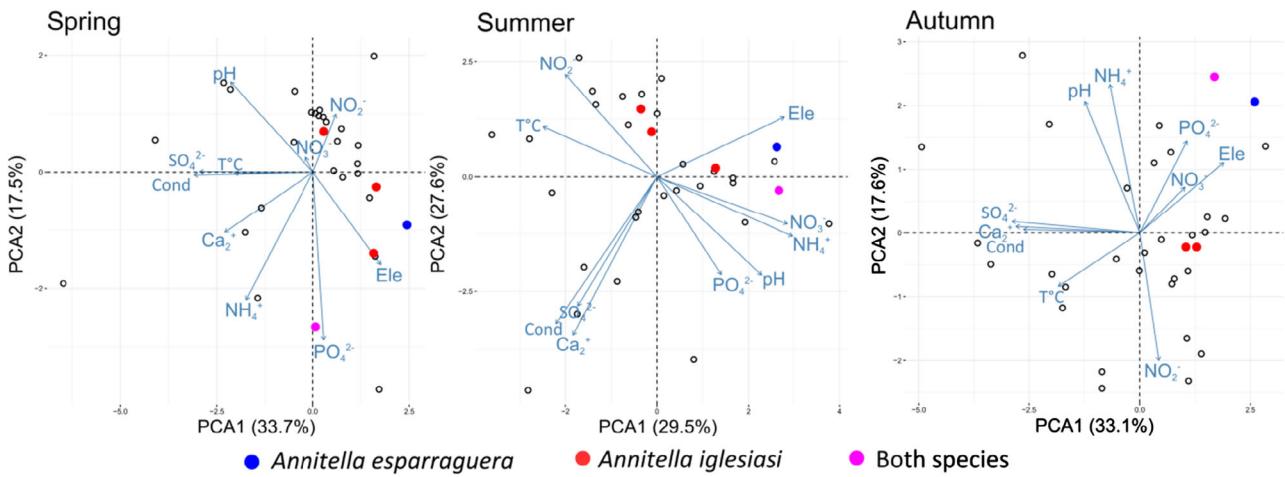
**Fig. 2.** Time-calibrated coalescent-based species tree of genus *Annitella* inferred in Beast of the mtDNA *cox1* gene. Numbers at nodes indicate the mean divergence time and the 95% high posterior density (HDP) interval of the time estimates, which are also represented in bars. Filled circles at nodes indicate posterior probability support values >0.95. [Color figure can be viewed at wileyonlinelibrary.com]

mountain ranges of Sierra de Baza and Sierra de Cazorla (Fig. 3a; Table 1). In the Sierra Nevada, these species were located only at 7 out of the 47 sampled sites, which indicated that *Annitella* are rare species and have a restricted range. The two species co-occurred at Río Alhama (AL); moreover, *A. iglesiasi* was captured in another four sites (MA, PA, LA and TE), whereas *A. esparraguera* was found in two other sites (MO, DI). The species were univoltine with autumnal flight period of adults. The first two axes of the PCA on the ecological local conditions explained a similar amount of variation in spring and autumn (33.7% and 17.5% of the total variation, respectively), whereas in summer, the first axes were equally informative

(29.5% and 27.6%, respectively) (Fig. 4). In the three seasons, the PCA1 axis mainly described changes in elevation but also water mineralisation and temperature, separating sites located in high elevations from low elevation sections characterised by higher conductivity and temperature. The second axis was mainly correlated with nutrient concentration and pH, but the contribution of ecological factors on this axis showed high variation across seasons. In general, the two species inhabited high-elevation stream reaches and were never captured at pristine sites located at lower elevations characterised by higher conductivity, temperature and concentration of sulphates, calcium, magnesium and chloride. In the ecological space determined



**Fig. 3.** (a) Maps showing localities and geographical distribution of studied specimens in the South of the Iberian Peninsula, and *cox1* haplotype networks with a 95% connection limit for (b) *Annitella iglesiasi* and (c) *A. esparraguera*. In the maps, colours indicate populations in the Sierra Nevada (yellow); Sierra de Baza (red); and Sierra de Cazorla (blue). Site codes as in Table 1. [Color figure can be viewed at wileyonlinelibrary.com]



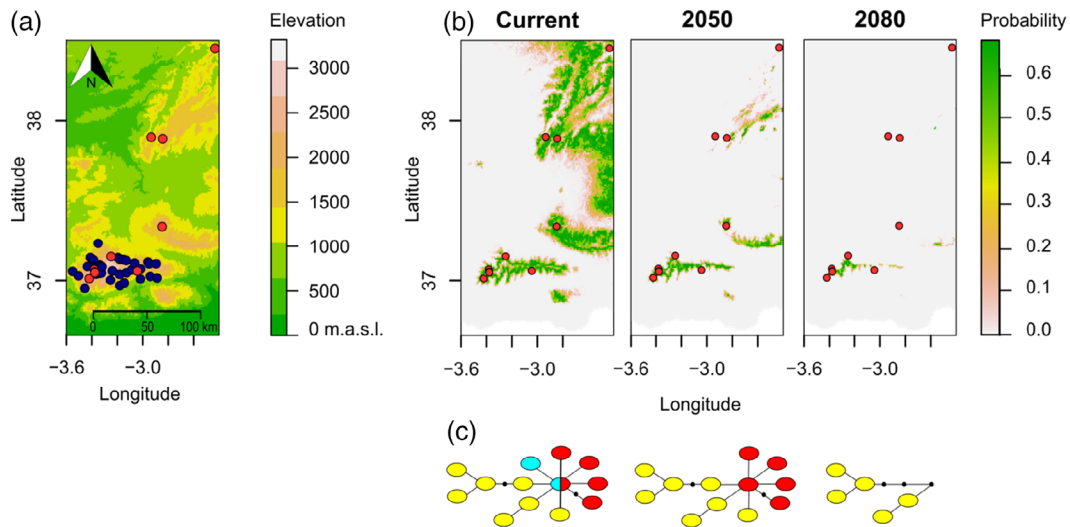
**Fig. 4.** Results of the PCA on environmental variables measured at the sites sampled in spring, summer and autumn in the Sierra Nevada. Open black circles indicate the sites where no *Annitella* species were found. Acronyms of environmental factors as follows: Ele, elevation; Cond, conductivity. [Color figure can be viewed at wileyonlinelibrary.com]

by PCA axes, several sites where *Annitella* was absent (open black circles, Fig. 4) were in close proximity to sites where the species were captured (blue, red and pink circles, Fig. 4), which reveals that these streams had potentially similar ecological conditions for larval development.

*Intraspecific genetic structure*

Despite the high sampling effort at each site, in four populations, the small number of individuals captured (1 or 2) revealed the low abundance and rarity of these species (Table 1). The 42 sequenced

individuals of *A. esparaguera* clustered in 13 unique *cox1* haplotypes, which included 10 polymorphic sites (1.62%) and a nucleotide diversity  $\pi$  of 0.00481. For *A. iglesiassi*, the 32 individuals sequenced resulted in 10 unique *cox1* haplotypes that contained eight polymorphic sites (1.3%) and a nucleotide diversity  $\pi$  of 0.00444. Haplotype composition displayed a significant genetic structure for *A. esparaguera* because most of the haplotypes were locally unique and only one out of 13 haplotypes (haplotype 10) was found in two populations (GO in Sierra de Baza and GA in Sierra de Cazorla). The absence of dominant haplotypes across populations suggested population isolation, especially between Sierra Nevada and the two nearby mountain ranges. For *A. iglesiassi*, a



**Fig. 5.** (a) Maps showing the current distribution of *Annitella esparaguera* in the studied area based on elevation. In red, sites where individuals of *A. esparaguera* were captured; in blue sites where the species was absent. (b) SDMs for *A. esparaguera* in the South of the Iberian Peninsula in the present, and predictions for years 2050 and 2080 using the RCP 2.6 climate scenario. (c) For each SDM, the future projection of the current haplotype network based on current haplotype composition for each population is shown. [Color figure can be viewed at wileyonlinelibrary.com]



non-significant genetic structure was revealed, mainly because haplotype 6 was present in almost all populations, and haplotypes 2 and 7 were common. For both species, the parsimony haplotype network was compact (Fig. 3b,c) with only two intermediate steps required to link the haplotypes of *A. esparaguera*, as visible in the star-like pattern, suggesting recent divergence.

### Species distribution models

The entire area of its current potential distribution was located at the highest elevations of Sierra de Cazorla, Sierra de Baza and mainly Sierra Nevada (Fig. 5a). The SDM results of *A. esparaguera* showed a map of the area with high probabilities of occurrence in current climatic conditions (maximum probability values of 0.7 on 1, Fig. 5b) and were broader than the area where the species has been recorded. Future potential distributions were similar for all RCP scenarios (Supporting Information Fig. S1), and only the climate model HadGEM2 ES with RCP 8.5 climatic scenario (the highest emissions) is described here. Precipitation seasonality (Bio15) revealed a homogenisation of the precipitation across the year, i.e. less seasonality, which together with a decrease in Mean Temperature of Wettest Quarter (Bio8), indicates a displacement of precipitation to colder months. In 2050, *A. esparaguera* species was predicted to lose all suitable habitats at Sierra de Cazorla and to be distributed in a much constrained range at Sierra de Baza and Sierra Nevada, for a total loss of 88.4% of the current distribution area (Fig. 5b). Range contraction will cause population extirpation and consequently one haplotype will be lost (Fig. 5c). In 2080, only three of the populations currently located at Sierra Nevada will persist as a result of losing 95% of the current distribution area (Fig. 5b), and the haplotype diversity will be reduced dramatically (Fig. 5c). The future habitat suitability predictions also showed a displacement of 41.5% of the current potential distributional area to new habitats located at higher elevations than its current distribution.

### Discussion

The two *Annitella* species endemic to the south of the Iberian Peninsula originated independently in two different lineages splitting from their respective closest relatives likely by long-term isolation between mountain ranges since the Pleistocene. Both species have a narrow and restricted distribution in cold high-elevation headwater streams, and particularly *A. esparaguera* shows local genetic subdivision indicating limited dispersion across streams. Given the predicted significant reduction and shift of suitable habitat to higher elevations, our study identifies the populations at risk (GA, GO, MA, PA and TE) and predicts high vulnerability of these species in future scenarios of climate change, as generally reported for species that are endemic to the summits of single mountain ranges (Hering *et al.*, 2009; Tierno de Figueroa *et al.*, 2010; Conti *et al.*, 2014).

In contrast to highly dispersive and medium-altitude taxa of caddisflies such as *Hydropsyche* Pictet 1834 that showed high genetic

homogeneity across nearby biogeographic regions (Múrria *et al.*, 2017), the genus *Annitella* has accumulated significant genetic divergence among the majority of species confined to different European high-elevation mountain ranges. Molecular analysis confirmed the two morphological groups within the genus *Annitella* differentiated by substantial genetic divergence, but the smaller phylogenetic distance between the subgenus *Preannitella* and *Chaetopterygopsis* than to the subgenus *Annitella* commends a taxonomic revision of this group. The genetic divergence among *Annitella* species supports the notion of long-time isolation, except for the highly similar *A. chomiensis*, *A. triloba* and *A. lateroproducta*, which can explain the cases of several fertile hybrids found between *A. chomiensis* and *A. lateroproducta* such as *A. dziedzielewiczii*, *A. kosciuszki* and *A. transylvanica* (Szczeny, 1979). Although the low genetic differentiation, *A. chomiensis* and *A. lateroproducta* showed clear morphological differences in the male genitalia along the main axis of the Dinarides, Carpathian and Balkan mountains (Botosaneanu, 1975a; Sipahiler, 1998; Szczeny, 1979). The Pleistocene Epoch was characterised by repeated glacial cycles that have deeply affected the patterns of biodiversity in the Palaearctic Region (De Lattin, 1967; Hewitt, 2004; Schmitt, 2007; Schmitt & Varga, 2012). Traditionally, the Mediterranean peninsulas have been interpreted as major Pleistocene glacial refuge areas and centres of endemism (Hewitt, 2004; Ribera & Vogler, 2004), which is supported also here by the high number of endemic Iberian *Annitella* species. Moreover, other genetic studies of cold-adapted stream insects, as many caddisfly species such as *Drusus discolor* Rambur 1842, revealed that several continental species persisted in multiple extra-Mediterranean refuges located in different regions (e.g. Pyrenees, Alps or Carpathians, Malicky, 2006; Pauls *et al.*, 2006; Theissinger *et al.*, 2013), rather than only in those located in Mediterranean areas. Our findings also support that *Annitella* endemic species survived in several ice-free regions of Central and East Europe from where taxa expanded (Fig. 1), as Malicky (1983) originally proposed for the Dinodal biome species. Thus, as suggested for arctic-alpine fauna (Schmitt, 2007), the distribution of *Annitella* species can be explained by bottlenecks followed by range expansion from southern and extra-Mediterranean refuges through elevational movements within each mountain systems and its periphery, which is consistent with the intraspecific genetic structure and distribution (a star-like haplotype network) found for *A. esparaguera*. Hence, cold-tolerance required for persistence in extra-Mediterranean periglacial refuges and long-term isolation may explain the diversification of *Annitella*, but now these relict species are vulnerable to climate warming if individuals cannot disperse to localities where environmental conditions fit their eco-physiological requirements, as previously suggested by Bálint *et al.* (2011).

The high number of unique and locally distributed *cox1* haplotypes indicates population subdivision for both *Annitella* species, although sequencing of more individuals is desirable for stronger support. Evidence of population isolation and limited gene flow is even more apparent for *A. esparaguera*, because all populations have several singleton haplotypes and only one haplotype is shared across two of the studied populations. Because there are unoccupied streams in the Sierra Nevada with similar ecological conditions to sites where *Annitella* was collected (Fig. 4), the fragmented

geographical range of *Annitella* species seems to be more determined by intrinsic limited dispersal and ubiquitous topographic barriers between suitable sites and among catchments, than by the scarcity of preferred habitats. However, unrecognised differences in micro-habitat or water conditions could add to the limited distributions. Larval dispersal of *Annitella*, and caddisflies in general, is restricted to short-distance movement along watercourses, while dispersion between streams would be accomplished mainly by the winged adults (Petersen *et al.*, 2004). However, adults of *Annitella* are poor fliers or are unable to fly given their short, rounded wings, but rather can crawl through riparian vegetation like other Chaetopterygini (Schmid, 1952; Botosaneanu, 1975b; Lehrian *et al.*, 2010), and often show brachyptery (Schmid, 1952; González & Malicky, 1988; Sáinz-Bariáin *et al.*, 2013). Moreover, adult emergence occurs during autumn, when dispersal capacity for poikilothermic insects generally is assumed to be lower than those emerging in the summer (Lehrian *et al.*, 2010). Hence, the structured intraspecific variation and the unoccupied optimal habitat for *Annitella* suggest that mainly short-distance compensatory elevational movement will occur in response to the modelled habitat shifts under climate warming. As a result, high vulnerability is predicted where isolated habitat no longer connected to areas with suitable conditions. Similar conservation concerns have been raised for other cold-adapted narrow endemic headwater specialist species such as *Chaetopterygopsis maclachlani* Stein, 1874, and *Drusus romanicus* Murgoci & Botosaneanu, 1953, by future range contractions and shifts towards higher elevations (Lehrian *et al.*, 2010; Bálint *et al.*, 2011).

Until recently, most studies assessing species vulnerability to climate change have used bioclimatic modelling to identify environmental limits and predict future species distribution, ignoring for instance their adaptive capacity or dispersal abilities for accessing predicted future climate space (Araújo & Peterson, 2012). Here, SDMs determined the threat of loss of almost 90% of current habitat of *A. esparraguera* and its displacement of ~40% of the current area to higher elevations at the Sierra Nevada. Although larvae of both species commonly prefer permanent and temporary headwater brooks, some larvae may have certain preference for permanent limnophilous microhabitats that ensure water flow in autumn (Sáinz-Bariáin & Zamora-Muñoz, 2015). The predicted future homogeneity in rainfall patterns and the eventually displacement of precipitation to colder months will likely shift habitat availability by summer drought. These changes will be critical in autumn if habitats vanish or the flow is too low before the adults emergence, which should complicate the success of pupation and further persistence of these species at the current elevations. By assessing population structure using genetic information, we can anticipate that *Annitella* species will be unable to track these range shifts, given the limited dispersion. Moreover, headwater specialists, including other species such as *Limnephilus obsoletus* Rambur, 1842, may be increasingly threatened by arrivals of dispersive, temperature-generalist species from lower river sections tracking favourable environmental conditions at higher altitudes (Domisch *et al.*, 2013). Already caddisflies species richness in the Sierra Nevada increased at 1800–2000 m altitude since 1989 by colonisation from mid-lowland species and also species coming from neighbouring mountain ranges (Sáinz-Bariáin *et al.*, 2016).

In conclusion, our integrative approach has identified two species with a high extinction risk because of the confined

distribution to the Sierra Nevada and nearby mountain ranges and the low dispersive potential for tracking range shift in response to global warming. Although *A. esparraguera* revealed a higher level of genetic differentiation and a larger distribution range than *A. iglesiasi*, an effective preservation of their current habitat considering all reaches where these species were found, must be a conservation priority. Although Sierra Nevada is a National Park, sites MO and DI are affected by skiing activities; site LA is affected by water extractions; and MO, DI and AL are used in summer for livestock raising (Zamora *et al.*, 2017). All these anthropogenic activities should be regulated for ensuring the preservation of these unique habitats. In addition, periodic sampling for assessing habitat persistence, particularly in autumn and measuring populations sizes may be needed to evaluate the strategy adopted. Beyond the conservation plans, if future habitat no longer provides suitable conditions, both species might profit from active translocation (i.e. assisted migration, Thomas, 2011) of populations to higher-elevation habitats within the native range where other local endemics are missing.

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### Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** SDMs using the precipitation seasonality (Bio15) for *A. esparraguera* in the South of the Iberian Peninsula in the present, and predictions for years 2050 and 2080 using the climate models EC-Earth (RCP 8.5), HadGEM2 ES (RCP 2.6, 4.5 and 8.5) and MRI-CGCM3 (RCP 2.6, 4.5 and 8.5).

**Appendix S1.** Supporting Information: Species used in the phylogenetic analyses including the GenBank accession numbers for the *cox1*, *EF1a* and *28S* genes. For each individual, the collector, site where it was collected and country of origin are indicated.

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