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Research

Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants

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Ecologists increasingly rely on molecular delimitation methods (MMs) to identify species boundaries, thereby potentially increasing the number of putative species because of the presence of morphologically cryptic species. It has been argued that cryptic species could challenge our understanding of what determine large-scale biodiversity patterns which have traditionally been documented from morphology alone. Here, we used morphology and three MMs to derive four different sets of putative species among the European groundwater crustaceans. Then, we used regression models to compare the relative importance of spatial heterogeneity, productivity and historical climates, in shaping species richness and range size patterns across sets of putative species. We tested three predictions. First, MMs would yield many more putative species than morphology because groundwater is a constraining environment allowing little morphological changes. Second, for species richness, MMs would increase the importance of spatial heterogeneity because cryptic species are more likely along physical barriers separating ecologically similar regions than along resource gradients promoting ecologically-based divergent selection. Third, for range size, MMs would increase the importance of historical climates because of reduced and asymmetrical fragmentation of large morphological species ranges at northern latitudes. MMs yielded twice more putative species than morphology and decreased by 10-fold the average species range size. Yet, MMs strengthened the mid-latitude ridge of high species richness and the Rapoport effect of increasing range size at higher latitudes. Species richness predictors did not vary between morphology and MMs but the latter increased the proportion of variance in range size explained by historical climates. These findings demonstrate that our knowledge of groundwater biodiversity determinants is robust to overlooked cryptic species because the latter are homogeneously distributed along environmental gradients. Yet, our findings call for incorporating multiple species delimitation methods into the analysis of large-scale biodiversity patterns across a range of taxa and ecosystems.

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Introduction

The species is a fundamental category of biological organization and serves as currency for analyses in multiple fields of biodiversity science including taxonomy, ecology, macroevolution and conservation. After centuries of debate, biologists finally reached a consensus in considering species as separately evolving segments of metapopulation lineages (Hey et al. 2003, de Queiroz 2005a). In this modern conception, criteria such as morphological distinguishability, reproductive isolation or monophyly are no longer considered necessary properties of species because they are acquired at different times and not necessarily in a fixed order along the extended process of speciation (de Queiroz 2005a). This paradigm shift in conceptualizing species has recently been embraced by taxonomy, which is increasingly using different species delimitation methods to formulate species hypotheses (SHs) (Dayrat 2005, Padial et al. 2010, Marshall et al. 2011). Usage of the term species hypothesis denotes that a species taxon like *Homo sapiens* serves as a hypothesis of a species as a separately evolving lineage in nature (Hey et al. 2003, p. 598). However, the use of different species delimitation methods is yet to be applied more broadly across biodiversity science, where any change in the number and distribution of SHs could have profound implications for understanding mechanisms driving biodiversity patterns (Isaac et al. 2004, Bickford et al. 2007, Schaefer et al. 2011, Marske et al. 2013, Vodă et al. 2015). In this study, we develop a general methodology that makes use of multiple species delimitation methods to integrate the heterogeneous nature of the speciation process into elucidating large-scale biodiversity patterns.

Species delimitation has been a very active field of research for the last decade (Flot 2015). Morphological methods to species delimitation are essentially phenetic in that they group similar individuals into morphologically distinguishable species. Molecular delimitation methods (MMs) can be roughly classified into three types (Flot 2015). Distance-based methods suppose that genetic variation should be smaller within species than between them, and use either a fixed threshold (Lefébure et al. 2006) or calculate the best-fitting threshold for the dataset at hand (Puillandre et al. 2012). Tree-based methods are rooted in the expectation that branching rates within species should be higher than branching rates between species. Then, within and between-species branching rates are modelled either respectively as a coalescent process and as a Yule process (Fujisawa and Barraclough 2013) or as two independent Poisson processes (Zhang et al. 2013). Allele sharing-based methods look at gene flow to delineate species, under the assumption that it is greater within species than between them and that this results in conspecific individuals sharing identical alleles whereas heterospecific individuals generally do not (Flot et al. 2010a).

Molecular methods to species delimitation do not replace morphology-based taxonomy. Rather, they provide SHs that may, or may not, be congruent with morphology-based SHs (Padial et al. 2010). Yet, incorporating multiple delimitation methods into macroecological analyses may foster our understanding of large-scale species richness patterns. The development of DNA sequencing revealed that morphological distinguishability, the criterion long used to delimit species, is not acquired in many lineages that are however highly genetically divergent (Pfenninger and Schwenk 2007). The term 'cryptic species' was coined to designate such lineages (Bickford et al. 2007). Comparing different species delimitation methods may unveil geographic variations in cryptic diversity, which could help us understand the determinants of species richness (Bickford et al. 2007, Pfenninger and Schwenk 2007, Trontelj and Fišer 2009, Schaefer et al. 2011). One popular hypothesis posits that morphological distinguishability is acquired more slowly across a physical barrier separating two ecologically similar regions than along a resource gradient with varying selection pressures (McCune and Lovejoy 1998, Rundle et al. 2000, Losos and Mahler 2010). Morphological and genetic differentiations in isolated regions with similar ecological conditions are mainly driven by chance events such as drift, polyploidization, allele incompatibility or founder effect (i.e. non ecological speciation, Rundle and Nosil 2005). On the contrary, resource gradients involve ecologically-based divergent selection (i.e. ecological speciation) which promotes character displacement and morphological differentiation as a by-product of niche partitioning (Rundle and Nosil 2005, Wagner et al. 2012, Hopkins et al. 2014). Based on this hypothesis, one would predict a proportionally higher number of cryptic species in regions where similar habitats are highly fragmented by strong topographic heterogeneity than in regions of high productive energy allowing further opportunities for niche partitioning and morphological differentiation.

Comparing different species delimitation methods may also refine testing of the factors shaping large-scale patterns of geographic range size. Predicting how the use of MMs instead of morphology may change our understanding of continental-scale variation in geographic range size is difficult because this change depends both on the number and relative range sizes of cryptic species (Isaac et al. 2004). Fragmentation of a morphological species range can either be symmetrical (i.e. even division of the morphological species range among cryptic species), asymmetrical (i.e. uneven division of the range among cryptic species), or it may not even occur if the ranges of cryptic species remain as large as that of the morphological species (Eme et al. 2013). Thus, the use of MMs to formulate SHs would affect both the average and variance of species range size across large geographical regions. There is now substantial evidence that the pattern of increasing range size of morphological species at higher latitudes in the Palearctic region (the Rapoport effect; Stevens 1989) is primarily driven by long-term temperature oscillations (Zagmajster et al. 2014). An important prediction of the historical climate variability hypothesis (Jansson and Dynesius 2002) is that the geographic range of morphological species at higher latitude should, in addition to being larger, be less subdivided genetically. This is because strong climatic oscillations

increase gene flow by selecting vagile and generalist species, thereby enabling those species to colonize higher latitude regions from a few distant refugia. An alternative hypothesis is that the effect of long-term climatic oscillations fragment geographic ranges into a number of spatially-isolated refugia containing genetically-divergent lineages, from which postglacial colonization proceeds locally (Provan and Bennett 2008, Stewart et al. 2010). These lineages may well correspond to cryptic species if stabilizing selection prevents the evolution of specialized phenotypes in regions with fluctuating climates. Comparing morphology and MMs into the study of large-scale patterns of geographic range size could help tease apart these alternative hypotheses. Asymmetrical fragmentation of large morphological species ranges at northern latitudes when shifting from morphology to MMs would lend additional support to the historical climate variability hypothesis because it would indicate that postglacial colonization proceeded from a few distant refugia. On the contrary, symmetrical fragmentation of large morphological species ranges into a series of small species ranges would seriously question the role of long-term climatic oscillations in selecting for vagility and generalism because it would indicate that multiple refugia contributed locally to postglacial colonization (Trontelj et al. 2009).

In this study, we applied morphology and three MMs commonly used in molecular taxonomy to obtain four different sets of SHs for groundwater crustacean specimens collected at multiple sites across Europe. For each set of SHs, regression models were used to assess the relative role of three broad mechanisms – spatial heterogeneity, climate/productivity and historical climate variability – in shaping European patterns of species richness and geographic range size. We tested three predictions. First, MMs would yield many more SHs than morphology because groundwater is considered a highly constrained environment allowing little morphological changes during speciation and promoting morphological convergence in non-sister lineages (Trontelj et al. 2009). Second, for species richness, MMs would increase the proportion of variance attributed to spatial heterogeneity because morphological differentiation may be slower in isolated habitats with similar ecological conditions than along resource gradients (McCune and Lovejoy 1998, Rundle et al. 2000, Losos and Mahler 2010). Third, for range size, MMs would increase the proportion of variance attributed to historical climate variability. Even though multiple microrefugia might have persisted at northern latitudes, it is unlikely that they equally contributed to the post-glacial colonization of Europe (Eme et al. 2013).

Methods

Generating sets of species hypotheses

Our data set for Europe comprised 2205 individuals of obligate groundwater Aselloidea (Isopoda) and Niphargidae (Amphipoda), collectively representing 263 morphologically distinguishable species (MDSs) collected at 1075 sites (Supplementary material Appendix 1 and Appendix 2 Fig. A1). We focused on these two taxa because they are among the most species rich in groundwater. For species delimitation, we used an additional set of 678 individuals representing 91 additional MDSs collected inside (surface water species) and outside Europe (surface water and groundwater species). Of a total number of 2883 individuals, 92% were analyzed by the authors as part of this study or previous studies, thereby minimizing incoherencies in species delimitation due to different practices in morphological taxonomy and molecular protocols (Supplementary material Appendix 1).

Individuals were collected from caves, springs, wells, and the hyporheic zone of streams. They were placed in 96% ethanol at ambient temperature for transportation back to the laboratory, then stored at –20°C. MDSs were delimited using well-established diagnostic characters and each individual was assigned a nominal species name or a provisional code for undescribed species. DNA was extracted using an optimized chloroform DNA extraction protocol for the Aselloidea (Calvignac et al. 2011) and Qiagen DNeasy kits for the Niphargidae (Flot et al. 2010b). We amplified DNA with primers targeting the mitochondrial cytochrome oxidase subunit I (COI) gene. The COI gene was selected because it is highly variable, straightforward to align and sequences were already available for several MDSs of Aselloidea and Niphargidae. Moreover, several studies have previously shown that species of both groups delimited based on the COI gene were supported by the distribution of haplotypes of the 28S and/or ITS nuclear markers (Meleg et al. 2013, Morvan et al. 2013, Flot et al. 2014). PCR reactions were done following published, optimized protocols (Flot et al. 2010b, Morvan et al. 2013). PCR products were sequenced in both directions using the same primers as for amplification (GATC Biotech, Konstanz; Eurofins MWG Operon, Ebersberg; SeqLab, Göttingen, Germany; BIOFIDAL, Vaulx-en-Velin, France). Chromatograms were visualized and cleaned using Finch ver. 1.5.0 (Geospiza, Seattle, USA) and Sequencher 4.1.4 (Gene Codes, Ann Arbor, USA). We submitted 1104 sequences to GenBank as part of this study (Supplementary material Appendix 1).

To delimit species using MMs, we chose one distance-based method, the fixed threshold (TH) implemented by Lefébure et al. (2006) for the crustaceans, and one tree-based method, the Poisson tree processes (PTP) proposed by Zhang et al. (2013). In addition to the original PTP method, we also used its Bayesian implementation (bPTP) in order to take into account the uncertainty inherent to phylogenetic reconstruction. No allele sharing-based method was used in the present study because they require nuclear markers (Flot et al. 2010a). Hereafter, we use the terms 'MM-based species' to refer to the three sets of species hypotheses delimited using the three molecular methods (TH, PTP and bPTP).

To avoid saturation artifact, we applied these methods to two COI alignments, one for Aselloidea and the other for Niphargidae (Supplementary material Appendix 1). For all

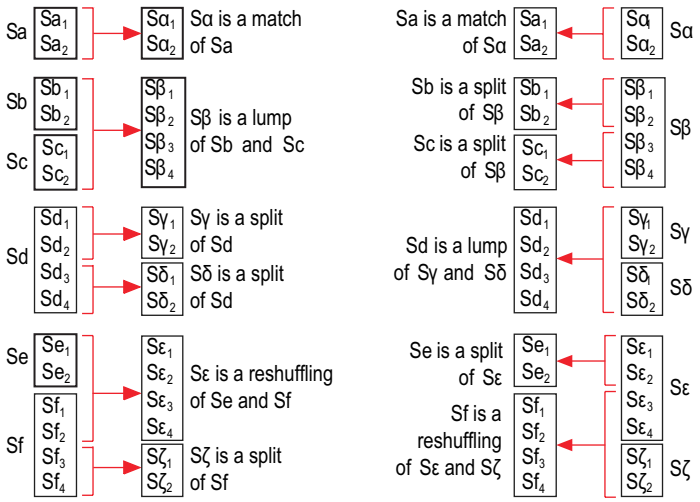

Figure 1. Schematic illustration of the four possible types of changes – match, lump, split and reshuffling – when shifting from one set of species hypotheses to another.

methods, duplicate haplotypes were removed using a custom Perl script (Eme et al. 2013) because including duplicate sequences in MDSs that received a high sampling effort could lead to oversplitting of MDSs that were comparatively less sampled (Zhang et al. 2013). Following Morvan et al. (2013), the best representative for any given haplotype was identified as the longest sequence containing the fewest ambiguities using R (R Development Core Team). Then, COI maximum-likelihood phylogenies were built using PhyML (Guindon et al. 2010) under the GTR + G + I model of evolution with 4 substitution rate categories and a gamma shape parameter ($\alpha = 0.519$) as well as a proportion of invariant sites (0.375) estimated by maximum likelihood in PhyML. From these phylogenies, we followed the procedure described in Eme et al. (2013) to delimit SHs according to the 16% divergence threshold. This threshold as defined by Lefébure et al. (2006) is based on the observation made from 1500 COI sequences belonging to 276 MDSs of crustaceans that two monophyletic groups diverging by more than 0.16 substitution per site, as measured by patristic distances, have a strong probability (ca 0.99%) of belonging to different species. To delimit SHs according to the PTP and the bPTP methods (Zhang et al. 2013), analyses were run on the bPTP web server (<http://species.h-its.org/ptp/>, last accessed 23 October, 2015) using 400 000 MCMC generations, with a thinning of 400 and 0.1 (10%) burn-in.

In the following of this article, the datasets for Aselloidea and Niphargidae were pooled together because we aimed to explore the implications of a change in the number and distribution of SHs for large-scale studies of biodiversity patterns within higher taxa.

Measuring biodiversity patterns

Taxonomic patterns

We documented the changes when shifting from one set of SHs (Sa, b, c, d, e, f) to another (Sα, β, γ, δ, ε, ζ) by distinguishing the following four cases (Fig. 1). 1) Match: the individuals in Sα are from a single SH Sa and include all the individuals of that SH. 2) Lump: the individuals in Sβ are from more than one SH (e.g. Sb and Sc) and include all the individuals of those SHs. 3) Split: the individuals in Sγ are from a single SH Sd but do not include all the individuals of that SH. 4) Reshuffling: the individuals in Sε are from more than one SH (e.g. Se and Sf) and do not include all the individuals of those SHs.

Patterns of species richness and range size

The maximum linear extent (MLExt) of each species – defined as the straight-line distance between its two most distant known localities – was used as a proxy for range size and measured in the ETRS89 Lambert Azimuthal Equal Area projection. For comparing patterns of range size among the four sets of SHs, we retained only those MDSs whose ranges were covered by localities with COI sequences (Supplementary material Appendix 2 Fig. A2). To analyze the latitudinal patterns of median range size and species richness, we used a grid of 0.9°-latitude equal-area cells (Supplementary material Appendix 2 Fig. A1). For each set of SHs, we computed the median range size of SHs contained in each cell and the species richness of cells. The species richness and median range size data sets consisted of 188 and 166 cells containing at least one SHs, respectively ($n = 263$ and 147 MDS for species richness and median range size, respectively).

Predictors of species richness and median range size

For each grid cell, we used five predictors to represent the three broad mechanisms – spatial heterogeneity, climate/productivity and historical climate variability – that were proposed to explain the geographic variation of species richness at continental scale (Field et al. 2009, Eme et al. 2015). Spatial heterogeneity was represented by habitat diversity and elevation range as a surrogate for topographic heterogeneity. Climate/productivity was represented by mean annual actual

evapotranspiration (AET). To test for the effect of history, we computed temperature and precipitation anomalies, defined as the differences in mean annual temperature and annual precipitation between present and the Last Glacial Maximum (Araùjo et al. 2008, Leprieur et al. 2011).

We used seven predictors to test for the role of habitat area/heterogeneity, climate seasonality and historical climate variability in shaping the pattern of range size (Morueta-Holme et al. 2013). Habitat area/heterogeneity was represented by elevation range, AET, aquifer area, and climatic rarity to account respectively for the climatic buffer hypothesis, the resource specialization hypothesis, the area availability hypothesis, and the climatic rarity hypothesis (Bonn et al. 2004, Ohlemüller et al. 2008). To test the climate seasonality hypothesis (Stevens 1989), we selected precipitation seasonality as a proxy for present intra-annual environmental variability because there is very little thermal seasonality in groundwater (Zagmajster et al. 2014). To test for the effect of historical climate variability on range size, we used the same two predictors as for the effect on species richness. Detailed explanations on how the predictors were computed are provided in the Supplementary material Appendix 3. We checked for multicollinearity among predictors using variance inflation factors (VIFs) and found them to be in an acceptable range (VIFs < 5; Zuur et al. 2009).

Determinants of spatial variation in species richness and median range size

We assessed the geographic patterns of species richness and median range size as well as their determinants for each set of SHs. The relationships between both the cell average of median range size and species richness per 0.9° latitudinal band and latitude were assessed using generalized additive models (GAMs) to account for non-linear relationships. For each set of SHs, we used ordinary least square (OLS) models to determine the relative importance of spatial heterogeneity, climate/productivity, and historical climate variability in shaping European patterns of species richness. Species richness data ($n = 188$ cells) were log-transformed to improve normality. Temperature anomaly was also log-transformed, and all predictors were normalized. To check for potential bias caused by the log-transformation of richness data, we also applied generalized linear models with negative binomial error (GLM.nb) to account for over-dispersion in non-transformed richness data. A two-step procedure was used to test for multi-causality. In step 1, we used variance partitioning to determine the independent and shared contributions of the three mechanisms to geographic variation in species richness (Legendre and Legendre 1998). Variance partitioning was computed using adjusted R^2 for OLS and pseudo-R^2 for GLM.nb, the latter being defined as the proportional increase in explained deviance (Zuur et al. 2009). In step 2, we used multimodel inference to evaluate the relative importance of predictors within each broad mechanism (Burnham and Anderson 2002). We ran all possible OLS/GLM.nb models and retained only those whose difference in the Akaike's information criterion corrected for small sample size (AICc) with the best model (lowest AICc) was ≤ 5 (Eme et al. 2015). In this subset of models, we measured the relative importance of each predictor as the sum of the AICc weights of the models in which the predictor occurred (Burnham and Anderson 2002). The observed AICc weight of each predictor was compared to the 95th percentile of a null distribution estimated using 1000 permutations of species richness data (Galipaud et al. 2014). For each permutation, the AICc weight of each predictor was computed using the same multimodel inference scheme as for the original data. To take into account spatial autocorrelation in the residuals of OLS models (which can affect both parameter estimates and their statistical significance), we applied generalized least square (GLS) models on log-transformed species richness data (Dormann et al. 2007, Beguería and Pueyo 2009). The statistical procedure used to perform GLS models is described in the Supplementary material Appendix 3.

For each set of SHs, we also applied OLS and GLS models to determine the relative importance of habitat area/heterogeneity, climate seasonality and historical climate variability in explaining geographic variation of median range size in Europe. The median MLExt of MM-based species were log-transformed to improve normality and all MLExt data were standardized to ensure comparability of regression parameters across the four sets of SHs ($n = 166$ cells). Precipitation seasonality, climatic rarity and temperature anomaly were log-transformed to satisfy normality assumption and all predictors were normalized. OLS and GLS models were performed as previously described for species richness.

GAMs were performed using the mgcv R package (Wood 2011). Variance partitioning was computed using the vegan R package (Oksanen et al. 2015) for OLS models and the set of equations provided by Legendre and Legendre (1998) for the GLM.nb and GLS models. GLS and GLM.nb models were implemented using the nlme R package (Pinheiro et al. 2015) and MASS (Venables and Ripley 2002) R packages, respectively. Multimodel inference and the null distribution of AICc weights were performed using the MuMIn R package (Barton 2015) and the R scripts of Galipaud et al. (2014).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.ns8v4> (Eme et al. 2017).

Results

Taxonomic comparison among the four sets of species hypotheses

TH and PTP delimited respectively, 1.97 and 2.5 more SHs than morphology (Fig. 2, Supplementary material Appendix 2 Table A1). In contrast, PTP and bPTP delimited only 1.25 more SHs than TH and there was almost no difference in the number of species between the two PTP models.

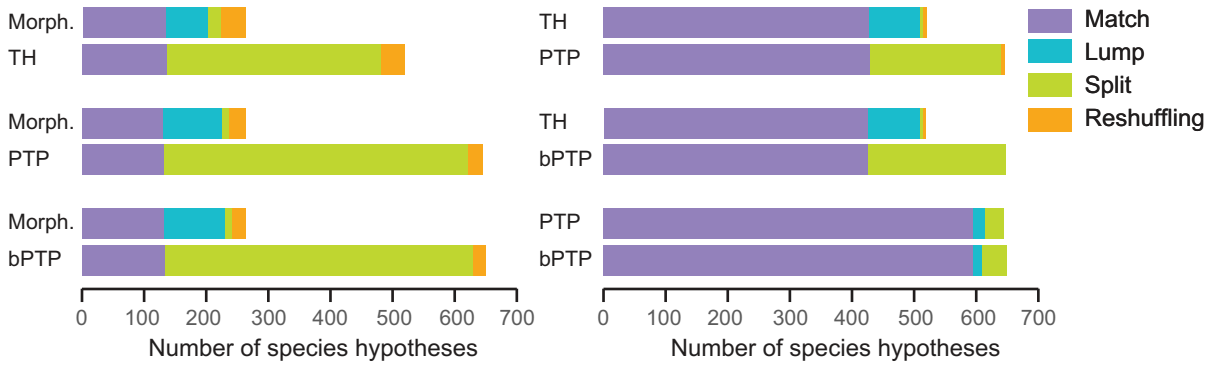

Figure 2. Pairwise taxonomic comparisons between the four different sets of species hypotheses delimited using morphology (Morph.), a COI divergence threshold (TH), the Poisson tree processes model (PTP) and its Bayesian implementation (bPTP). See text and Fig. 1 for definitions of match, lump, split and reshuffling.

TH and PTP split MDSs into smaller clusters of individuals but reshuffling cases were rare (Fig. 2): of the 519 and 646 SHs respectively delimited by TH and PTP, only 36 (6.9%) and 22 (3.4%) fell in that category (Supplementary material Appendix 2 Table A1). Although the two PTP methods yielded almost the same number of SHs, they slightly differed in the way they delimited species. Of a total of 650 species delimited by bPTP, respectively 13 and 40 resulted from the lumping and splitting of species delimited by PTP.

Patterns and determinants of species richness

All four different sets of SHs revealed a ridge of high species richness at latitudes ranging from ca 42° to 46°N and a smaller more northern ridge at latitudes ranging from ca 49° to 51°N (Fig. 3). The main ridge of high species richness became increasingly pronounced as species delimitation shifted from morphology to TH, then to PTP.

GLM.nb models explained on average 10% more variance in species richness than the OLS and GLS models, indicating substantial overdispersion in the species richness data (Table 1). Yet, the proportion of explained variance was remarkably stable across the four sets of SHs, regardless of the model used to fit species richness data. Also, the proportion of variance attributed to the three broad mechanisms varied little across the four sets of SHs (Fig. 4a, Supplementary material Appendix 2 Table A2). Productive energy made by far the highest independent contribution to the overall variance (9.6 ± 1.6%), followed by spatial heterogeneity (4.8 ± 0.6%). Productive energy also shared a substantial amount of variance with spatial heterogeneity (5.5 ± 2.2%). Historical climate variability alone was a poor predictor of species richness, explaining only 0.3 ± 0.7% of the overall variance. Actual evapotranspiration and elevation range were the only predictors showing a significant positive relationship with species richness across all model types and sets of SHs (except elevation range for GLS model performed on MDSs; Table 1, Supplementary material Appendix 2 Fig. A3). Their AICc weights systematically differed from the null distribution, showing that both predictors contributed to explain geographic variation of species richness across the four sets of SHs. The slope parameters and AICc weights of all other predictors were in most cases not significant.

Patterns and determinants of median range size

The average MLExt of SHs was 170 ± 316, 41 ± 136, 22 ± 96 and 17 ± 63 km for morphology (n = 147 SHs), TH (n = 379), PTP (n = 477) and bPTP (n = 478), respectively. A pattern of increasing median range size at higher latitudes was more evident with MMs than with morphology (Fig. 5). Below 43°N, most MDSs, including the most widely distributed ones, were symmetrically split into a series of narrow-range MM-based species. This decreased the average range size per band and resulted in a flat distribution of average range sizes across latitudinal bands with no dispersion around the band averages. Above 43°, MDSs were asymmetrically split in fewer MM-based species, some of which retained wide geographic ranges. This resulted in a lower decrease in the average range size per band and a higher dispersion around the band averages than in southern latitudes (i.e. below 43°).

The proportions of variance in median range size explained by the OLS and GLS models were 18.8 ± 0.9, 33.7 ± 0.4, 40.9 ± 1.1 and 41.8 ± 1.3% for morphology, TH, PTP and bPTP, respectively (Table 2, Fig. 4b). Among the three broad mechanisms, historical climate variability had by far the highest independent contribution (15.2–31.3%) to spatial variation in range size, regardless of the models and sets of SHs considered (Fig. 4b, Supplementary material Appendix 2 Table A3). The variation explained by the independent components of habitat area/heterogeneity and climate seasonality were consistently below 4 and 3%, respectively. Historical climate variability increasingly contributed to geographic variation in range

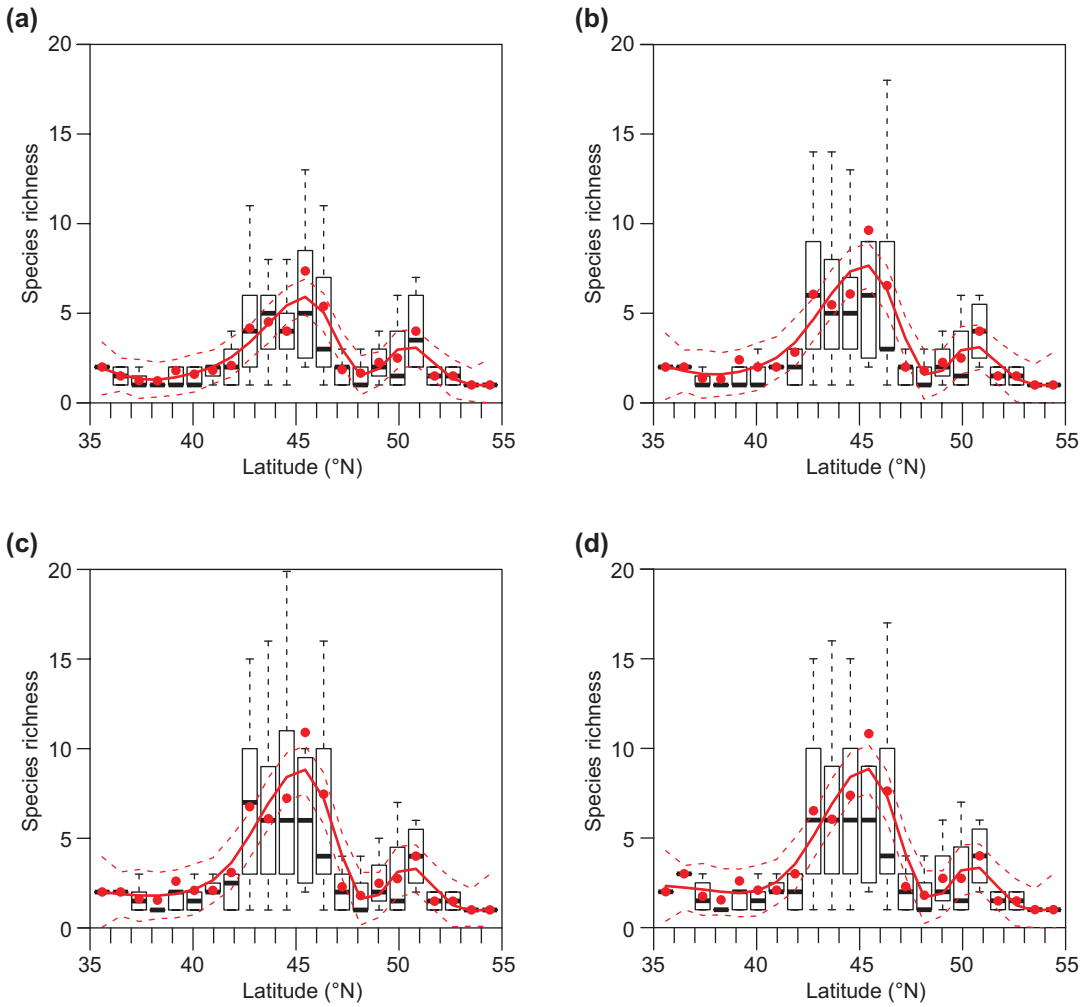

Figure 3. Relationships between species richness per latitudinal band and latitude for the four different sets of species hypotheses delimited using (a) morphology; (b) a COI divergence threshold (TH); (c) the Poisson tree processes model (PTP); (d) the Bayesian implementation of the Poisson tree processes model (bPTP). Black horizontal bars, red dots and boxes show the median, average and interquartile range, respectively, for 0.9° latitudinal bands. The maximum length of each whisker is up to 1.5 times the interquartile range. Continuous red lines represent the fit of a generalized additive model to the averages of latitudinal bands and dashed red lines show 95% confidence intervals.

size when SHs were delimited using MMs, especially PTPs. The shared variance between historical climate variability and climate seasonality as well as among the three broad mechanisms also increased when shifting from MDSs to MM-based species, but their contribution to the overall variance remained substantially lower than that of historical climate variability alone (Fig. 4b, Supplementary material Appendix 2 Table A3). Temperature anomaly was the only predictor showing a significant positive relationship with median range size as well as a significant AICc weight across all model types and sets of SHs. The slope of the relationship was steeper for OLS models performed on MM-based species, especially PTP and bPTP, while it varied little across sets of SHs for GLS models (Table 2, Supplementary material Appendix 2 Fig. A4). There was also a weak, negative but significant relationship between AET and median range size for PTP and bPTP.

Discussion

A new generation of biodiversity databases

Integrating the heterogeneous nature of the speciation process into the analysis of large-scale biodiversity patterns requires species distributional databases that include 'information on the diverse properties of species' (de Queiroz 2005b). Here, we developed for the first time such a multi-criteria database by attributing individual specimens to multiple SHs using three distinct criteria: morphological distinguishability; a distance threshold hypothesized to separate intraspecific and interspecific variation (i.e. TH); and the shift in branching rate expected to take place in phylogenetic trees at the point of transition from species-level to population-level processes (i.e. PTP and bPTP). This general methodology differs from that of previous studies which explored how historical

Table 1. Summary results of the generalized linear models with negative binomial error (GLM.nb), and ordinary and generalized least squares models (OLS and GLS) for testing predictors of species richness of obligate groundwater crustaceans in Europe. The four data sets correspond to four different sets of species hypotheses generated using morphology (Morph.), a COI divergence threshold (TH), the Poisson tree processes model (PTP) and its Bayesian implementation (bPTP). Ex_Var: proportion of explained variance (%); AET: actual evapotranspiration; Elevr: elevation range; Hab_Div: habitat diversity; Ano_T: temperature anomaly; Ano_P: precipitation anomaly; Coeff: coefficient parameters are from model averaging using a set of models whose difference in the Akaike's information criterion corrected for small sample size (AICc) with the best model was ≤ 5 (in bold, p-value < 0.05); wAICc: summed AICc weight of the predictor (in bold, values outside the 95 percentile of the null distribution).

			AET		Elevr		Hab_Div		Ano_T		Ano_P	
Dataset	Model type	Ex_Var (%)	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc
Morph.	GLM.nb	28.54	**0.354**	**1**	**0.183**	**1**	–0.004	0.24	**0.142**	0.73	–0.032	0.29
	OLS	16.61	**0.251**	**1**	**0.165**	**1**	0.028	0.28	0.090	0.50	–0.040	0.31
	GLS	18.11	**0.221**	**1**	0.118	**0.74**	0.035	0.23	0.033	0.22	0.021	0.26
TH	GLM.nb	27.54	**0.376**	**1**	**0.228**	**1**	–0.053	0.27	0.087	0.38	–0.019	0.32
	OLS	16.46	**0.287**	**1**	**0.194**	**1**	0.018	0.24	0.050	0.3	–0.038	0.28
	GLS	18.33	**0.255**	**1**	**0.149**	**0.81**	0.015	0.23	0.004	0.23	–0.007	0.23
PTP	GLM.nb	26.58	**0.370**	**1**	**0.245**	**1**	–0.055	0.32	0.088	0.38	–0.004	0.27
	OLS	16.44	**0.291**	**1**	**0.217**	**1**	0.015	0.25	0.034	0.27	–0.014	0.25
	GLS	18.39	**0.252**	**1**	**0.175**	**0.94**	0.013	0.23	–0.003	0.23	0.008	0.23
bPTP	GLM.nb	26.80	**0.374**	**1**	**0.237**	**1**	–0.074	0.38	0.087	0.37	–0.006	0.27
	OLS	16.32	**0.292**	**1**	**0.211**	**1**	0.006	0.24	0.031	0.26	–0.026	0.26
	GLS	18.31	**0.261**	**1**	**0.162**	**0.94**	0.001	0.23	–0.002	0.23	–0.003	0.23

changes in taxonomy affected testing of macroecological and macroevolutionary hypotheses (Isaac and Purvis 2004, Vodă et al. 2015). It could be extended further to include multilocus/genome-scale data as well as ecological and behavioral criteria (Marshall et al. 2011, Guillot et al. 2012).

In accordance with our first prediction, we found that MMs more than doubled the number of SHs in our data set. This large increase in SHs number compared to morphology-based delineations may result in very different inferences about the determinants of biodiversity patterns

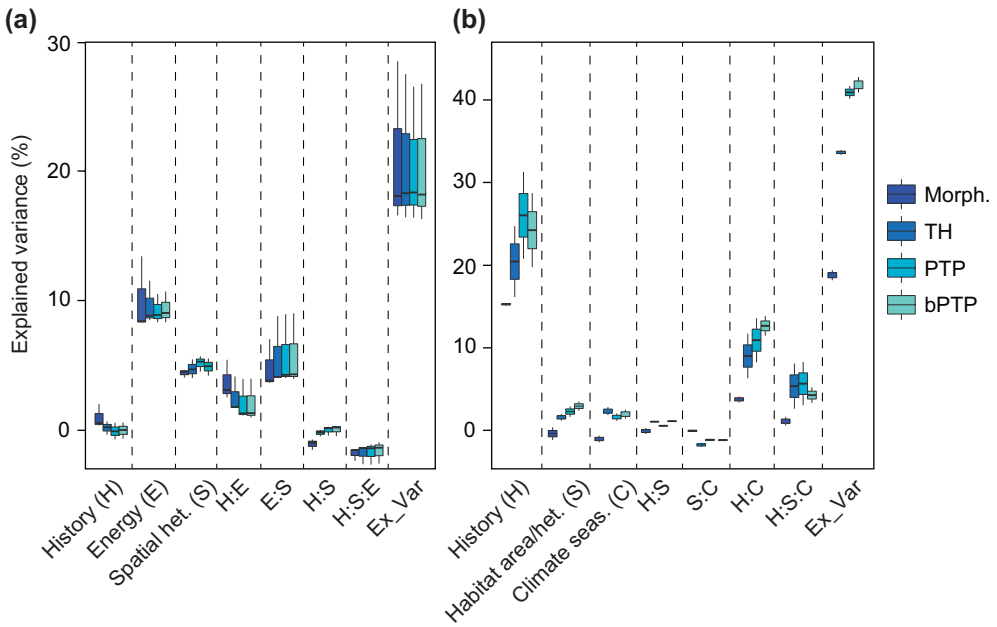

Figure 4. (a) Independent and shared contributions of historical climate variability (H), productive energy (E) and spatial heterogeneity (S) to variation in species richness of obligate groundwater crustaceans in Europe. In abbreviations, colons denote shared variance between mechanisms. Ex_Var: total proportion of variance explained by the models. Variance partitioning is shown for the four different sets of species hypotheses delimited using morphology, a COI divergence threshold (TH), the Poisson tree processes model (PTP) and its Bayesian implementation (bPTP). For each of the four species sets, black horizontal bars and boxes show the median and interquartile range of variances computed using generalized linear models with negative binomial error, ordinary least squares models (OLSs) and generalized least squares models (GLSs). (b) Independent and shared contributions of historical climate variability (H), habitat area/heterogeneity (S) and climate seasonality (C) to variation in median range size. Black horizontal bars and boxes show the median and interquartile range of variances computed using OLS and GLS models.

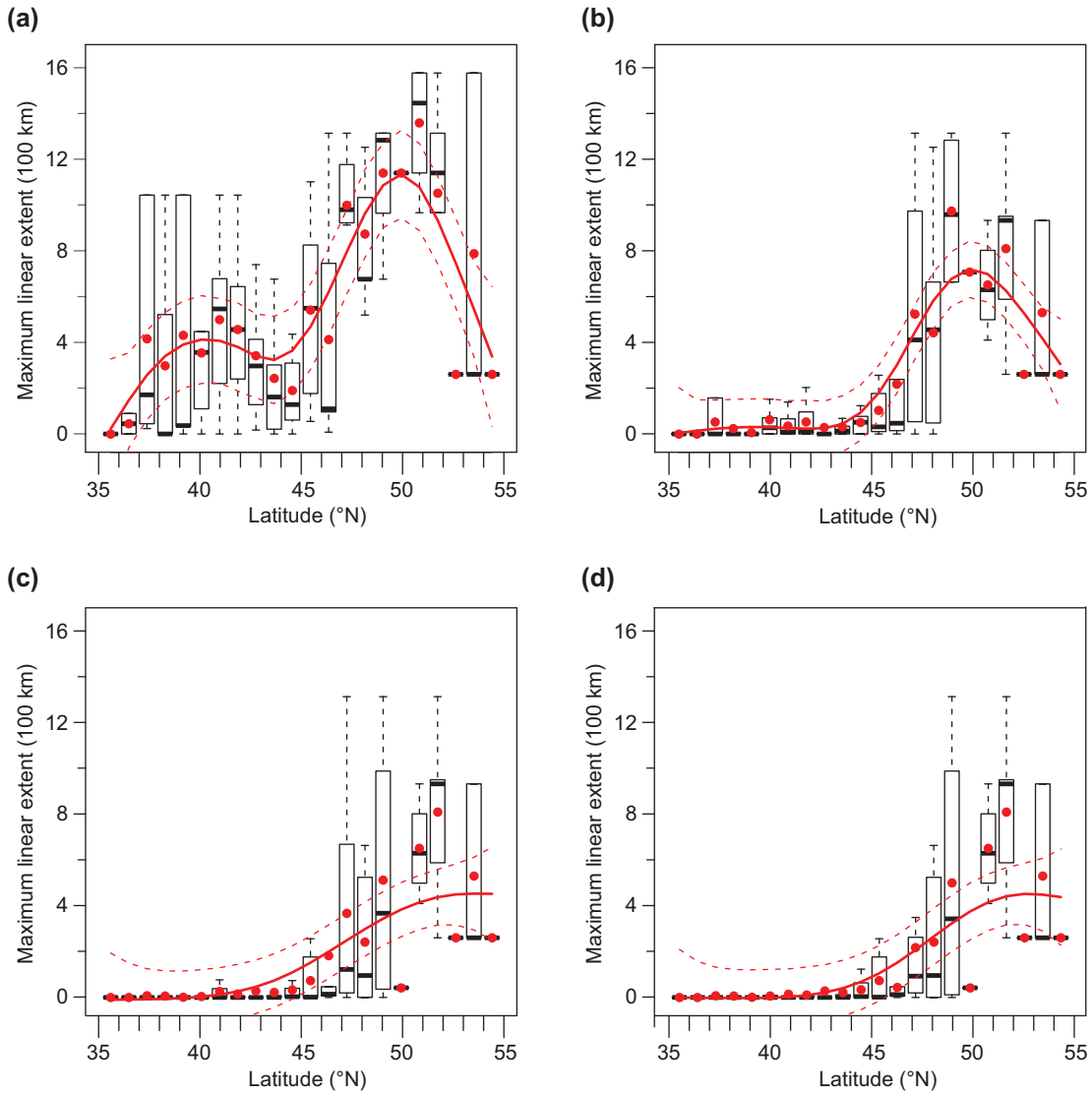

Figure 5. Relationship between median range size (maximum linear extent) per latitudinal band and latitude for the four different sets of species hypotheses delimited using (a) morphology, (b) a COI divergence threshold (TH), (c) the Poisson tree processes model (PTP), (d) the Bayesian implementation of the PTP (bPTP). Black horizontal bars, red dots and boxes show the median, average and interquartile range, respectively, for 0.9° latitudinal bands. The maximum length of each whisker is up to 1.5 times the interquartile range. Continuous red lines represent the fit of a generalized additive model to the averages of latitudinal bands and dashed red lines show 95% confidence intervals.

(Marske et al. 2013), which calls for integrating MMs into the analysis of large-scale studies of the groundwater fauna. Some level of differences between the numbers of species delimited by different MMs is inevitable since they use different criteria (Dellicour and Flot 2015). For instance, species delimitation methods that are based on allele sharing (Flot et al. 2010a) or on multilocus coalescence (Yang and Rannala 2014) could change the number of SHs in our dataset by splitting divergent entities that have not yet reached reciprocal monophyly or by lumping entities that are still connected by gene flow (Knowles and Carstens 2007, Leaché et al. 2014). Yet, the three MMs used in the present study provided similar richness estimates, as in previous groundwater studies showing that the variation in the number of SHs among different MMs is typically smaller than between them and morphology-based taxonomy (Eme et al. 2013, Morvan et al. 2013). This matches the result of a recent simulation study showing that single-locus tree-based, distance-based and allele sharing-based approaches give congruent species hypotheses when dealing with highly variable markers in species of small effective population size and low speciation rate (Dellicour and Flot 2015). Yet, several studies found that the ability of MMs to delimit species accurately declined as the mean or among-species variance in effective population size (Ne) increased (Esselstyn et al. 2012, Fujisawa and Barraclough 2013, Dellicour and Flot 2015, Ahrens et al. 2016).

Table 2. Summary results of the ordinary and generalized least squares models (OLS and GLS) for testing predictors of median range size of obligate groundwater crustaceans in Europe. The four data sets correspond to four different sets of species hypotheses generated using morphology (Morph.), a COI divergence threshold (TH), the Poisson tree processes model (PTP) and its Bayesian implementation (bPTP). Ex_Var: proportion of explained variance (%); Ano_T: temperature anomaly; AET: actual evapotranspiration; Ano_P: precipitation anomaly; Elevr: elevation range; Aq_Area: aquifer area; Clim_rar: climatic rarity; Sea_P: precipitation seasonality; Coeff: coefficient parameters are from model averaging using a set of models whose difference in the Akaike's information criterion corrected for small sample size (AICc) with the best model was ≤ 5 (in bold, p-value < 0.05); wAICc: summed AICc weight of the predictor (in bold, values outside the 95 percentile of the null distribution).

	Model		Ano_T		AET		Ano_P		Elevr		Aq_Area		Clim_rar		Sea_P	
Dataset	type	Ex_Var (%)	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc	Coeff	wAICc
Morph.	OLS	19.42	**0.1336**	**1**	**–0.0327**	**0.77**	0.0060	0.27	0.0029	0.21	–0.0024	0.22	–0.0016	0.22	0.0011	0.18
	GLS	18.19	**0.1803**	**1**	–0.0169	0.23	0.0397	0.26	–0.0109	0.22	–0.0259	0.43	0.0281	0.32	**0.0710**	**0.75**
TH	OLS	33.37	**0.1921**	**1**	–0.0383	0.53	–0.0044	0.21	–0.0125	0.24	–0.0528	0.66	0.0118	0.22	**–0.0653**	**0.83**
	GLS	33.95	**0.1858**	**1**	**–0.0622**	0.72	0.0230	0.26	–0.0211	0.29	–0.0623	0.63	0.0404	0.47	–0.0514	0.38
PTP	OLS	40.14	**0.2261**	**1**	**–0.0591**	**0.97**	–0.0472	0.6	0.0029	0.21	–0.0298	0.35	0.0197	0.28	**–0.0570**	**0.79**
	GLS	41.70	**0.2017**	**1**	**–0.0661**	**0.92**	–0.0276	0.23	–0.0042	0.20	–0.0271	0.29	0.0437	0.55	–0.0371	0.30
bPTP	OLS	40.90	**0.2172**	**1**	**–0.0631**	**1**	–0.0481	0.66	–0.0014	0.21	–0.0241	0.33	0.0035	0.21	**–0.0591**	**0.84**
	GLS	42.78	**0.1883**	1	**–0.0618**	**0.86**	–0.0287	0.21	–0.0015	0.18	–0.0298	0.29	0.0259	0.30	–0.0465	0.37

However, larger Ne was either found to result in over-splitting or lumping depending on the method used, differences in Ne between the common ancestor and descendant species and most importantly on the relative change of speciation rate. Thus, even under the assumption of a large-scale geographic variation in Ne (e.g. Ne hypothetically increases with increasing productive energy), it is not yet possible to assess the extent to which this variation might have influenced the continental-scale pattern of species richness as determined with MMs.

Patterns and determinants of species richness

We showed that the latitudinal pattern of species richness in the European groundwater crustacean fauna was robust to variation in the number of SHs inferred by morphology and MMs. Our results confirmed recent studies revealing a ridge of high species richness at latitudes ranging from ca 42° to 46°N in both the terrestrial and aquatic subterranean European fauna (Culver et al. 2006, Zagmajster et al. 2014, Eme et al. 2015). Zagmajster et al. (2014) demonstrated for the groundwater crustaceans that the ridge was robust to spatial variation in sampling effort. Here, we showed that the ridge became increasingly pronounced using MMs because the number of MM-based species increased proportionally to the number of MDSs present in the different latitudinal bands (number of SHs using PTP = 1.38 × number of MDS, $r^{2=}$ 0.93; n = 22 bands). This indicates that cryptic species are evenly distributed across large geographic regions. This brings support at the European scale to the idea that the proportion of cryptic species may be invariant across biogeographic regions, as first suggested by Pfenninger and Schwenk (2007) and subsequently criticized by Trontelj and Fišer (2009) but recently confirmed for Mediterranean butterflies (Vodă et al. 2015).

Contrary to our second prediction, using MM-based species instead of MDSs did not increase the contribution of spatial heterogeneity to geographic variation in species richness. This prediction was based upon the assumption that MMs would unveil a disproportionately higher number of cryptic species in regions of high topographical heterogeneity. Moreover, the fragmentation of the range of widely distributed MDSs into smaller ranges was expected to increase the contribution of topographic heterogeneity because physical barriers to dispersal are major determinants of species richness (Rahbek and Graves 2001, Davies et al. 2007, Svenning et al. 2009) especially for narrow-range species (Jetz and Rahbek 2002, Bregović and Zagmajster 2016). No species richness model performed on MM-based species brought support for a disproportionate increase in the number of cryptic species in regions of higher topographic heterogeneity. Productive energy was constantly the best predictor of overall species richness, indicating a strong dependence of subterranean communities on food supply from the surface environment (Eme et al. 2015). The stability in the proportion of variance

attributed to productive energy and spatial heterogeneity in our study indicates that the proportion of cryptic species among groundwater crustaceans is evenly distributed along gradients of topographic heterogeneity and productive energy. However, our findings do not imply that the mechanisms causing cryptic species are invariant across space or environmental gradients. The lack of morphological distinguishability between species may be due to recent divergence, niche conservatism or morphological convergence, three possibilities that are well documented among groundwater crustaceans (Trontelj et al. 2012, Meleg et al. 2013). Cryptic species may predominantly be young in energy-rich regions with high speciation rate, whereas they may result from morphological stasis or convergent morphological evolution in regions of reduced environmental heterogeneity (Trontelj and Fišer 2009). Clearly, further research is needed to determine whether mechanisms shaping cryptic diversity can compensate each other across space and environmental gradients, thereby yielding the even distribution of cryptic species observed in our study.

Patterns and determinants of median range size

If wide-range MDSs are composed of narrow-range cryptic species (Trontelj et al. 2009, Eme et al. 2013), this would challenge the interpretation of wide MDS ranges as evidence of increased dispersal. Instead, MMs provided additional support for increased dispersal at higher latitudes: the disproportionate and symmetrical fragmentation of the ranges of MDSs at southern latitudes reinforced the pattern of increasing species range size with latitude documented in earlier distribution studies of groundwater crustaceans (Stoch and Galassi 2010, Zagmajster et al. 2014). Also, the asymmetrical fragmentation of the range of widely-distributed MDSs and resulting high variance of range sizes of MM-based species at higher latitudes are consistent with the view that some northern refugia contributed more than others to the postglacial colonization of regions severely affected by the cold Pleistocene climate (Schmitt 2007, Eme et al. 2013). Asymmetrical fragmentation of the range of northern European groundwater species was first observed by Eme et al. (2013), who found that several molecularly defined cryptic species of asellids retained geographic ranges almost as large as that of the corresponding MDSs.

In accordance with our third prediction, MMs provided increasing support for the overriding influence of long-term temperature oscillations in causing a groundwater Rapoport effect. Obligate groundwater species differ from surface species in that they are not exposed to increasing temperature seasonality at higher latitudes (Zagmajster et al. 2014). Consequently, the increase in species range size at higher latitudes should reflect the effect of long-term climatic oscillations, precipitation seasonality and/or habitat area/heterogeneity. The disproportionate importance of temperature anomaly across all sets of SHs and regression models used in this study strongly supports the importance of climate variability at time-scales well beyond seasonal changes in shaping range-size patterns (Leprieur et al. 2011, Zagmajster et al. 2014). Our findings that the present-day distribution of range sizes among groundwater crustaceans has been shaped by long-term temperature oscillations and that the geographic range of many southern species might be much smaller than previously thought also have important conservation implications in the current context of fast-occurring climate changes: if range sizes are negatively correlated with extinction rates (Hugueny et al. 2011), then the southern European groundwater fauna may be facing greater risks of extinction than previously thought.

Conclusion

Confronting different SHs is slowly gaining momentum across biodiversity science as it can substantially improve the testing of eco-evolutionary theories (Isaac et al. 2004, Bickford et al. 2007, Padial et al. 2010, Marske et al. 2013, Vodă et al. 2015). This study is the first to develop a general methodology that makes use of different species delimitation methods in hypothesis testing of large-scale biodiversity patterns. Using MMs instead of morphology not only doubled the number of SHs of groundwater crustaceans but also decreased drastically their average range size. Our finding calls for a more cautious use in macroecology/macroevolution of phylogenies that are often derived from a few specimens which cover only a restricted fraction of the range of morphological species (Schaefer et al. 2011, Etienne and Rosindell 2012, Marske et al. 2013). The prevalence of cryptic species may become even more problematic when such phylogenies are combined with morphological species occurrence databases to document and interpret large-scale patterns of phylogenetic and phylogenetic beta diversities (Eiserhardt et al. 2013). However, we showed that the prevalence of cryptic species strengthened the mid-latitude ridge of high species richness and the Rapoport effect of geographic range size instead of qualitatively affecting these patterns. Shifting from morphology to molecules as a basis for macroecological analysis did not affect the relative contribution of climate/energy, spatial heterogeneity and historical climates to geographic variation in species richness of European groundwater macrocrustaceans, indicating that morphologically cryptic species were homogeneously distributed along environmental gradients. Yet, MMs provided increasing support for the overriding influence of historical climates in causing the Rapoport effect. The multi-criteria species database and comparative analyses developed as part of this study pave the way for integrating the heterogeneous nature of the speciation process into our understanding of large-scale biodiversity patterns and can be easily extended when additional genes and species delineation methods become available for large-scale community datasets. They also provide a general methodology to document whether cryptic speciation variably contributes to species richness and geographic range size across space and environmental gradients for a broader range of taxa and ecosystems.

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References

Ahrens, D. et al. 2016. Rarity and incomplete sampling in DNA-based species delimitation. – Syst. Biol. 65: 478–494.
Araújo, M. B. et al. 2008. Quaternary climate changes explain diversity among reptiles and amphibians. – Ecography 31: 8–15.
Barton, K. 2015. MuMIn: multi-model inference. – R package ver. 1.15.1, <https://cran.r-project.org/web/packages/MuMIn> accessed 5 November 2015.
Beguería, S. and Pueyo, Y. 2009. A comparison of simultaneous autoregressive and generalized least square models for dealing with spatial autocorrelation. – Global Ecol. Biogeogr. 18: 273–279.
Bickford, D. et al. 2007. Cryptic species as a window on diversity and conservation. – Trends Ecol. Evol. 22: 148–155.
Bonn, A. et al. 2004. Structure of the species–energy relationship. – Proc. R. Soc. B 271: 1685–1691.
Bregović, P. and Zagmajster, M. 2016. Understanding hotspots within a global hotspot – identifying the drivers of regional species richness patterns in terrestrial subterranean habitats. – Insect Conserv. Divers. 9: 268–281.
Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. – Springer.
Calvignac, S. et al. 2011. Preventing the pollution of mitochondrial datasets with nuclear mitochondrial paralogs (numts). – Mitochondrion 11: 246–254.
Culver, D. C. et al. 2006. The mid-latitude biodiversity ridge in terrestrial cave fauna. – Ecography 29: 120–128.
Davies, R. G. et al. 2007. Topography, energy and the global distribution of bird species richness. – Proc. R. Soc. B 274: 1189–1197.
Dayrat, B. 2005. Towards integrative taxonomy. – Biol. J. Linn. Soc. 85: 407–415.
de Queiroz, K. 2005a. Ernst Mayr and the modern concept of species. – Proc. Natl Acad. Sci. USA 102: 6600–6607.
de Queiroz, K. 2005b. A unified concept of species and its consequences for the future of taxonomy. – Proc. Calif. Acad. Sci. 56: 195–215.
Dellicour, S. and Flot, J.-F. 2015. Delimiting species-poor datasets using single molecular markers: a study of barcode gaps, haplowebs and GMYC. – Syst. Biol. 64: 900–908.
Dormann, C. F. et al. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. – Ecography 30: 609–628.
Eiserhardt, W. L. et al. 2013. Dispersal and niche evolution jointly shape the geographic turnover of phylogenetic clades across continents. – Sci. Rep. 3: 1164.
Eme, D. et al. 2013. Bayesian phylogeographic inferences reveal contrasting colonization dynamics among European groundwater isopods. – Mol. Ecol. 22: 5685–5699.
Eme, D. et al. 2015. Multi-causality and spatial non-stationarity in the determinants of groundwater crustacean diversity in Europe. – Ecography 38: 531–540.
Eme, D. et al. 2017. Data from: Do cryptic species matter in macroecology? Sequencing European groundwater crustaceans yields smaller ranges but does not challenge biodiversity determinants. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.ns8v4>.
Esselstyn, J. A. et al. 2012. Single-locus species delimitation: a test of the mixed Yule-coalescent model, with an empirical application to Philippine round-leaf bats. – Proc. R. Soc. B 279: 3678–3686.
Etienne, R. S. and Rosindell, J. 2012. Prolonging the past counteracts the pull of the present: protracted speciation can explain observed slowdowns in diversification. – Syst. Biol. 61: 204–213.
Field, R. et al. 2009. Spatial species-richness gradients across scales: a meta-analysis. – J. Biogeogr. 36: 132–147.
Flot, J.-F. 2015. Species delimitation's coming of age. – Syst. Biol. 64: 897–899.
Flot, J.-F. et al. 2010a. Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus *Pocillopora* in Clipperton. – BMC Evol. Biol. 10: 372.
Flot, J.-F. et al. 2010b. Unsuspected diversity of *Niphargus* amphipods in the chemoautotrophic cave ecosystem of Frasassi, central Italy. – BMC Evol. Biol. 10: 171.
Flot, J.-F. et al. 2014. *Niphargus–Thiothrix* associations may be widespread in sulfidic groundwater ecosystems: evidence from southeastern Romania. – Mol. Ecol. 23: 1405–1417.
Fujisawa, T. and Barraclough, T. G. 2013. Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. – Syst. Biol. 62: 707–724.
Galipaud, M. et al. 2014. Ecologists overestimate the importance of predictor variables in model averaging: a plea for cautious interpretations. – Methods Ecol. Evol. 5: 983–991.
Guillot, G. et al. 2012. A unifying model for the analysis of phenotypic, genetic and geographic data. – Syst. Biol. 61: 897–911.
Guindon, S. et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. – Syst. Biol. 59: 307–321.
Hey, J. et al. 2003 Understanding and confronting species uncertainty in biology and conservation. – Trends Ecol. Evol. 18: 597–603.
Hopkins, R. et al. 2014. Strong reinforcing selection in a Texas wildflower. – Curr. Biol. 24: 1995–1999.
Hugueny, B. et al. 2011. Habitat fragmentation and extinction rates within freshwater fish communities: a faunal relaxation approach. – Global Ecol. Biogeogr. 20: 449–463.
Isaac, N. J. B. and Purvis, A. 2004. The 'species problem' and testing macroevolutionary hypotheses. – Divers. Distrib. 10: 275–281.

Isaac, N. J. B. et al. 2004. Taxonomic inflation: its influence on macroecology and conservation. – Trends Ecol. Evol. 19: 464–469.
Jansson, R. and Dynesius, M. 2002. The fate of clades in a world of recurrent climatic change: Milankovitch oscillations and evolution. – Annu. Rev. Ecol. Evol. Syst. 33: 741–777.
Jetz, W. and Rahbek, C. 2002. Geographic range size and determinants of avian species richness. – Science 297: 1548–1551.
Knowles, L. L. and Carstens, B. C. 2007. Delimiting species without monophyletic gene trees. – Syst. Biol. 56: 887–895.
Leaché, A. D. et al. 2014. The influence of gene flow on species tree estimation: a simulation study. – Syst. Biol. 63: 17–30.
Lefébure, T. et al. 2006. Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. – Mol. Phylogenet. Evol. 40: 435–447.
Legendre, P. and Legendre, L. 1998. Numerical ecology, 2nd ed. – Elsevier.
Leprieur, F. et al. 2011. Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. – Ecol. Lett. 14: 325–334.
Losos, J. B. and Mahler, D. L. 2010. Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. – In: Bell, M. A. et al. (eds), Evolution since Darwin: the first 150 years. Sinauer Associates, pp. 381–420.
Marshall, D. C. et al. 2011. Hybridization, mitochondrial DNA phylogeography, and prediction of the early stages of reproductive isolation: lessons from New Zealand cicadas (genus *Kikihia*). – Syst. Biol. 60: 482–502.
Marske, K. A. et al. 2013. Phylogeography: spanning the ecology-evolution continuum. – Ecography 36: 1169–1181.
McCune, A. R. and Lovejoy, N. R. 1998. The relative rate of sympatric and allopatric speciation in fishes: tests using DNA sequence divergence between sister species and among clades. – In: Howard, D. J. and Berlocher, S. H. (eds), Endless forms: species and speciation. Oxford Univ. Press, pp. 172–185.
Meleg, I. N. et al. 2013. Can environment predict cryptic diversity? The case of *Niphargus* inhabiting western Carpathian groundwater. – PLoS One 8: e76760.
Morueta-Holme, N. et al. 2013. Habitat area and climate stability determine geographical variation in plant species range sizes. – Ecol. Lett. 16: 1446–1454.
Morvan, C. et al. 2013. Timetree of Aselloidea reveals species diversification dynamics in groundwater. – Syst. Biol. 62: 512–522.
Ohlemüller, R. et al. 2008. The coincidence of climatic and species rarity: high risk to small-range species from climate change. – Biol. Lett. 4: 568–572.
Oksanen, J. et al. 2015. vegan: community ecology package. – R package ver. 2.3-1, <https://cran.r-project.org/web/packages/vegan/> accessed 5 November 2015.
Padial, J. M. et al. 2010. The integrative future of taxonomy. – Front. Zool. 7: 16.
Pfenninger, M. and Schwenk, K. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. – BMC Evol. Biol. 7: 121.
Pinheiro, J. et al. 2015. Nlme: linear and nonlinear mixed effects models. – R package ver. 3.1-122, <http://cran.r-project.org/web/packages/nlme/index.html> accessed 5 November 2015.
Provan, J. and Bennett, K. D. 2008. Phylogeographic insights into cryptic glacial refugia. – Trends Ecol. Evol. 23: 564–571.
Puillandre, N. et al. 2012. ABGD, Automatic barcode gap discovery for primary species delimitation. – Mol. Ecol. 21: 1864–1877.
Rahbek, C. and Graves, G. R. 2001. Multiscale assessment of patterns of avian species richness. – Proc. Natl Acad. Sci. USA 98: 4534–4539.
Rundle, H. D. and Nosil, P. 2005. Ecological speciation. – Ecol. Lett. 8: 336–352.
Rundle, H. D. et al. 2000. Natural selection and parallel speciation in sympatric sticklebacks. – Science 287: 306–308.
Schaefer, H. et al. 2011. The Linnean shortfall in oceanic island biogeography: a case study in the Azores. – J. Biogeogr. 38: 1345–1355.
Schmitt, T. 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. – Front. Zool. 4: 11.
Stevens, G. C. 1989. The latitudinal gradient in geographical range – how so many species co-exist in the tropics. – Am. Nat. 133: 240–256.
Stewart, J. R. et al. 2010. Refugia revisited: individualistic responses of species in time and space. – Proc. R. Soc. B 277: 661–671.
Stoch, F. and Galassi, D. M. P. 2010. Stygobiotic crustacean species richness: a question of numbers, a matter of scale. – Hydrobiologia 653: 217–234.
Svenning, J.-C. et al. 2009. Plio-Pleistocene climate change and geographic heterogeneity in plant diversity–environment relationships. – Ecography 32: 13–21.
Trontelj, P. and Fišer, C. 2009. Cryptic species diversity should not be trivialised. – Syst. Biodivers. 7: 1–3.
Trontelj, P. et al. 2009. A molecular test for cryptic diversity in ground water: how large are the ranges of macro-stygobionts? – Freshwater Biol. 54: 727–744.
Trontelj, P. et al. 2012. Ecomorphological convergence of cave communities. – Evolution 66: 3852–3865.
Venables, W. N. and Ripley, B. D. 2002. Modern applied statistics with S, 4th ed. – Springer.
Vodă, R. et al. 2015. Cryptic matters: overlooked species generate most butterfly beta-diversity. – Ecography 38: 405–409.
Wagner, C. E. et al. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. – Nature 487: 366–369.
Wood, S. N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. – J. R. Stat. Soc. B 73: 3–36.
Yang, Z. and Rannala, B. 2014. Unguided species delimitation using DNA sequence data from multiple loci. – Mol. Biol. Evol. 31: 3125–3135.
Zagmajster, M. et al. 2014. Geographic variation in range size and beta diversity of groundwater crustaceans: insights from habitats with low thermal seasonality. – Global Ecol. Biogeogr. 23: 1135–1145.
Zhang, J. et al. 2013. A general species delimitation method with applications to phylogenetic placements. – Bioinformatics 29: 2869–2876.
Zuur, A. F. et al. 2009. Mixed effects models and extension in ecology with R. – Springer.

Supplementary material (Appendix ECOG-02683 at <www.ecography.org/appendix/ecog-02683>). Appendix 1–4.