

Climatic context shapes contrasting insect richness responses to elevation across Europe

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Climatic context shapes contrasting insect richness responses to elevation across Europe

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Abstract

Elevational gradients have long been used to study biodiversity responses, yet insect richness–elevation relationships remain difficult to generalize. Here, we used standardized Malaise trap metabarcoding across elevational gradients in seven European countries to examine whether regional differences in insect richness responses reflect the climatic position of each gradient. Across the studied gradients, insect richness showed elevational responses ranging from monotonic declines to increases and unimodal patterns. These regional curves were broadly aligned with a study-wide unimodal climate–richness relationship, indicating that each elevational transect captures a different segment of a broader curve, thereby helping to explain why regional richness–elevation relationships differ in direction and shape. Joint species distribution models revealed that these richness patterns reflected region-specific balances of positive and negative species responses, indicating turnover in insect communities along the elevational gradient. Closely related taxa responded similarly within regions, and their responses were correlated across regions occupying comparable climatic contexts. Our results suggest that the local climatic context and evolutionary history jointly shape insect community assembly along environmental gradients, highlighting that forecasting insect biodiversity under climate change should account for both climatic position of the regional gradients and the phylogenetic structure of taxa.

Introduction

Mountains serve as natural laboratories for examining biodiversity responses to climate change because gradients in temperature and precipitation occur over relatively short distances¹. Elevational gradients therefore offer a robust framework for examining how biodiversity varies in response to changing climatic conditions. This is particularly relevant given that understanding species responses to diverse environmental conditions holds significant implications for conservation².

Research on species richness and community composition along elevational gradients has been one of the central focuses of biogeography since the field's inception³. Across taxa, richness–elevation relationships typically display four main patterns: decreasing richness with increasing elevation, low plateaus with subsequently decreasing richness, low plateaus with mid-elevational peaks, and unimodal patterns with mid-elevational peaks⁴. The richness of small mammals predominantly peaks at mid-elevations, whereas bats exhibit patterns that are divided between decreasing and mid-elevational peaks¹. In contrast, studies on bird and reptile richness in response to elevation have identified all four patterns¹. For plants, unimodal patterns and a decline in richness with increasing elevation have been most frequently reported^{5,6}. Global syntheses of literature on insect richness have revealed an overall pattern of low-elevation plateaus with a subsequent decrease in richness with increasing elevation⁷. However, the richness–elevation relationships from the individual studies included in this meta-analysis demonstrated highly variable patterns. This large variability in insect richness responses to elevation may reflect land-use and biogeographical differences among sampled regions, focus on different taxonomic groups as well as from heterogeneous research methods used in different studies^{1,5,7-9}.

Understanding what explains variation in insect responses to elevation is of fundamental importance, as insects are a critical component of biodiversity and ecosystem functioning, playing important roles in pollination, decomposition, and trophic interactions^{10,11}. However, insect assemblages are undergoing widespread global change, with many studies reporting declines in biomass and species richness¹²⁻¹⁵. To understand how insect diversity is structured by climate, elevational gradients provide a useful framework by allowing insect richness and species responses to be compared across varying climatic conditions. Standardized, multi-country studies across mountain gradients remain scarce, particularly those assessing entire insect communities. This lack of standardized data across studies limits our ability to determine whether contrasting insect richness–elevation relationships reflect idiosyncratic regional patterns or predictable responses to climatic context.

Here, we examine insect community responses to elevation by applying a standardized Malaise-trap sampling protocol across seven European countries over a common season, thereby generating directly comparable data across distinct biogeographic regions with varying climatic contexts. We leveraged a DNA metabarcoding approach to characterize whole insect communities from more than 650 samples and analyzed richness–elevation patterns coupled with joint species distribution modeling. Additionally, we tested whether insect responses to elevation are phylogenetically conserved, asking whether closely related species exhibit similar responses to elevation-associated conditions. We hypothesized that contrasting regional richness–elevation relationships are not idiosyncratic outcomes of individual gradients but are driven by the climatic conditions of each regional gradient within a broader climate–richness relationship. To our knowledge, this is one of the first large-scale, standardized assessments of insect communities across multiple elevational gradients. Our results indicate that climatic context helps explain contrasting insect responses to elevation and suggest that forecasts of insect biodiversity change should account for both the climatic position of regional gradients and the phylogenetic structure of the taxa.

Results

Insect responses to elevation vary among regions

Per-region generalized additive models (GAMs), which also included seasonal terms, revealed that insect richness responses to elevation differed among regions. Species richness declined monotonically with elevation in Poland and Portugal, while Norway and Italy showed significant non-linear richness–elevation relationships (Fig. 1A; Supplementary Table 1). In Germany, Spain and Greece, richness–elevation relationships were non-significant, with a slight negative tendency in Germany, a positive tendency in Spain and an almost flat relationship in Greece. The family-level models showed the same main structure (Fig. 1B; Supplementary Table 1). At least one seasonal term was significant for all regions except for Italy and Greece (Supplementary Table 1, Supplementary Fig. 1). Thus, richness–elevation relationships differed among regions, ranging from monotonic declines to unimodal responses and weak, non-significant relationships, even after accounting for seasonal variation.

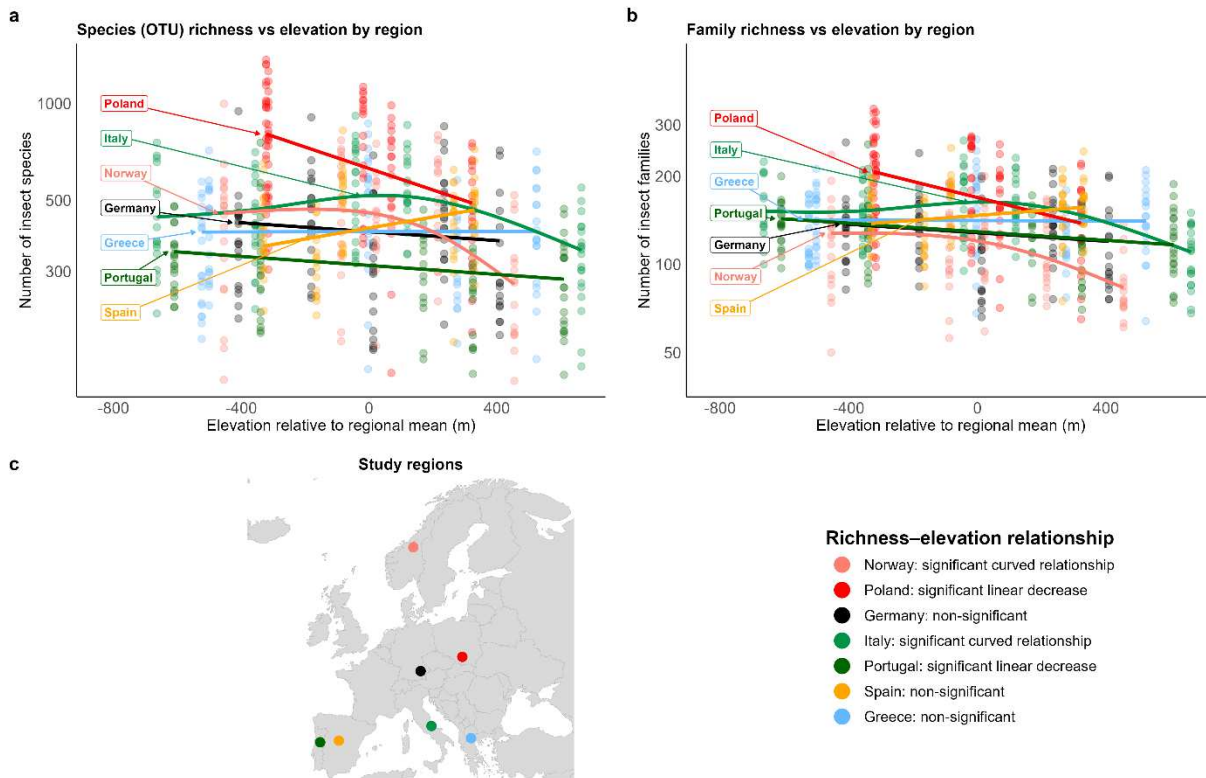


Figure 1. Regional variation in insect richness–elevation relationships across seven European countries. a–b, Relationships between elevation (centered within each region, unit: meter) and insect species richness (A) and family richness (B). Points represent individual samples and lines show fitted relationships from region-specific GAMs. Elevation responses are drawn as curved when the elevation smooth had $\text{edf} > 1.5$ and $P < 0.05$, and as linear otherwise (Supplementary Table 1). c, Map of study regions and summary for species-level richness–elevation relationship inferred for each region.

Results from the joint species distribution models demonstrated that many insect species showed statistically supported responses to elevation (Fig. 2). Across most regions, the mean species response was negative, indicating that occurrence probabilities generally declined with increasing elevation (Fig. 2A). However, positive and negative responses often occurred within the same region (Fig. 2B), revealing species turnover along elevational gradients. The most pronounced negative effects on species were observed in Poland, where 29.5% of the modeled species exhibited a negative response to increasing elevation, while 5.9% showed a positive response (Fig. 2B). Species in Norway, Germany, Italy, and Portugal datasets displayed a similar pattern, but with a slightly lower proportion of species with statistically supported responses to elevation. In contrast, species responses to elevation were mostly positive in Spain, where 21.9% of the modeled species responded positively, while 1.7% negatively. In Greece, species-level responses were weak overall, with only one modelled species showing a statistically supported negative response to increasing elevation. Thus, elevational gradients reshape insect assemblages not only through changes in richness but also through shifts in species identity, with the ratio of elevation-associated gains and losses differing among regions. Consistent with per-region GAMs, the variation partitioning of joint species distribution models showed that seasonality explained a substantial part of variation in species occurrences across all datasets (27.4–57.6%; Supplementary Table 2).

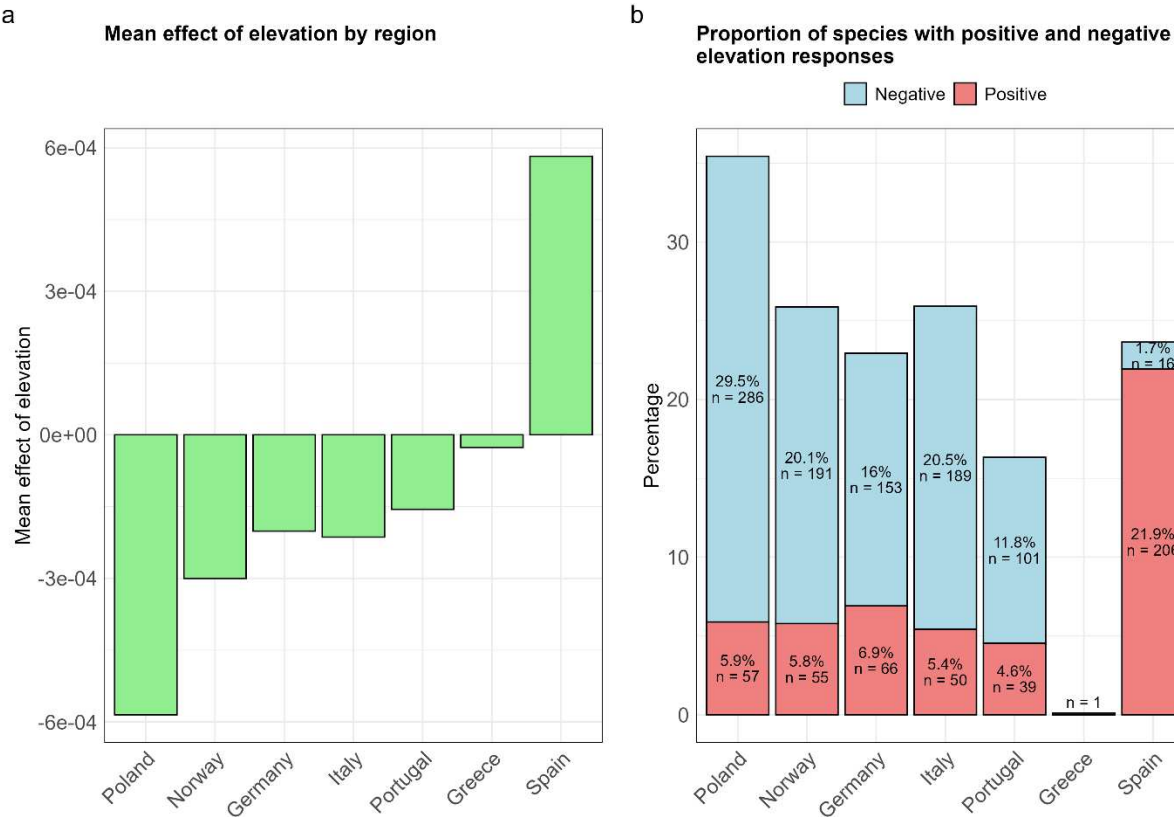


Figure 2. Region-specific insect species responses to elevation. a, Mean effect of elevation on species occurrence estimated from region-specific joint species distribution models, expressed as the change in the linear predictor per meter increase in elevation. Negative values indicate that occurrence probability decreases with increasing elevation, and positive values indicate that occurrence probability increases towards higher elevations. B, Proportions of species with statistically supported (at least 95% posterior probability) negative (blue) and positive (red) responses to increasing elevation. All species' responses to elevation are listed in Supplementary Table 3.

Regional richness–elevation patterns reflect climatic context

As insect richness responses to elevation differed among regions, we tested whether these differences could be explained by the breadth of climatic conditions sampled within each elevational gradient. Climatic breadth was quantified as climate PC1 range, and regional richness–elevation pattern strength as the range of predicted link-scale richness (η) along the elevational curve [$\max(\eta) - \min(\eta)$]. Across the seven regions, climatic breadth was not associated with richness–elevation pattern strength (linear regression: $\beta = 0.098$, $SE = 0.292$, $t_s = 0.335$, $R^2 = 0.022$, $P = 0.751$). Thus, differences among regions in the strength (amplitude) of richness–elevation relationships were not simply explained by some gradients spanning broader climatic ranges than others.

We then examined study-wide and regional insect richness patterns along the sampled climate gradient. Study-wide species richness showed a significant unimodal association with the study-wide climate PC1 axis (quadratic term: $\beta = -0.060$, $SE = 0.019$, $z = -3.18$, $P = 0.002$) where a quadratic climate term improved fit over a linear term (likelihood-ratio test: $\chi^2_1 = 8.94$, $P = 0.003$; AIC 8545 vs. 8551.9). After removing among-region differences in mean log-richness, regional residual-richness trends were broadly consistent with their position along the study-wide unimodal climate–richness curve (Fig. 3). For example, low-elevation sites in Poland extended towards the climatic optimum (according to the study-wide richness–climate relationship), whereas higher-elevation sites extended towards colder and wetter conditions. As increasing elevation shifted communities away from the optimum, the positioning of this gradient was associated with predominantly negative species responses (Fig. 2). In contrast, sites in Spain occupied the warmest and driest segment of the climatic gradient, so that increasing elevation moved communities towards intermediate climatic conditions (Fig. 3). Although the regional richness–elevation relationship was not significant in Spain (Fig. 1A), species-level responses were predominantly positive (Fig. 2), consistent with movement toward more favorable climatic conditions. Residual-richness trends in Italy and Norway were unimodal, indicating that these gradients did not simply move towards or away from the study-wide optimum, but instead showed curved responses within climate space (Fig. 3).

For each region, we compared the observed richness–elevation curve with the climate-based expectation obtained by projecting the regional elevation gradient onto the study-wide climate–richness relationship. Regional responses generally followed the expected pattern of richness change, but the magnitude of change often differed (Supplementary Fig. 2). Here, the magnitude of change was defined as the range of fitted richness (maximum - minimum) along each regional gradient, and amplitude ratios represent the observed magnitude of change relative to the expected magnitude. The observed richness–elevation curves in Poland and Portugal closely matched climate-based expectations in both the pattern of richness change along the elevation gradient (Spearman's $\rho = 1$ for both) and the magnitude of change (amplitude ratios = 1.08 and 1.22, respectively). Spain also showed a high correspondence with the expected pattern (Spearman's $\rho = 1$), but the observed change in richness was larger than predicted from climate alone (amplitude ratio = 2.58). The observed changes were also stronger than expected in Italy and Norway (amplitude ratios = 2.78 and 2.62, respectively). In Italy, the climate-based expectation was only a shallow decline because the gradient was positioned close to the peak of the study-wide climate–richness curve, whereas the observed richness–elevation relationship was unimodal (Fig. 3); consequently, the observed and expected curves showed weak rank agreement (Spearman's $\rho = 0.36$). In Norway, the observed response was also stronger than expected, but the overall pattern remained broadly similar to the climate-based expectation despite significant curvature in the richness–elevation relationship (Spearman's $\rho = 0.84$). Germany and Greece showed high rank agreement between observed and expected curves (Spearman's $\rho = 1$ and 0.94, respectively), but the observed richness change was weaker than expected from climate alone (amplitude ratios = 0.45 and 0.04, respectively). Thus climate (PC1: mean annual temperature and precipitation) captured the broad direction of regional richness–elevation responses but did not fully explain their magnitude or shape (Supplementary Fig. 2). Correspondingly, adding an elevation term to the study-wide quadratic climate GLMM improved the model fit relative to the climate-only model (LRT $\chi^2_1 = 5.02$, $P = 0.025$; AIC 8541.9 vs. 8545), and allowing the elevation effect to vary with climate improved the fit further ($\chi^2_1 = 5.09$, $P = 0.024$; AIC 8538.9).

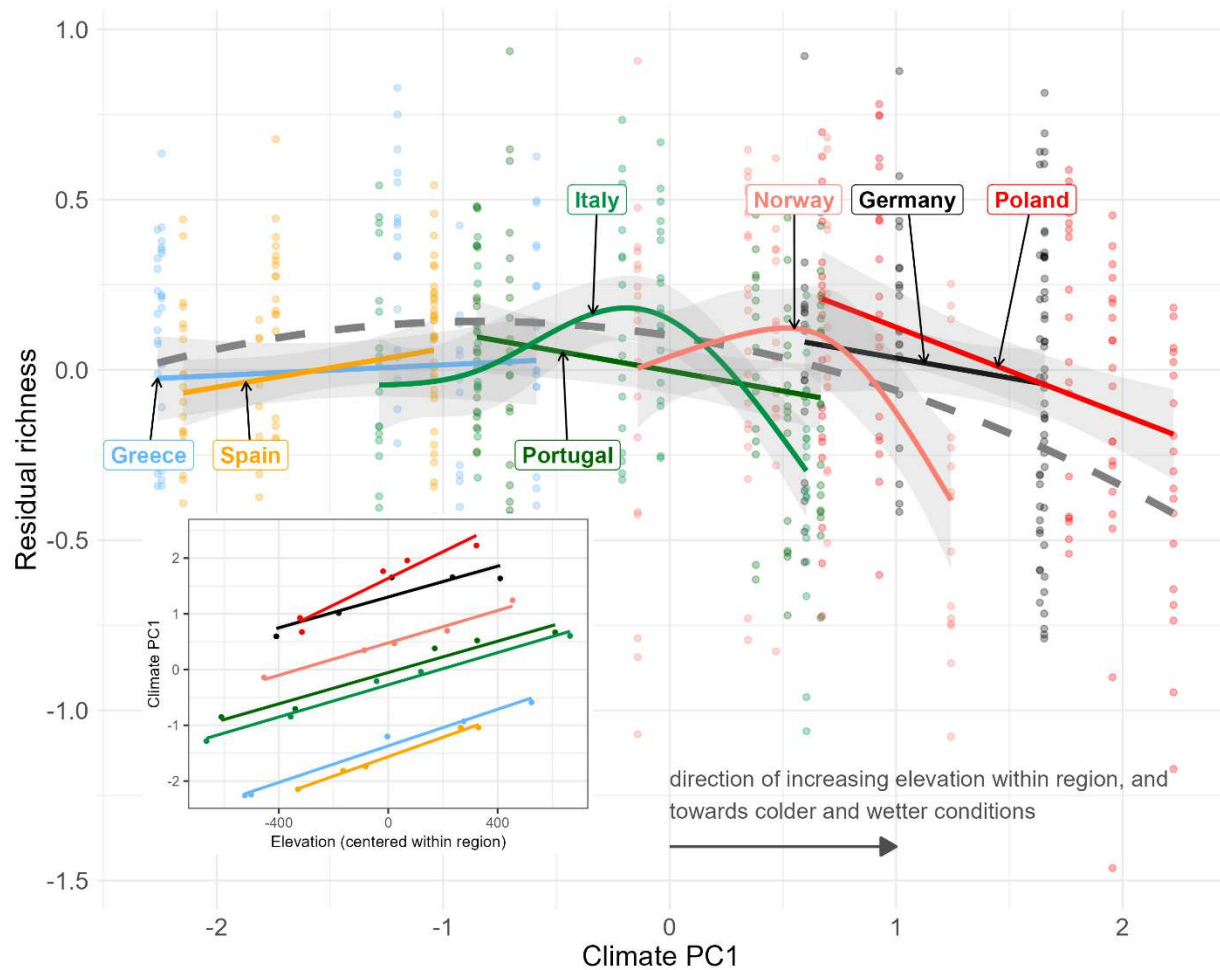


Figure 3. Relationship between residual species richness and climate after accounting for regional variation. The climate PC1 (x-axis) represents the first principal component of the mean annual temperature and mean annual precipitation, with higher values indicating colder and/or wetter conditions. The y-axis represents residual log-richness extracted from a study-wide linear model with log-transformed species richness as the response variable and region as the only fixed effect. Points are sample-level residuals, and solid lines with shaded 95% confidence intervals represent regional trends of these residuals along climate PC1: for regions where the richness–elevation GAM indicated a curved relationship, trends were fitted with a GAM smooth; otherwise, with a linear smooth. The dashed grey curve represents the study-wide climate–richness relationship from a negative binomial GLMM with linear and quadratic climate PC1 terms and random intercepts for region and Malaise trap. The inset figure (bottom left) represents the relationship between climate PC1 and elevation within each region, where regional linear fits illustrate how elevation covaries with climate along each regional gradient.

Phylogenetic structure and cross-regional consistency in species responses

Next, we examined whether the species contributing to the observed responses were phylogenetically structured. Species responses to elevation (i.e., conditions associated with elevation) showed significant phylogenetic structure in all regions, with the exception of Greece. Phylogenetic GLS models outperformed

null models in Norway, Poland, Germany, Italy, Spain and Portugal ($P < 0.001$), indicating that closely related insect taxa tended to show more similar responses than expected by chance. The strength of this phylogenetic signal varied among regions, being highest in Italy ($\lambda = 0.69$), followed by Portugal ($\lambda = 0.44$), Germany ($\lambda = 0.39$), Poland ($\lambda = 0.31$), Norway ($\lambda = 0.28$), and Spain ($\lambda = 0.24$).

After accounting for phylogenetic relatedness, posterior mean elevation response estimates of shared or closely related taxa were positively correlated among several region pairs, indicating a degree of consistency in the direction of insect elevational responses across regions (Fig. 4). Among statistically supported correlations, the strongest positive correlations were generally observed between regions occupying broadly similar parts of climatic space, suggesting that taxa decreasing or increasing with altitude in one region often show similar directional responses under comparable climatic contexts. However, a weak but statistically supported positive correlation was also detected between Greece and Poland (Fig. 4). The correlations in this analysis included posterior mean elevation-response estimates for all modelled taxa, not only responses with strong posterior support as shown in Fig. 2. In Greece, posterior mean responses were nearly balanced, with 429 positive and 532 negative estimates, but only one was statistically supported (Supplementary Table 3). Thus, the Greece–Poland correlation reflects subtle similarity in the relative ordering of many weak response estimates across shared or closely related taxa, rather than a strong shared community-wide negative response comparable to Poland. The only statistically supported negative correlation occurred between Poland and Spain, indicating weak but significant opposition in the responses of shared or related taxa between these two gradients (Fig. 4). This contrast is consistent with species-level response patterns, where elevation was associated predominantly with negative responses in Poland but predominantly with positive responses in Spain (Fig. 2), reflecting their different positions within the broader climate–richness curve (Fig. 3).

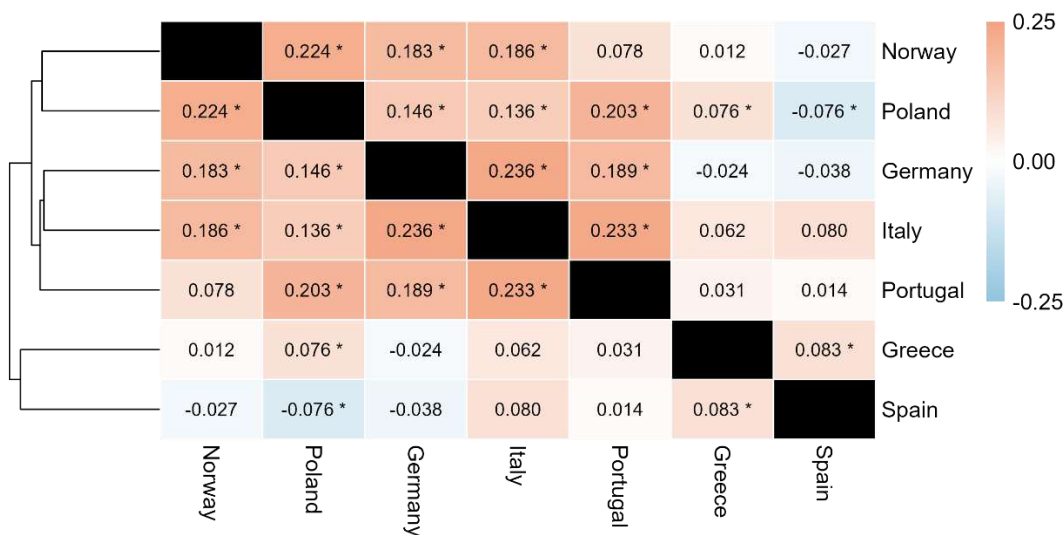


Figure 4. Cross-regional consistency in insect responses to elevation. The heatmap shows pairwise correlations in the elevation responses (HMSC elevation-response coefficients) of shared or closely related insect taxa across regions (see methods). Cell values are weighted Pearson correlations; warm colors indicate concordant elevational responses, and cool colors indicate opposite responses. Asterisks (*) mark correlations stronger (positive) or more opposing (negative) than expected under a phylogenetic null ($P \leq 0.05$ and $P \geq 0.95$, respectively; Supplementary Table 4). Rows and columns are ordered by average-linkage hierarchical clustering of the correlation matrix.

Discussion

Using standardized sampling across elevational gradients in seven European countries, we show that insect richness does not respond to elevation in a uniform way. We found that across the studied elevational gradients, insect richness declined steeply or weakly, tended to increase towards higher elevations, or showed unimodal responses. Such variation has often made insect richness–elevation relationships appear inconsistent, obscuring general inferences about how insect diversity responds to environmental gradients. Here, we show that these apparent inconsistencies correspond generally to distinct climatic contexts in which each regional gradient is embedded. Regional gradients represented different segments of a broader unimodal climate–richness relationship, generating predominantly negative or positive richness–elevation responses depending on their position in climate space. However, additional elevation-associated conditions can shape regional deviations from the broader climate-based expectation. Furthermore, we found that insect species responses were phylogenetically structured and demonstrated correlated responses across climatically similar regions. These results suggest that climatic context and evolutionary history jointly shape insect community assembly along environmental gradients, highlighting that forecasting insect biodiversity under climate change should account for both the climatic position of the regional gradients and the phylogenetic structure of the taxa.

The richness–elevation patterns observed here help to explain why previous studies have reported inconsistent elevational diversity patterns for insects^{7,16,17}. A recent global synthesis highlighted substantial variation among individual insect richness–elevation relationships, but showed that overall proportional richness was best explained by mean annual temperature and temperature seasonality, each explaining more than half of the variation in proportional richness in separate models⁷. Our results align with this climate-centered interpretation but add a regional perspective: richness–elevation relationships are context-dependent, reflecting the position of each gradient within broader climatic space. Seasonality also contributed to variation in richness and species occurrence in our models, indicating that insect assemblages are also shaped by temporal variation in their activity. This is consistent with previous studies showing seasonal changes in insect richness along elevational gradients^{18–20}. Importantly, because all regions were sampled during a common season and seasonal effects were included in the models, the contrasting regional richness–elevation relationships observed here cannot be attributed to seasonal sampling differences. Placing regional richness–elevation patterns within a climatic context revealed that regional gradients generally represent ‘pieces of a curve’, where each transect captures only part of a broader unimodal climate–richness relationship (Fig. 3).

The idea that local climatic conditions can shape species responses to elevation has previously been suggested for butterflies. Fleishman et al.²¹ found opposite richness–elevation patterns in two adjacent mountain ranges and suggested that gradient-specific climatic context may underlie these contrasting patterns. A related climate-based explanation for elevational richness patterns has been more explicitly tested in bats, where richness was found to be highest in an optimal combination of temperature and water availability²². Beyond elevational-gradient studies, Boucher-Lalonde et al.²³ showed that temperature and precipitation explained much of the variation in bird and mammal richness across the Americas. Our results are consistent with this broader view of climate as a major correlate of richness patterns but also show how such climate dependence is expressed within regional elevational gradients.

If insect responses to elevation are largely governed by a region’s position along a climate–richness curve, then climate warming is unlikely to have uniform effects across mountain systems. In regions positioned on the colder/wetter side on the climate axis, warming may shift communities toward more favorable

conditions, potentially weakening the negative richness–elevation gradients. In contrast, in regions positioned on the warm/dry side, warming may shift especially low-elevation sites further away from the favorable climatic conditions, which could steepen the richness–elevation relationships. Therefore, predicting insect biodiversity change requires understanding how each region’s climate is shifted relative to the optimal climatic conditions. The changes in biodiversity patterns also depend on how warming alters precipitation, seasonality, microclimate, and biotic interactions¹⁷, as well as on habitat continuity and land use^{9,24–26}. Indeed, although climate provided the predominant framework for interpreting regional richness–elevation relationships, it did not fully account for elevation-associated variation. Adding elevation to the study-wide climate model slightly improved the model fit, indicating that elevation retains explanatory information beyond the combined mean annual temperature and precipitation gradient. Correspondingly, in several cases the observed regional richness–elevation curves demonstrated deviations from the climate-based expectations: the elevational richness direction often followed climate, but the magnitude of change was stronger or weaker than predicted from climate alone (Supplementary Fig. 2). These residual elevation effects likely reflect finer-scale environmental variation that co-varies with elevation, such as habitat structure, vegetation, resource availability, and microclimate, that is not captured by macroclimate alone. For example, the occurrence of herbivorous insects depends not only on the ambient climate but also on the distribution and phenology of their host plants, which are shaped by local microclimate, soil conditions and vegetation structure²⁷. Thus, our results suggest that macroclimate sets the broad direction of regional insect richness responses, whereas fine-scale environmental variation may shape local deviations from this climate-based expectation.

Beyond richness, species-level responses revealed substantial turnover along elevational gradients. This indicates that elevation-associated environmental change shapes not only how many species occur, but also which species occur. This is consistent with the broader view that elevational gradients alter community composition as species are filtered and replaced along environmental gradients^{1,3,27}. In our study, the species responses exhibited a phylogenetic structure, indicating that closely related insect taxa tend to demonstrate similar responses. Corresponding to this, it has been demonstrated that insect responses to climate and land-use change are mediated by species traits⁹. Since closely related taxa may share traits linked to climatic tolerance, phenology or habitat use, they may respond similarly to elevation-associated conditions. Moreover, the response correlations among regions indicated that shared or closely related taxa tended to show consistent responses when gradients occupied comparable climatic space, whereas opposing responses emerged between gradients positioned in contrasting climatic contexts (Fig. 4). This suggests that evolutionary history contributes to the reorganization of insect assemblages across elevational gradients, whereas the direction of species responses depends on the location of each gradient in climate space.

In conclusion, our study shows that contrasting insect richness–elevation relationships and species-level responses across studied regions in Europe represent context-dependent expressions of a broader climate–richness relationship. A region’s position in climate space provides a first-order expectation for the direction of insect responses, whereas local environmental constraints additionally determine the realization of these responses. The phylogenetic structure of species-level responses further indicates that evolutionary history contributes to the reorganization of insect assemblages along the elevational gradients. Predicting insect biodiversity responses to climate change therefore requires understanding how climate change moves regional assemblages relative to the climatic optimum, and which lineages are most likely to respond along those shifting gradients.

Methods

Sampling

Sampling was conducted following the standardized protocol within the Biodiversity Genomics Europe project for Malaise-trap sampling²⁸, hosted publicly in WorkflowHub²⁹. In each region, sampling was conducted at five sites along the elevational gradient. At each site, two Townes-style Malaise traps were installed. One trap was placed for metabarcoding, and the second trap served as a backup and as a source of material for potential morphological examination or DNA barcoding of individual specimens. Specimens were collected into 96% ethanol. Malaise trap samples were collected weekly at each site (that is, after each 7 days; average of 7.3 ± 1.2 SD days) for 19-22 weeks from April to November 2023 (depending on region; Supplementary Table 5). The number of samples per region was 96, 92, 100, 89, 106, 103 and 96 for Germany, Greece, Italy, Norway, Poland, Portugal and Spain, respectively.

DNA extraction and amplicon library preparation

Bulk arthropod samples were processed using the Biodiversity Genomics Europe laboratory workflow for invertebrate characterization from Malaise-trap samples³⁰. DNA extraction protocol was based on the FAVIS non-destructive metabarcoding protocol³¹ with project-specific modifications detailed in the protocol file³⁰. Before DNA extraction, ethanol was decanted and samples were processed in batches of 22, together with one lysis blank as a negative control. Lysis was performed directly in the original collection bottles to minimize specimen handling. Lysis buffer and bottles containing specimens were pre-heated separately to 56°C, after which buffer and proteinase K were added to the samples. Samples were then incubated at 56°C in a shaking dry incubator. A lysate aliquot (1.8 ml) was transferred to 2 ml tubes and stored at -20°C as a working stock for DNA purification. DNA was purified from 200 µl of lysate using the Omega Mag-Bind Blood and Tissue DNA HDQ 96 Kit on a KingFisher Flex 96 automated extraction platform. DNA was eluted in a 100 µl elution buffer. Extraction plates included negative controls to monitor potential contamination.

Amplicon libraries were prepared using a two-step PCR strategy. In the first PCR, we amplified a 418 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene using the BF3-BR2 primer pair³² modified with Illumina-compatible overhangs. Resulting PCR products were purified and indexed with a second round of PCR for sequencing using Illumina NovaSeq 6000 platform (2 × 250 bp). Details of the PCR procedures are outlined in an open-source protocol hosted in WorkflowHub³⁰ and ReadTheDocs³³.

Bioinformatics

Raw sequences per sample were processed as follows: primers were removed with cutadapt v4.9³⁴ by allowing a maximum of 2 mismatches in the primer search and a minimum overlap of 16 bp. The corresponding R1 and R2 sequences were discarded if both read pairs did not contain a primer sequence. Quality filtering (maxEE = 2), denoising (errorEstimationFunction=loessErrfun), merging of paired-end reads (default settings) and chimera filtering (method = “consensus”) were performed using DADA2 v1.30³⁵. Potential tag-jumps per sequencing run were removed using UNCROSS³⁶ with $f = 0.03$ (expected tag-jumps rate), as implemented in PipeCraft³⁷. Followingly, the ASV tables from multiple sequencing runs were merged using the DADA2 *mergeSequenceTables* function, and ASVs with no internal mismatches were collapsed using the *collapseNoMismatch* function. Taxonomy annotation of ASVs was performed using SINTAX³⁸, as implemented in vsearch v2.28.1³⁹. Sequences in COIClassifier v5.1.0⁴⁰ supplemented with COI reads from newly barcoded species by the Biodiversity Genomics Europe (BGE)

consortium (accession numbers are provided in Supplementary Table 5) served as a reference database for SINTAX. ASVs with a bootstrap value of at least 0.8 to kingdom Metazoa were subjected to metaMATE v0.4.3⁴¹ to filter out potential nuclear mitochondrial pseudogenes (NUMTs). ASVs that did not pass the invertebrate mitochondrial code (--table 5) check and were shorter or longer than 418±2 bp were discarded. Additional sequence abundance-based filtering of metaMATE included a clade-based strategy for which the ASVs were clustered with a 90% similarity threshold using VSEARCH (--cluster_fast) to obtain abundance information per 'clade' (herein cluster). Based on metaMATE-find results, the ASV table was filtered with metaMATE-dump to remove >95% of the estimated verified non-authentic ASVs (vnaASVs). The reference database for metaMATE was the same as for taxonomy annotation.

In this study, the analyses were performed at the operational taxonomic unit (OTU) level. ASVs were clustered using the OptimOTU R package⁴² (version 0.9.3.9000). This operation consisted of two phases: optimizing cluster thresholds based on the taxonomically annotated reference database (as above) and clustering the ASVs using their taxonomic identifications to determine the optimal cluster thresholds. Reference sequences were dereplicated and curated in several rounds to facilitate the threshold optimization step. In the initial dereplication round, all but one of each set of fully redundant reference sequences – those which were identical in both sequence and taxonomic annotation – were removed. In the second dereplication round, remaining sequences that were identical in taxonomic annotation and only partially overlapped, but were identical in the overlapping region, were merged. This was performed using the --cluster_fast command in VSEARCH, with a 100% identity cutoff. The VSEARCH dereplication was performed separately for each taxonomic annotation present in the reference database. The resulting dereplicated sequences were then aligned to the nucleotide Hidden Markov Model (HMM) of the Folmer barcoding region presented in Roslin, et al.⁴³, using the "hmmalign" command in HMMER v3.4⁴⁴. Insertions relative to the HMM were removed, and positions 241-658, corresponding to the BF3-BR2 amplicon, were extracted. Reference sequences with more than 50 gaps in this region were removed. After alignment and trimming, any additional fully redundant sequences – i.e., those which were identical after trimming to the BF3-BR2 region – were again dereplicated. The taxonomic annotations were then processed by the OptimOTU clean_taxonomy function, which identifies common indicators of 'missing' taxa, such as those including terms linked to uncertainty in identification ('sp.', 'aff.', or 'unknown'), as well as those linked to uncertainty in the taxonomic classification itself, such as 'incertae sedis'. Although these terms may be useful for taxonomic identification, they are excluded for cluster threshold optimization because they cannot be assumed to have intra-group sequence variation similar to other taxa at the same rank. Thresholds were then optimized for the curated references using the OptimOTU optimize_thresholds function, which calculates clustering thresholds for each taxonomic group in the reference that lead to clusters that closely approximate the actual 'children' of that taxonomic group. Distances were calculated using the Hamming distance on the aligned reference sequences. The ASV sequences were then aligned to the same HMM and trimmed as above. The resulting sequences were clustered using the OptimOTU optimotu function, which first groups sequences by known taxonomy, in this case, the SINTAX identifications with 0.9 bootstrap support, and then clusters them within each taxon based on the optimized thresholds calculated in the first step. This results in clusters at each taxonomic rank in the reference – in this case, domain, kingdom, phylum, class, order, family, genus, and species. Clusters based on taxa that were present in the database are given the taxon name from the database, but clusters based on sequences that were all unidentified at a given rank are given a 'pseudotaxon' identifier, such as 'pseudogenus_00625' for an approximately genus-level cluster. The clusters at the species level, i.e., named species and pseudospecies, were used as OTUs for further analysis.

In addition to length and stop codon filtering, metaMATE implements sequence abundance-based filtering. However, ASVs that were excluded solely because of low abundance were incorporated into the OTU

clustering. This decision was based on the observation that many low-abundance ASVs exhibit high classification confidence against the utilized database. Therefore, a different sequence abundance-based filtering (SAM) was applied to OTUs; calculated as follows:

$$SAM = \max\left(0, \min\left(\frac{SA_{OTU} - avgSA_{off}}{avgSA_{pass} - avgSA_{off}}, 1\right)\right)$$

where SA_{OTU} denotes the sequence abundance (number of sequences) of an OTU; $avgSA_{pass}$ denotes the average number of sequences for OTUs whose representative sequences belong to ASVs that passed through full metaMATE filter (herein it was 10,695 sequences); $avgSA_{off}$ denotes the average number of sequences for OTUs that are found in negative control samples, represent non-land taxa and Chordata (off-targets; herein it was 302 sequences). OTUs that passed the full metaMATE filtering received SAM of 1 and off-target OTUs 0. For the remaining OTUs, SAM was calculated as above, and those with a SAM value of 0 were discarded.

Additional data filtering

We assessed sequencing completeness per sample using rarefaction curves (*rarecurve* function with `step = 5000` in the *vegan* v2.6-10 package⁴⁵ in R v4.4.3⁴⁶) and excluded samples that did not reach asymptote. Samples with a rarefaction curve slope of > 0.001 between the last two points were considered not to have reached an asymptote and were excluded. Samples with insufficient sequencing depth were removed, retaining 663 samples for analysis (mean sequencing depth: 628,882 sequences per sample; Supplementary Table 6, Supplementary Fig. 3). Subsequent analyses focused on OTUs assigned to the class Insecta (Supplementary Table 7), which represented 95.2% (15,854) of the total number of OTUs.

Because we applied species-level clustering thresholds that optimize OTUs to represent the closest species proxies achievable with sequence-based methods, OTUs are referred to as species throughout the results and discussion sections for simplicity.

Study-wide richness models

We fitted three study-wide (including all samples) negative-binomial generalized linear mixed models (GLMMs) with species richness (number of OTUs per sample) as the response: (i) an elevation-only model with elevation in units of meters, centered by region; (ii) a model including elevation and a quadratic climate term (climate PC1 + climate PC1²); and (iii) an interaction model including elevation $\times$ climate PC1 + the quadratic climate term. All models included region and Malaise trap per elevation point as random effects. Climate PC1 represents the first principal component of the mean annual temperature (MAT) and precipitation (MAP) data (obtained from the CHELSA database⁴⁷). Models were compared using likelihood-ratio tests (LRT) and Akaike information criterion (AIC) to determine whether adding climate terms (ii) improved the elevation-only model (i) and whether an elevation $\times$ climate interaction (iii) further improved fit relative to the climate + elevation model (ii). Models were fitted using *glmmTMB*⁴⁸ v1.1.11 in R.

Per-region richness–elevation models

Per-region generalized additive models (GAMs) were fitted separately with species richness (number of OTUs per sample) and family richness (number of families per sample). MAT and MAP were significantly correlated with elevation in each region (Spearman $R = -0.75$ to -1 , and $R = 0.66$ to 1 , respectively, $P < 0.001$; Supplementary Fig. 4); thus, we included only elevation in the per-region models. All per-region models included elevation (centered by region) and seasonality as predictor variables and Malaise trap per

elevation as a random effect. Elevation was represented by a smooth term, $s(\text{elevation_centered}, k = 4)$, allowing flexible linear or curved regional trends. Effective degrees of freedom (edf) of the elevation smooth were used to classify relationships as approximately linear ($\text{edf} \approx 1$) or curved ($\text{edf} > 1.5$). Seasonality terms were modeled as periodic functions to capture annual $[\cos(2\pi t/365), \sin(2\pi t/365)]$ and biannual $[\cos(4\pi t/365), \sin(4\pi t/365)]$ cycles, where t is the Julian day. Models were fitted using mgcv⁴⁹ v1.9.1 (function gam(); family nb()) in R.

Climate range test

To test whether the sampled climatic range within a region explained the magnitude of richness variation along elevational gradients, we quantified climatic breadth for each region as the range of the first principal component of MAT and MAP across sampling sites (climate PC1 range = maximum – minimum). Using the same per-region GAMs of species richness as above, we summarized the amplitude of the fitted richness–elevation curve in each region. From each fitted model, we predicted richness on the link scale (η) at 80 evenly spaced elevations between the lowest and highest sampled in that region, using the fitted elevational pattern. At each of these elevations, predicted η was averaged over the Julian days present in the regional data. We then calculated the fitted richness amplitude as the difference between the highest and lowest predicted values along the curve $[\max(\eta) - \min(\eta)]$. These regional amplitude values were related to climate PC1 range using linear regression across regions, asking whether regions spanning broader climatic ranges showed larger changes in richness along elevation.

Climate context of regional richness–elevation patterns

To visualize regional species richness patterns along study-wide climate axis (first principal component axis of MAT and MAP) while accounting for region-level variation, we extracted residual richness from study-wide linear model with log-transformed species richness as the response variable and region as the only fixed effect. The study-wide climate–richness relationship (dashed curve in Fig. 3) was fitted from a study-wide negative binomial GLMM with a quadratic climate term. This model was selected over a model with only linear climate term after comparing the two GLMMs using likelihood-ratio test. For the regional trends along the climate axis, we fitted a GAM smooth for regions whose richness–elevation relationship was curved according to per-region GAMs, and a linear regression otherwise.

To test whether regional richness–elevation patterns are consistent with a study-wide climate–richness relationship, we compared, for each region, the observed species richness pattern with the profile expected if elevation acted only through climate (PC1). Observed profiles (η_{obs}) were the same link-scale GAM predictions of species richness along the 80-point elevational sequence described above in the climate range test section. For the climate-expected profile (η_{exp}) for each region, we first modelled the relationship between climate PC1 and elevation (centered) using a linear model. From this regression we predicted climate PC1 at the same 80 elevations, i.e. the climate expected at each elevation given the observed climate–elevation relationship in that region. These predicted climate PC1 values were entered into the already fitted study-wide quadratic climate–richness GLMM (above, in this section) using the *predict()* function on the log (link) scale, with random effects omitted (`type = "link", re.form = NA`). Per-region climate-expected richness (η_{exp}) at each elevation was therefore the study-wide climate–richness curve evaluated at the climate PC1 predicted for that elevation. Both profiles were obtained on the link (log) scale and mean-centered so that the comparison concerned shape, not baseline richness. We summarized shape agreement with the Spearman correlation of the centered curves (shape r), and the relative magnitude of elevational change with the amplitude ratio $[\max(\eta_{\text{obs}}) - \min(\eta_{\text{obs}})] / [\max(\eta_{\text{exp}}) - \min(\eta_{\text{exp}})]$. High shape r indicates that the two curves rise and fall together along the transect; an amplitude ratio of 1 indicates that they span the same range of fitted link-scale richness.

Joint species distribution models

To analyze species responses to elevation within a region, we applied the Hierarchical Modelling of Species Communities (HMSC) framework^{50,51} of joint species distribution modelling. As the response variable, we used the presence-absences (0/1) of the OTUs (species proxy) in each sample. Predictor variables were sequencing depth (log-transformed number of sequences per sample), seasonality (as above), sampling duration (log-transformed number of days a sample was in the field; average of 7.3 ± 1.2 days), and elevation (centered around the region's mean elevation). Sequencing depth and sampling duration were included as covariates to control for the methodological effects. Random effects were specified for both sample and Malaise trap levels.

Prior to HMSC model fitting, we filtered the data using a prevalence threshold to exclude both rare and ubiquitous species, and to reduce computational intensity, retaining a maximum of 1,000 species per region. The prevalence-absence threshold ranged from 10-18% depending on the dataset, meaning species were retained when present in $\geq \text{threshold}\%$ and absent in $\geq \text{threshold}\%$ of samples. We used thresholding symmetrically for both presences and absences because the lack of either leads similarly to limited variation in the model. Models were fitted separately for each region to allow for region-specific responses using a probit distribution for presence-absence data. We assumed the default prior distribution of Hmsc⁵⁰. The posterior distributions were sampled using five Markov chain Monte Carlo (MCMC) chains with 375,000 iterations for each chain (125,000 burn-in + 250,000 sampling iterations, thinned by 1000 to yield 250 samples per chain). Models were fitted using Hmsc-HPC⁵², which serves as a high-performance computing (HPC) enhancement of the R package Hmsc⁵³. We assessed the convergence of MCMC by analyzing the potential scale reduction factors⁵⁴ for the model parameters (Supplementary Table 8).

Phylogenetic structure of species-level elevation responses

To test whether species responses to elevation exhibited a phylogenetic signal, we estimated Pagel's λ parameter⁵⁵ for the species-level elevation coefficients (posterior mean beta parameters) derived from the HMSC models. Pagel's λ estimates the strength of a phylogenetic signal, where λ of 0 indicates no signal (responses are phylogenetically independent) and λ of 1 indicates a perfect phylogenetically structured signal (closely related species respond similarly). Generalized least squares (GLS) models were fitted with a Pagel correlation (phylogenetic model) in R. We approximated phylogeny with a taxonomic tree, which was constructed from nested taxonomic ranks of OTUs (phylum, class, order, family, genus, species; Supplementary Table 7) using *as.phylo* function in ape v5.8.1 package⁵⁶ in R, with equal branch lengths assigned to each taxonomic rank. AIC values of the phylogenetic models were compared with those of the null models (without Pagel correlation) to assess whether phylogenetic relationships improve the model fit. Phylogenetic models were compared against the null models with a likelihood ratio test using the *lrttest* function in lmttest v0.9.40 R package⁵⁷ to test the statistical significance of a phylogenetic signal.

Next, we tested whether the same or related species show similar responses to elevation in different regions. Because regions differed in which species were detected and thus included into the HMSC models, the missing responses were imputed using elevation response estimates from taxonomically related species. This imputation was justified as the region-specific phylogenetic signal analyses (Pagel's λ) showed taxonomically conserved responses to elevation (related species tended to have more similar responses than non-related ones). For a species that was not included in the analysis for a specific region, the missing response was imputed as the average response among those species that belonged to the same genus (level 2 imputation). If no such species were available, family (level 3), order (level 4), class (level 5), or phylum (level 6) level response was used, whichever was first available. Then, the correlation in elevation responses

between two region sets (ρ_{C_1, C_2} , where C1 and C2 denote the regions) were computed as a weighted Pearson correlation between the posterior mean estimates (elevation responses from the HMSC models) for each species. To account for the increased uncertainty of the estimate for imputed data, less weight was given to species where imputation was used, and especially so, if data on close relatives were not available. One over the product of the two imputation levels was used as a weight. For example, weight was 1 if the species was included in the models of both regions, and it was $1/(2 \times 3) = 1/6$ if genus-level imputation was used in one region and family-level imputation was used in another region. A phylogenetic null model approach was applied to test whether the observed correlation was statistically significant. The motivation for this is that the species-specific responses are not independent of each other, as related species tended to have more similar responses, as tested with the Pagel's λ approach. To account for this, random (null) responses were generated from a multivariate normal distribution with mean zero and covariance-variance matrix $\lambda C + (1 - \lambda)I$, where λ is Pagel's λ estimated for the region in question, C is the species-by-species taxonomic correlation matrix, and I is the identity matrix. The C matrix was compiled with the *vcv.phylo* function of the ape R-package, assuming equal branch lengths between taxonomic levels. The null distribution of ρ_{C_1, C_2} was computed by computing the weighted correlation for the null data (imputed identically as the original data) using 999 simulations, and significance was evaluated by examining the proportion of the null distribution for which ρ_{C_1, C_2} was greater than or equal to the observed value.

All figures were generated using ggplot2 v4.0.0⁵⁸.

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Data availability

The raw sequencing data were deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under the accession number PRJEB108401.

Supplementary Files

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