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Màster en Ecologia, Gestió i Restauració del Medi Natural

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Abstract

Community assembly processes are difficult to observe in the field, hence the considerable need to infer processes from observed patterns of species distributions, traits distributions and phylogenies. Patterns observed in nature may be consistent with multiple explanations, such as habitat filtering, competitive exclusion and biogeographical processes that act differently in distinct spatial and organizational scales. To elucidate the role that historical and current factors have been playing in establishing patterns of diversity, we assessed how species, functional traits and phylogenetic composition of caddisflies (Trichoptera, Insecta) changed among 180 communities in 18 ecoregions (α- and β-diversity) and among those ecoregions (γ- and β-diversity) across a large latitudinal gradient from the Mediterranean Peninsulas to northern Scandinavia. Results showed that regional taxonomic and phylogenetic diversities decrease as latitude increases, whereas functional diversity displays a unimodal relationship with latitude. Northern species pools were found to be phylogenetically clustered while southern overdispersed, but this was not reflected in their phenotypic structure, as functional niche was only partially conserved. Patterns of regional taxonomic and phylogenetic β-diversity partitioned into turnover and nestedness, revealed that the northern species pools are subsets of species pools in central but not in southern Europe. This is consistent with the hypothesis that northern Europe was recolonized by species of southern regions after the Pleistocene glaciations, but contradicts the widely accepted idea that recolonization initiated from Mediterranean refugia. At the local scale, the patterns detected suggested similar local carrying capacity across Europe and the dependence of community assembly mechanisms on stochastic or local abiotic factors that were not considered in this study. Overall, the combined use of decoupled taxonomic, phylogenetic and functional diversity highlights that Pleistocene refugia in Central Europe are critical for explaining current latitudinal patterns as well as the importance of environmental filtering in limiting the distribution range of species across Europe.

Resum

Donada la complexitat d'observar, *in situ,* aquells processos que intervenen en l'establiment de comunitats biològiques, resulta imprescindible investigar els patrons de distribució d'espècies, de trets funcionals i de filogènies. Els patrons observats en la natura poden estar explicats per diversos factors —filtratge ambiental, exclusió competitiva i processos biogeogràfics. Per esbrinar el paper d'aquest factors, tant històrics com actuals, en la configuració dels patrons, hem avaluat la variació de la diversitat específica, funcional i filogenètica de tricòpters (Trichoptera, Insecta) entre 180 comunitats de 18 eco-regions (diversitat α i β) i, entre aquestes eco-regions (diversitat γ i β), al llarg d'un gradient latitudinal (de les Penínsules del Mediterrani fins al nord d'Escandinàvia). Per una banda, a escala regional, podem determinar que la diversitat taxonòmica i filogenètica disminueixen en incrementar la latitud, mentre que la diversitat funcional hi presenta una relació unimodal. Les espècies de latituds majors presenten agregació filogenètica, mentre que al sud del continent presenten més dispersió. Els patrons de beta diversitat taxonòmica i filogenètica, revelen que les espècies situades al nord provenen d'espècies del centre del continent, coincidint amb la hipòtesis que el nord d'Europa es va recolonitzar des de menors latituds, després de les Glaciacions del Pleistocè. Però es contradiu amb la idea que la recolonització es va iniciar en el Mediterrània. Per altra banda, a escala local, no hem detectat patrons, suggerint que l'establiment de les comunitats té lloc per factors estocàstics o abiòtics, no considerats en el següent treball. Així doncs, l'anàlisi combinat de la diversitat taxonòmica, filogenètica i funcional en permet destacar el paper tant de las refugis centre-europeus, durant les Glaciacions del Pleistocè, com la principal explicació dels actuals patrons latitudinals, com del filtratge ambiental alhora de determinar la distribució de les espècies.

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Introduction

The combination of Taxonomic, Phylogenetic and Functional Diversity

Biodiversity is defined as the variety and variability of life on Earth and is often quantified by the total number of species (Gaston, 2000). However, species richness does not capture neither reflect sufficiently the differences in the evolutionary history of species and their ecological function in the ecosystem. For this reason, phylogenetic diversity (PD, the sum of branches lengths on a phylogenetic tree among all species within a community) and functional diversity (FD, the value and the range of variability of functional traits within a community) are increasingly being quantified and studied to inform ecological theory and conservation prioritisation (Cadotte et al., 2009; Wong et al., 2018). Especially in the field of conservation biology, it has been documented that the single use of either taxonomic diversity (TD), PD or FD produces biases when establishing protected areas, as one component can result to be over-represented while another under-represented in the area. For instance, different species can be similar or even identical in terms of life-history traits and thus performing an overlapping ecological function, therefore their co-occurrence would add to the taxonomic and phylogenetic but not to the functional richness of a community (Devictor et al., 2010). For this reason, encompassing the three facets of diversity when describing patterns of diversity, assessing ecosystem functioning and defining conservation areas has been highly suggested (Le Bagousse-Pinguet et al., 2019). Nonetheless, studies based on an integrative approach of quantifying and merging TD, PD and FD in extended spatial scales are yet scarce. Here, I aim to combine these three attributes of diversity to assess the patterns of diversity of caddisflies (Trichoptera, Insecta) across a broad latitudinal gradient in Europe and to elucidate the causing mechanisms underlying these patterns.

Species are characterized by functional traits such as respiration and feeding strategies, body size, emergence or flight period that have been acquired over the evolutionary time (Múrria et al., 2018). The functional space (FS), which is a common approach to FD, orders all the species of a community or spatial scale (e.g. local, regional, continental) on a few independent functional axes that capture the range of trait variability among organisms (Mason et al., 2005; Villéger et al., 2008).The values of FD should integrate the variation of traits among all organisms within a community, whereas the functional niche of a single species would be defined as the exact position which this occupies on the FS (Rosenfeld, 2002). Under this conceptual framework, the functional richness (FRic) of a given community is the hypervolume that all species in the community occupy in the FS (Villéger et al., 2008). Another component of the FS is the functional dispersion/divergence (FDis) which represents the mean dispersion or trait differentiation in the FS of a given set of taxa (Laliberté & Legendre, 2010). A high value of FDis is caused by the clustering of species at the edges of the FS. Commonly, FDis indicates the degree of resource differentiation and thus competition (Mason et al., 2005). Nowadays, the applicability of functional approaches is being expanded to answer questions related to community assembly rules (Kraft et al., 2008; Cornwell & Ackerly, 2009). One of the reasons why the FD approach is becoming increasingly popular is that FD influences ecosystem processes more than TD does (Hooper et al., 2005; Cadotte et al., 2011).

On community assembly and diversity patterns

Community assembly processes, which are conceptualized as the accumulation of species diversity over time in a given habitat, are difficult to observe. Hence there is a considerable need to infer the ecological and evolutionary mechanisms underlying the pattern observed using several perspectives based on trait and species distributions and phylogenies, among others (Mouquet et al., 2012). Diversity and community assembly patterns can be shaped by both stochastic and deterministic processes, which include the simultaneous action of ecological and evolutionary processes. The effect of stochastic processes would lead to communities that are random subsets of the regional species pool with the exclusive action of the ecological drift and isolation-by-distance (Hubbell, 2001). On the other hand, deterministic processes such as environmental filtering, competitive exclusion and limited dispersion assume that there is certain species sorting on the regional pool during the assembly of local communities (Vellend, 2016). However, since community assembly occurs via multiple processes including eco-evolutionary feedbacks, patterns observed in nature may be consistent with multiple explanations (Vellend, 2010), such as habitat filtering, competitive exclusion and biogeographical processes.

Environmental filtering implies that species can arrive in a site but fail to establish or persist due to the inability to tolerate the abiotic conditions (Cornwell et al., 2006). This filtering process acts upon the biological traits of species. For instance, in areas characterized by extreme climates (e.g. cold temperature or aridity) only species characterized either by highly adapted individuals with a low combination of characters or generalists that can cope with fluctuating environments are expected to survive (Clavero et al., 2004). Hence, when the effect of environmental filtering on the community assembly is strong, communities should be composed by species that have acquired particular traits that permit them to survive under the extreme conditions, displaying low values of FRic and FDis (Lamanna et al., 2014). Moreover, if these characters are phylogenetically conserved, communities should be also clustered in terms of phylogenetic diversity displaying low values of PD (Emerson & Gillespie, 2008).

Competitive exclusion states that if two competing life forms attempt to occupy the same niche, the one should drive out the other. Strong competitive exclusion can lead to communities characterized by high FDis and also phylogenetic overdispersion and high values of PD if the functional niche is conserved (Emerson & Gillespie, 2008).

Habitat instability favours high dispersion and forces cladogenesis to forming weakly structured communities (Papadopoulou et al., 2009; Ribera et al., 2003). In contrast, low dispersion is commonly found across stable habitats, resulting in strongly structured populations and communities (Múrria et al., 2015; Papadopoulou et al., 2009).

Also important in determining the community assembly processes are the extrinsic dispersal barriers such as high mountain chains, which should explain the patterns of diversity observed (Field et al., 2009; Leprieur et al., 2009). Focusing in Europe, basins are considered historically isolated when they lay in one of the areas which are geographically isolated from Central Europe by the main European mountain chains: the Iberian Peninsula (isolated by the Pyrenees), the Italian Peninsula (isolated by the Alps) and the Hellenic-Western Balkans (isolated by the Dinaric Alps) (Bănărescu, 1989; Leprieur et al., 2009). In this direction, for aquatic insects, the high topographic barriers between catchments at headwaters sections have increased the populations structuring more than at the lowland river sections, which results in higher diversity in headwaters compared to the other river sections (Múrria et al., 2013).

Finally, it would be important to mention that many theories on community assembly and the derived patterns across the latitudinal gradient had remained up until now only verbalized. This has been constantly changing owing to the introduction of null models, which have been applied to a diverse set of questions and have yielded new insights into patterns and mechanisms in community ecology. Null models could be briefly described as thought experiments that allow us to explore the range of possible worlds and patterns where certain ecological mechanisms do not operate (Gotelli & Graves, 1996).

On the spatial scale

The ecological and evolutionary processes may act differently in distinct temporal and spatial scales. The regional species' pools (γ-diversity) as well as the differences in community composition between localities (β-diversity) are determined by large-scale historical and biogeographical processes, such as dispersal limitation, speciation and extinction (Ricklefs, 2004). Local patterns of α- and β-diversity are influenced not only by the regional species' pool but also by local abiotic conditions and species' interactions (Lessard et al., 2012). As a result, biodiversity should be studied in multiple spatial scales from local sites through regional and continental to the global scale. Resolving the relative contributions of local vs regional processes may be the key to understanding global patterns of species diversity (Huston, 1999). For instance, hump-shaped species–energy relationships can be observed on local scales, whereas positive relationships on regional ones (Rosenzweig & Abramsky, 1993). When exploring global variation in biodiversity, it is crucial to understand not only the importance of differences in spatial scale for the patterns that are observed, but also how diversity on one scale might relate to and affect that on another (Gaston, 2000). It is important to take into consideration that ecological patterns observed at one scale often do not extrapolate to other scales (Būhning-Gaese, 1997).

Objectives

(1) To assess patterns of local and regional diversity across Europe to understand how biogeographical and ecological processes have driven the formation of regional species' pools as well as the differences between them within and across 18 ecoregions (ERs).

(2) To elucidate how distinct communities within each ER are assembled, across a latitudinal gradient spreading from the Mediterranean peninsulas to northern Scandinavia.

(3) To examine phylogenetic community structure (clustering or overdispersion) on both regional and local scale and understand how TD and FD could have determined these patterns.

Focusing on the study

The studied gradient is unique for elucidating the contribution of the (i) environmental filtering; (ii) competitive exclusion; and (iii) neutral processes on current patterns of diversity, because it covers a broad range of environmental and biogeographic conditions.

Environmental heterogeneity is expected to decrease, and climatic stress associated to temperature is expected to increase in parallel to increasing latitude, whereas aridity should be spatially limited to Mediterranean regions. The cyclic Pleistocene glaciations have strongly affected the biogeographic history of the continent, since they provoked species extinctions in the north, restricting the regional biodiversity (Hewitt, 2004). The northern regions are expected to have been subsequently colonized by high-dispersive and cold-tolerant species from the Mediterranean and extra-Mediterranean refugia located in several high mountain ranges in central Europe such as the Pyrenees, Alps, Carpathians and Dinaric Alps (Malicky, 1983). Moreover, the biogeographic isolation of the Mediterranean peninsulas by the aforementioned high topographic barriers should increase their community differentiation compared to more connected Central-European ERs. Given all these historical and environmental factors, different patterns were expected within and across regions. Patterns of diversity will be explored assessing i) α- and β-diversity within regions to disentangle how biodiversity is distributed across local communities and how these intra-regional patterns resemble or differ across the latitudinal gradient, and ii) β- and γ-diversity across regions in order to elucidate the mechanisms and possible pathways through which recolonization could have possibly taken place.

TD and PD should explain differences in speciation, extinction and the hypothesized colonization processes across regions, whereas FD and PD are crucial for inferring the effects of deterministic processes such as environmental filtering, competitive exclusion and limited dispersion on the community composition patterns and their variation across the latitudinal gradient. Briefly, I will try to comprehend processes that have shaped biodiversity patterns across the latitudinal gradient in Europe by incorporating insights offered by the complementary use of TD, PD and FD at two relevant spatial scales.

Hypotheses

Within a community/region (α- and γ- diversity)

On the regional scale, a prominent influence of past climatic history, biogeography and contemporary environmental filtering on diversity patterns is expected. TD and PD should decline in parallel to increasing latitude; high TD and PD in the south would be driven by greater habitat heterogeneity (Pianka, 1966), higher speciation rates and diversity accumulation in the Mediterranean due to long-term habitat stability during the Pleistocene, reflected in high endemism (Blondel et al., 2010). Contrarily, TD and PD in the north should be a consequence of the LGM extinctions and the current harsh environmental conditions, i.e. climatic stress. Given the short time period that has passed since the LGM, only a small if any contribution of speciation is expected to the current biota of northern areas, which should have originated mainly by re-colonization (Pinkert et al., 2018). Furthermore, an inverse relationship of FRic with latitude would be expected. The high levels of landscape heterogeneity and the predictable seasonality of the Mediterranean climate result in a significant variability of habitats, that should be occupied by species of many different combinations of traits (Bonada et al., 2007). As latitude increases, habitats become more homogenous and the environmental filtering is so strong that only species with certain characters would survive (Kerkhoff et al., 2014).

What is more, due to the decreasing competitive exclusion expected to be acting on local scale within each region, following decreasing taxonomic richness, an overall decreasing functional dispersion (FDis) should be expected. This would entail lower PD than expected by chance in northern regions and higher in southern ones, should functional niche be conserved for caddisflies in Europe (Lamanna et al., 2014).

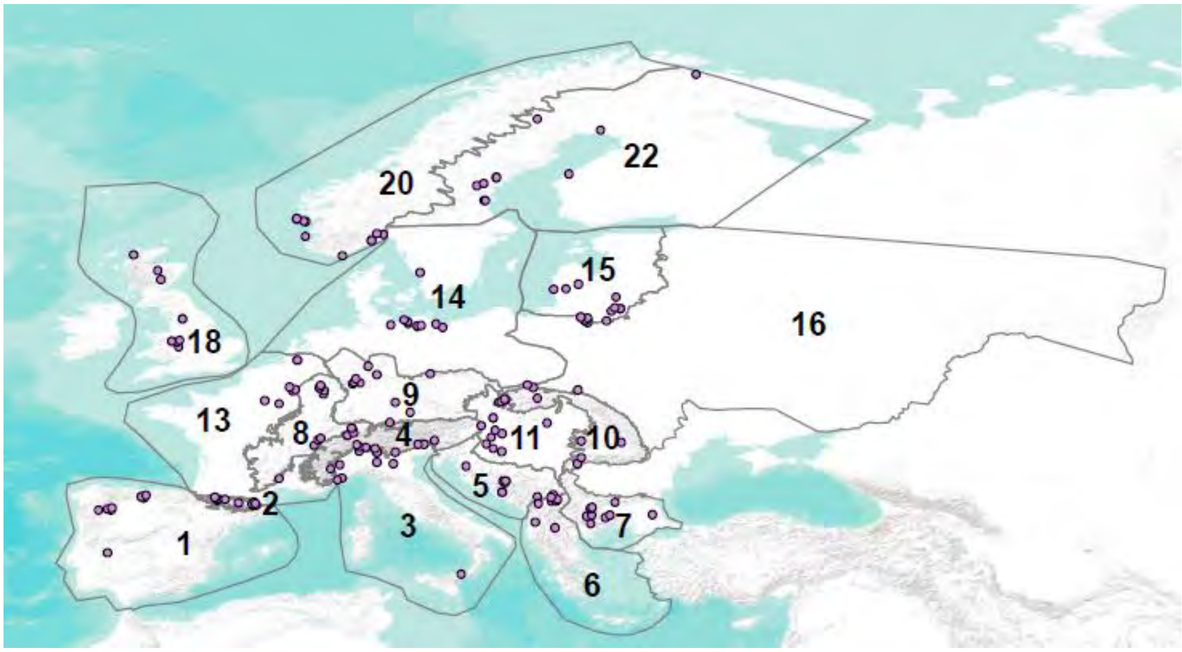

Figure 1. The 10 communities in each of the 18 ecoregions based on Illies, 1978. 1. Iberian Peninsula, 2. Pyrenees, 3. Italy and Corsica, 4. Alps, 5. Dinaric Western Balkan, 6. Hellenic western Balkan, 7. Eastern Balkan, 8. Western Highlands, 9. Central Highlands, 10. Carpathians, 11. Hungarian lowlands,13 Western plains, 14. Central plains, 15. Baltic province, 16. Eastern plains, 18. England, 20. Borealic uplands, 22. Fenno-scandian shield.

Across communities/regions (β-diversity)

If northern regions have been recolonized by species from the southern refugia that managed to pass through the harsh environmental filtering following the LGM, then the northern communities are expected to be a partial subset of species from southern species pools or the Pleistocene extra-Mediterranean refugia. Therefore, a strong contribution of the nestedness component on latitudinal patterns of β-TD and β-PD is expected across regions. However, values of β-diversity should be low across communities of northern regions because these areas should share a high number of cold-tolerant and high-dispersive species. Contrarily, in southern and central Europe, higher turnover is expected between communities of the same ER, driven by habitat heterogeneity. For instance, in the south, there are more high mountains as well as greater temperature variability than in the north.

The ERs that hosted possible Pleistocene refugia and which have been connected to the north by large river basins that favour dispersion, such as high elevation regions found in ER4, 8, 9 and 10 (Fig. 1) should have acted as species sources and thus be characterized by high nestedness with respect to northern ERs. On the other hand, southern regions that were not covered by ice are expected to display an equal contribution of the turnover as well as nestedness components on the β-TD, PD and FD between them. The contribution of each component in the overall dissimilarity between ERs would be driven by their spatial, in the case of the dominance of the nestedness components, as well as environmental filtering, if the turnover is the main process. Values of turnover should be high between more isolated main peninsulas of the Mediterranean and central or northern regions.

As far as the FD is concerned, functional nestedness between southern and northern regions is expected to increase in parallel to their geographic distance, due to the post-glacial recolonization and limited speciation in the north. This prediction assumes that the greatest trait variability in the south already includes trait combinations that can persist in the extreme climatic conditions in the north.

Materials and methods

Species data acquisition

The European EcoRegions (ERs) were defined by Illies (1978) based on the distributions of 75 different taxonomic groups and are still widely used in aquatic ecology such as in lake or river typologies (e.g. Moog et al., 2004). Additionally, the ERs are adopted for applied purposes and serve as typological entities for the assessment of European running waters according to the Water Framework Directive (WFD; Directive

2000/60/EC)(Schmidt-Kloiber et al., 2017). In order to study the taxonomic composition of local communities, at the ecoregion level the species list was compiled from presence-absence data of 1425 species available in the Freshwater Ecology website (Schmidt-Kloiber & Hering, 2015) (Table ES1a, electronic supplementary material). At the local scale, the species' abundances data from 180 communities (10 communities in each of the 18 ERs in Europe) were provided by the Distribution Atlas of European Trichoptera (Schmidt-Kloiber et al., 2017) (Table S1b, electronic supplementary material). The remaining 5 ERs were excluded from the analyses because of the scarce data available. The criteria for community selection was that communities should contain at least five different taxa and 50 individuals collected on a specific date and on the same geographic coordinates. The final dataset for the local communities included 340 species. Data selection and subsetting were conducted in R.

Compilation of DNA sequences and phylogenetic analyses

DNA sequences of the most frequently sequenced gene, the mitochondrial cytochrome *c* oxidase subunit I gene (*coi;* 658 bp, DNA barcode) were compiled from GenBank and the DNA-barcode project "Trichoptera barcode of Life" (Zhou et al., 2016). Given that adding more genes increases phylogenetic accuracy and robustness by enhancing information on the different lengths of the tree branches, because the degree of genetic conservation and variation in the substitution rates differs among genes (San Mauro & Agorreta, 2010), other genes were downloaded from GenBank. Finally, the molecular data included also the 18S ribosomal RNA gene (*18s;* 538 bp), and three nuclear genes: wingless (*wg;* 472 bp), cadherin (*cad;* 850 bp) and elongation factor-1 alpha (*ef-1α;* 1087 bp). This genetic dataset contained 512 European species representing 23 families and 4 species of Lepidoptera as outgroup taxa (Table S2. electronic supplementary material).

DNA sequences were aligned in MAFFT 7 (Katoh & Standley, 2013) and were manipulated using Bioedit (Hall, 2011) in order to be cut on the same length. Genes concatenation was executed using custom Python scripts. To select the best-fit partitioning schemes and models of molecular evolution for phylogenetic analyses, Partition Finder 2 (Lanfear et al., 2017) was used. The models of sequence evolution were implemented for different partitions of the genes, starting at different codon positions, in case of protein coding genes ("fullcodon" code), but not of the *18s* which was treated as a single partition. Individual models of nucleotide substitution were specified for each partition and the best model of substitution for each partition was determined using the AIC (Akaike Information

Criterion). Custom R, Python and Perl scripts were used for generating relevant input and output files for the various steps.

The RaxML (Randomized Axelerated Maximum Likelihood) method for maximum likelihood based phylogenetic inference (Stamatakis, 2014) was used to reconstruct phylogenetic relationships. The likelihood of a phylogenetic tree is the probability of observing the data given the resulting tree and the used GTRGAMMA model of evolution. 999 bootstraps were used to show how strongly the data supports each of the relationships depicted in the maximum likelihood tree. As conservative measures of node support, bootstrap values of 80% or greater might indicate substantial confidence for a group.

Functional traits

All available biological traits at the species taxonomic level covering all major families of European freshwater Trichoptera were extracted from the website www.freshwaterecology.info (Schmidt-Kloiber & Hering, 2015). After data compilation, a total of 4 biological characteristics as functional traits, including 15 categories (Table S3) were selected. The traits finally considered were respiration strategy, feeding type, emergence period and maximum forewing length of males and females, which is considered to be indicative of dispersion capacity. These traits should determine where a species lives or can potentially live (habitat preference). The final traits dataset included 236 species belonging to 23 families.

Intraspecific trait variability for each species was captured and summarized using a fuzzy coding approach (Chevene et al., 1994), which captures the affinity of species for each trait category considering the variability across its distribution range. However, only the traits ‘feeding type’ and ‘emergence period’ revealed intraspecific variability, and the values for each category were standardized to 1 using the ‘prep.fuzzy’ function of the *ade4* package (Dray & Dufour, 2007). The trait ‘maximum forewing length’ was a numerical continuous variable, while ‘respiration’ was codified as a multichoice nominal variable that was transformed into a binary one by the ‘prep.binary’ function. A dissimilarity matrix based on the Gower distance, which was adapted to handle fuzzy-coding-traits (Pavoine et al., 2009), was derived using the trait matrix. Then the FS was built using a Principal Coordinate Analysis (PCoA) carried out with the ‘dudi.pco’ function of the *ade4* package. The three most explanatory axes were retained. Pearson correlation coefficients (r) were used to identify which traits were correlated with each axis in the FS.

Species in the FS were grouped by family using the function 's.class' in the package *adegraphics* (Siberchicot et al., 2017). To estimate the occurrence probability of different trait combinations in the FS, the Kernel density estimation method was used through the package *ks* (Duong, 2018) in R. The Kernel densities for the different combinations of the functional axes 1 to 3, which captured the greater part of the total variance were estimated for illustration purposes.

Phylogenetic signal of traits and niche conservatism

To assess the phylogenetic conservatism of the individual traits as well as of the three functional PCoA axes, the Blomberg's K-statistic was used. This metric seeks to quantify the degree to which variation in a trait is explained by the structure of a given phylogenetic tree. This value is then standardized by an expectation derived from Brownian Motion trait evolution on the observed phylogenetic tree. Values of $K \approx 1$ indicate that the observed variation in the trait data is predicted by the structure of the phylogenetic tree under a Brownian Motion model of trait evolution. Values of $K > 1$ indicate more phylogenetic signal than expected from Brownian Motion (i.e., trait conservatism), whereas values $K < 1$ indicate less phylogenetic signal than expected (i.e., trait composition unrelated to evolutionary history). Blomberg's K was estimated with the function 'multiphylosignal' available in picante in R (Kembel et al., 2010). Traits with $\text{PIC.variance.P} < 0.05$ have non-random phylogenetic signal.

Taxonomic diversity

There are two key components of TD: Species richness and evenness. Species richness measures the number of species present in a particular community (α-diversity) or region (γ-diversity), while evenness measures the relative abundance of the different species making up the richness. The study was restricted to using species richness (TRic) as a proxy to TD because available data at the regional scale included only the presence-absence of species, thereafter TD is referred to species richness. β-diversity between communities of a region and across regions were measured using the Sorensen dissimilarity index, which was partitioned into its turnover (otherwise, Simpson diversity) and nestedness components, based on the relationship '$\beta_{Sor} = \beta_{Sim} + \beta S_{ne}$' (Baselga, 2010), by the 'bpair' function of the package *betapart* (Baselga et al., 2018).

Phylogenetic diversity

Values of PD incorporate phylogenetic difference between species such as the minimum total length of all the phylogenetic branches required to span a given set of taxa on the phylogenetic tree (Faith´s PD; Faith, 1992) and the mean of all pairwise phylogenetic distances in a community or sample (MPD; Webb et al., 2002). Faith's PD consistently increases with species richness in an assemblage, therefore it is considered biased when measuring PD within a community, while MPD is independent of species richness or species turnover. Moreover, it has been shown that MPD is sensitive to deep branching structure in phylogenetic trees and therefore MPD is less sensitive to poorly resolved phylogenies (Cadotte & Davies, 2016). Hence, only the MPD metric was used to measure α-PD within localities and γ-PD within regions. However, Faith's PD was used to calculate the Sorensen dissimilarity across localities or regions, because of the advantage that it can be partitioned into its turnover and nestedness components, which was relevant to compare the results with the taxonomic and functional approach.

In order to assess the phylogenetic community structure at the local and regional scales, i.e., the phylogenetic clustering or overdispersion, the standardized effect sizes of MPD metrics within communities and within ERs were calculated by the function ses.mpd using picante in R (Kembel et al., 2010). The SES.MPD metric expresses the mean difference between the MPD of the observed communities and the mean MPD of random communities generated by a given null model, in standard deviation units. The 'taxa.labels' null model was used, which shuffles a distance matrix labels across all taxa included the matrix, in 999 iterations. To account for phylogenetic β-diversity between localities and ERs, the β Phylogenetic Sorensen index based on Faith's PD was discomposed using the function 'phylo.beta.pair' of the *betapart* package.

Functional diversity

Functional traits data was available for 236 out of 1425 species. Moreover, this subset was mainly located in central Europe, where the taxonomists carry out more extensive observations and species descriptions. Therefore, to overcome a possible bias in the calculation of the regional FD, the FRic and FDis of each ER were calculated using a loop of 999 iterations in R. In each of the iterations, a subset of 109 species (minimum number of species that have associated trait information across ERs) was sampled from each ER. Then, the FRic was calculated by the function 'fric_3d', which computes the hypervolume defined by three PCoA axes. To estimate how each community fills the functional space, the FDis was calculated using the function ‘fdisp_k_sub’ in R (Gutiérrez-Cánovas et al., 2015). The final estimates of FRic and FDis per ER were the mean values of the iterations. Loops were also used in the calculation and partition of β-FD between ERs, using the functions ‘functional.beta.core’ and ‘functional.beta.pair’ of the *betapart* package. At the local scale, the number of species with associated trait value was comparable across communities, and therefore only the observed vales for FRic and FDis were considered.

To test for functional community structure at both scales, i.e., the functional clustering or overdispersion, the standardized effect sizes of the FRic and FDis metrics within communities and within ERs were calculated, similarly as for the SES.MPD., but using custom-made functions following Swenson (2014). The ‘independent-swap’ null model was used in 999 iterations.

Statistical analyses

Abiotic predictor variables

The bioclimatic variables of average annual temperature (BIO1) and annual precipitation (BIO12) for the years 1970-2000 were used as descriptors of current climate. For each local community, these variables were obtained in a raster format from the WorldClim v.2 website (Fick and Hijmans, 2017) on a spatial resolution of 30 seconds (~1 km^2). The mean values for each ecoregion were calculated in ArcGIS 10.6. Elevation values for each locality were extracted from digital elevation models of a 25 m resolution, which were obtained from the Copernicus Land Monitoring Service website of the European Environment Agency (https://www.eea.europa.eu/). Elevation was used as a predictor variable only in models at the local scale. The coordinates of the centroid of each ER, as well as all spatial analyses and intersections were calculated in ArcGIS 10.6, too.

A significant collinearity between temperature and latitude was detected at the regional scale ($r=-0.84$, $p<0.001$), but not at the local scale because of greater variability of local values of temperature and latitude in temperate regions. As a result, the mean ecoregion temperature was included only in the analyses of β-diversity at the local scale.

To test the dissimilarity distance increase between localities within each ER and between the centroids of each pair of ERs, the straight-distance matrices were calculated using the function *GeoDistanceInMetresMatrix*, which accounts for the curvature of the earth (Rosenmai, 2014)*,* while environmental and elevation euclidean distance matrices were calculated using the function ‘dist’ in R.

Diversity Patterns Within Regions

Local TRic, MPD, FRic and FDis (α-diversity) were correlated to the latitude, mean annual temperature, annual precipitation and elevation values of each locality. At the regional scale, TRic, MPD, FRic and FDis (γ-diversity) were correlated to latitude and precipitation. Local and regional SES.MPD, SES.FRic and SES.FDis were correlated to latitude. Various linear models were tested and fitted with the function 'lm' in R (R Core Team, 2019) in both cases.

Diversity Patterns Across Regions

The relative contribution of turnover and nestedness to the Sorensen dissimilarity of local β-TD, PD and FD was estimated and represented in barplots. Mann-Whitney-Wilcoxon tests were conducted to test if there is a difference on the contributions of turnover and nestedness between the communities of the northern and southern ERs.

Variation partitioning analyses of the β-diversity between communities were carried out in order to evaluate which variables account for the variance between local communities. Principle coordinate analysis (PCoA) was conducted separately on the Sorensen and the turnover and nestedness components of the β-diversity, employing the Lingoes correction to account for negative eigenvalues (Legendre, 2014), using the function 'pcoa' in the package *ape* (Paradis & Schliep, 2018). The PCoA eigenvectors (principle coordinates) for the three matrices were used as input response variables in separate variance partitioning analyses. As predictor variables were used the environmental and spatial variables as well as the values of the regional TRic, after standardization (scaling).

Separate dbRDA analyses employing a forward selection procedure were undertaken using the functions 'capscale' and 'ordiR2step' in vegan (Oksanen et al., 2018), to identify the variables influencing the components of β-diversity, the statistical significance of which was tested by the function 'anova'. The forward selection method employed three stopping rules: 1) when the adjusted R^2 begins to decrease; 2) when the significance of the pre-selected permutational level was exceeded ($p < 0.05$); and 3) when the adjusted R^2 of the full model was exceeded. To identify the pure and shared contribution of local environmental and spatial variables on patterns of diversity for all communities, variance partitioning (Borcard et al., 2016) based on partial linear regression was performed including all significant predictor variables identified by the forward selection, using the 'varpart' function in the *vegan* package. Adjusted R^2 fractions are presented as they provide unbiased estimations of explained variation which corrects for the number of explanatory variables (Peres-Neto et al., 2006).

At the regional scale, the Sorensen, turnover and nestedness dissimilarity matrices were correlated to the geographic and environmental distance matrices between regions using Mantel tests (‘mantel.test’ function, package *ape*). When there was a significant correlation between matrices, these were vectorized and models of linear or quadratic regressions were fitted.

Results

Phylogenetic tree

The phylogenetic tree was reconstructed using the maximum-likelihood method (Fig. S1, electronic supplementary material). Although not all basal nodes were highly supported, the phylogeny was appropriate for testing the hypotheses. The phylogenetic tree was concordant with previous taxonomic classifications (e.g. Malm et al., 2013), and the three main suborders were highly supported (Annulipalpia, Spicipalpia and Integripalpia, Kjer et al., 2001). Integripalpia can be divided into two infraorders (Plenitentoria and Brevitentoria), both well-supported. Plenitentoria and Spicipalpia did not appear to be monophyletic groups, as each one consisted of two branches, as reported before (Holzenthal et al., 2007, Kjer et al., 2001).

Functional space

The three PCoA axes captured a cumulative proportion of variance of the functional traits of 62% (PC1: 26%, PC2: 18%, PC3: 18% of total variance, respectively). The categories of traits that were significantly correlated to the first axis of the PCoA were *gill* as a respiration strategy, *maximum forewing size* and the feeding type *shredder*, to the second axis of the PCoA were the feeding types *grazer* and *predator*, and finally the *summer* and *autumn* as emergence periods were correlated to the third axis (Pearson correlation, $r>0.5$, $p<0.001$, Table S4). These variables were plotted as arrows in the Kernel Density Estimation plot (Fig. 2a). Each point on the FS represents a different species. Species were grouped by taxonomic families, representing overall functional niches for 23 families of caddisflies (Fig. 2b).

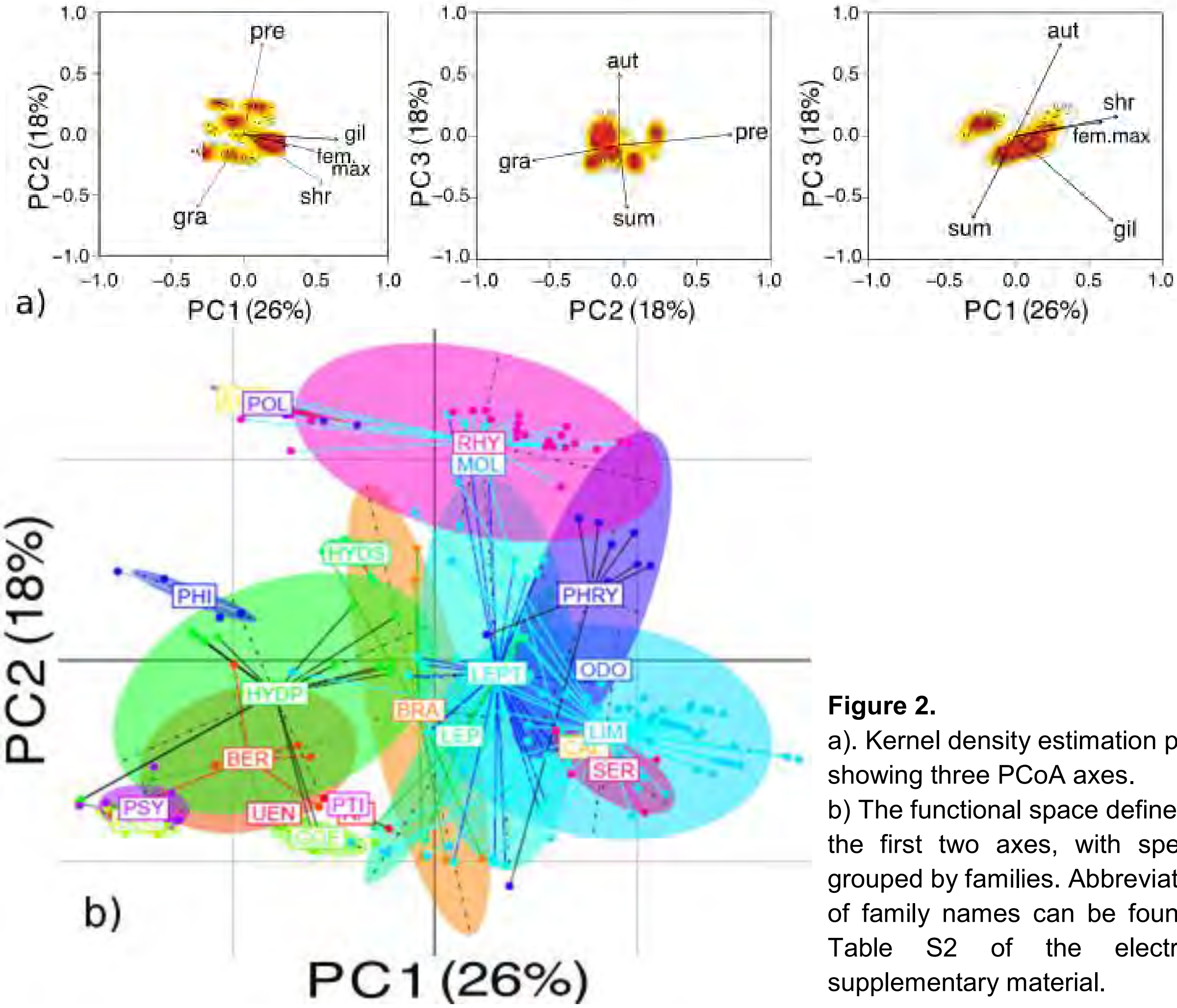

Figure 2.
a). Kernel density estimation plots, showing three PCoA axes.
b) The functional space defined by the first two axes, with species grouped by families. Abbreviations of family names can be found in Table S2 of the electronic supplementary material.

Phylogenetic niche conservatism

The respiration character *gills* was highly conserved (K=2.837, Table 1), whereas forewing length and categories of emergence period were not at all conserved. The feeding categories *passive filter feeder* and *predator* were highly conserved, while *grazer* and *xylophagous* showed some degree of conservatism.

The position of species on the first axis was not significantly conserved (which is inconsistent with gills' high conservatism but consistent with the rest of the traits that correlate to this axis). The position of species on the second axis showed some degree of conservatism (inconsistent with high conservatism of *predator*, consistent with intermediate conservatism of predator). The position on the third axis is not at all conserved, this being consistent with random evolution of the individual traits correlating to this axis. P values show significance in all cases of moderate and high conservatism (Table 1).

Table 1. Bloomberg's K and PIC.variance of P.

Traits/ PCoA axes	Categories	K	PIC.variance.P
PCoA axes	A1	0.683	0.001
	A2	0.819	0.001
	A3	0.071	0.133
Respiration	tegument	0.273	0.256
	gills	2.837	0.001
Maximum forewing length	male max.	0.363	0.001
	female max.	0.163	0.008
Emergence period	winter	0.171	0.193
	spring	0.017	0.786
	summer	0.022	0.665
	autumn	0.055	0.323
Feeding type	grazer	0.995	0.001
	xylophagous	1.025	0.001
	shredder	0.645	0.001
	gatherer	0.282	0.001
	passive filter feeder	2.412	0.001
	predator	1.534	0.001
	other	1.031	0.001

Diversity Patterns at the Local Scale

At the local scale, α-diversity was unrelated to latitude for all the estimated attributes of diversity, TRic, MPD, FRic or FDis (Fig. 3a-d,i). The observed MPD, FRic and FDis were not significantly different than the expected values according to the null models (Fig.3b-d,i). Correlations of the above values of diversity with the mean annual temperature, annual precipitation and elevation of each locality did not yield any clear linear relationship, either.

β-TD and β-PD between communities were low and mostly driven by turnover, whereas β-FD was driven by nestedness (Fig. 3a-d,ii). No difference on the contributions of turnover and nestedness between the communities of the northern and southern ERs was detected by the Mann-Whitney-Wilcoxon tests. Variation partitioning revealed an important role of elevation and regional species richness in the variation between communities (Fig. 3,iii). The predictor variables chosen by the forward selection process were latitude, elevation and regional TRic, while annual precipitation and mean annual temperature were discarded.

The selected variables were fitted in a dbRDA model and their contribution in explaining the overall variation was proven significant by the 'anova' function. More specifically, with regard to β-TD among communities, differences in elevation explained 13% of total variation of the Sorensen index (adj.R^2=0.132, $p<0.001$), differences in the regional TRic 2% (adj.R^2=0.020, $p<0.001$), while latitudinal differences 4% (adj.R^2=0.043, $p<0.001$) (Fig. 3a,iii). Since the Sorensen index is, in this case, mainly composed of turnover, the variation partitioning of the turnover component yields very similar values as well (Fig. 3a,iii). When it comes to the β-PD between communities, 12% of its variance is explained by elevation (adj.R^2=0.116, $p<0.001$), 2% by the regional TRic (adj.R^2=0.020, $p<0.05$) while 8% by latitude (adj.R^2=0.077, $p<0.001$). Nonetheless, none of the above variables was proved significant by the 'anova' function and the forward selection process for explaining the variance of the nestedness component of β-TD and PD, neither any of the components of β-FD.

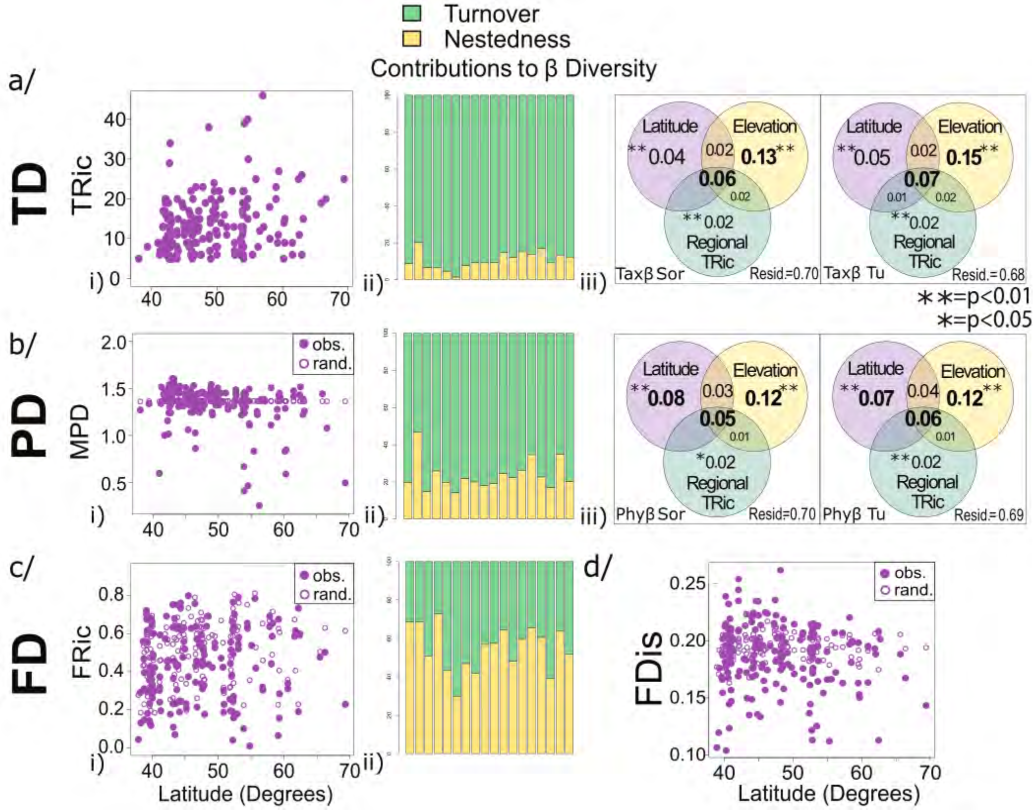

Figure 3. Local patterns of diversity. In each row, a different facet of diversity is shown. In the first column (i), local α-diversity with respect to latitude and in (d), the FDis also with respect to latitude. Empty circles are the expected values deriving from a null model, while filled circles are the observed values. The barplots in (ii) show the contributions of turnover and nestedness to the β-diversity between communities of the same ER. Each column of a barplot represents an ER; ERs are depicted in ascending index order. The third column (iii) contains the results of variation partitioning; the Venn diagrams and labels of each intersection (individual fraction) with the adjusted R^2, the residual variance of each model and the corresponding p-values.

Diversity Patterns at the Regional Scale

At the regional scale, all patterns of diversity revealed a significant distribution across latitude (Fig. 4a-c,i). Regional values of TD and MPD linearly declined with increasing latitude ($r^2=0.35, p<0.01$ and $r^2=0.76, p<0.01$, respectively). Observed values of MPD were significantly higher than expected in the Mediterranean regions 1, 2, 3, 6, 7 indicating phylogenetic overdispersion, whereas these values were lower than expected in the northern ERs 20 and 22 indicating phylogenetic clustering (Fig. 4b.i, orange and green dots respectively) (Table S5, mpd.obs.z, mpd.obs.p). In contrast, estimates of FRic displayed a unimodal relationship with latitude (Fig. 4c.i, significant linear and quadratic terms, $r^2=0.41, p<0.05$). This unimodal relationship was also found for the number of genera (richness) per region (Fig. 4e).

β-TD across regions increased in parallel to increasing geographic distance ($r^2=0.56$, $p<0.001$) and was driven mostly by turnover ($r^2=0.48$, $p<0.01$) (Fig. 4a.ii,iii). β-PD followed the same pattern as β-TD ($r^2=0.60$, $p<0.001$), being driven by turnover as well ($r^2=0.63$, $p<0.001$) (Fig. 4b.ii,iii). Finally, β-FD across regions also increased in parallel to increasing geographic distance ($r^2=0.45$, $p<0.001$), despite its very low values. The nestedness component of β-FD was more prominent, but did not follow any clear pattern, while turnover increased with increasing distance ($r^2=0.45, p<0001$) (Fig. 4c.ii,iii).

When examining the β-TD and β-PD between a particular ER and all the other ERs, patterns of turnover and nestedness were different. Considering the Iberian Peninsula as a reference point, turnover displays a strong direct relationship with geographic distance ($r^2 = 0.84$, $p<0.01$) while nestedness follows a unimodal one ($r^2 = 0.37$, $p<0.01$) (Fig. 5). ERs 4, 8 and 9 (central Europe) have the largest residuals in this linear model, meaning that the Iberian Peninsula (1) shares species with those regions, but as distance from central Europe increases, these species are replaced by others. Very similar patterns of dominant turnover over nestedness are observed when Italy (3) or the Hellenic-Western Balkans (6) are taken as reference points (Fig. S2). Contrarily, departing from the Central Highlands (9, Fig.5), the Alps or the Western Highlands (4 and 8 respectively, Fig.S2), there is increasing nestedness with respect to all of the ERs, except for the closest neighbouring ones. Departing from the Borealic uplands (Fig.5), or the Fenno-scandian shield (22), there is increasing turnover except for the cases of ERs 14, (northern Europe) 4, 8 and 9 (central Europe).15

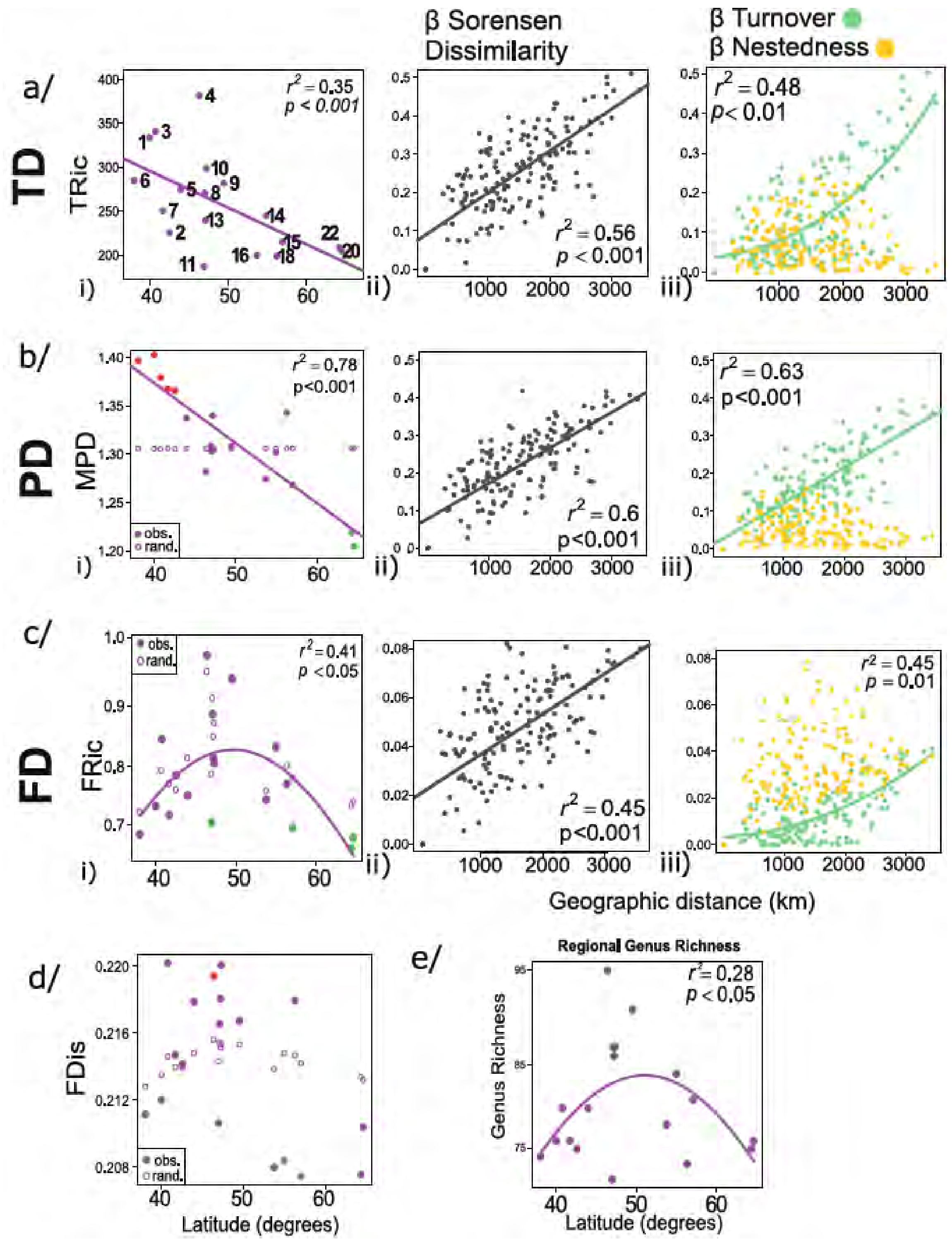

Figure 4. Regional patterns of diversity. In each row, a different facet of diversity is shown. In the first column (i), linear and quadratic regressions of the regional γ-diversity with respect to latitude are presented. Empty circles are the expected values deriving from a null model, while filled circles are the observed values. In the second column of a-c, linear regressions of the β Sorensen dissimilarity with respect to geographic distance are shown, while in the third column can be seen the relationship of turnover and nestedness with geographic distance. In d-e, the relationship of FDis and regional genus richness with latitude are shown.

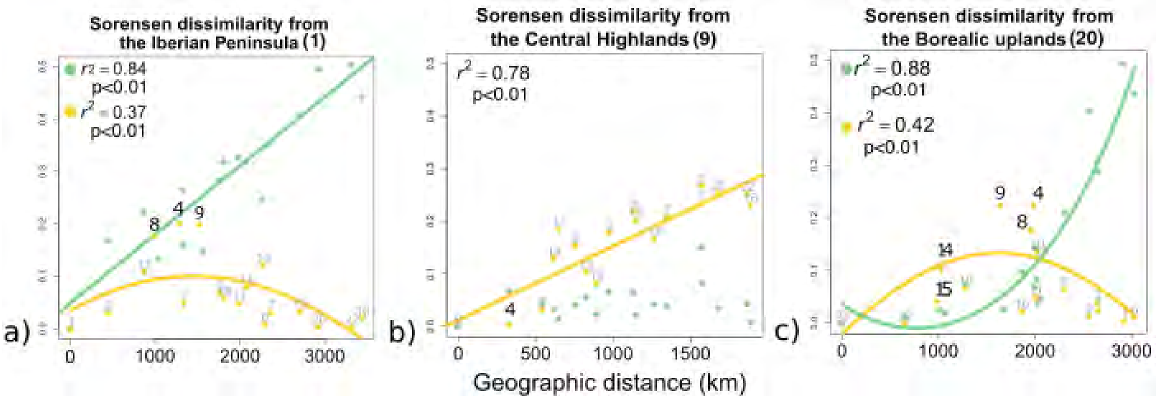

Figure 5. Sorensen β-TD discomposed into the turnover (green) and nestedness (yellow) components as a function of geographic distance, starting from three different ERs, in the south (a), centre (b) and north of Europe (c), respectively. Values in black will serve in the discussion.

Discussion

Patterns were utterly different between the local and the regional scale, suggesting therefore that the spatial scale is critical when studying patterns of all facets of diversity. This discordance at the two spatial scales is likely associated to the local carrying capacities across the latitudinal gradient. Apparently, the results suggest that local communities can accumulate similar local species richness independently of the ecoregion and latitude. However, regional diversity is extremely correlated to latitude, indicating the combined effect of biogeography and climatic conditions on the community composition. This is also reflected in patterns of β-diversity between regions.

Given the patterns detected at the local scale, results suggest that community composition of caddisflies is defined, apart from the regional species pool, by more local factors. Elevation was the most significant factor determining and contributing to the β-diversity. Moreover, and especially for Mediterranean environments, factors such as stream longitudinal zonation related to slope and bed stability, water temperature, current velocity, stream hydraulics, substratum composition, width and depth are critical in determining community composition (Bonada et al., 2005; Clarke et al., 2006). All these factors are related to elevation, justifying its profound and continental influence on β-diversity between communities irrespectively of the latitude.

Phylogeny, functional space and phylogenetic niche conservatism

The derived phylogenetic tree was not fully bifurcating, as it contained various polytomies. The lineages Annulipalpia and Integripalpia were mainly supported, but, among the paraphyletic group of Spicipalpia, only the Rhyachophilidae were well supported. Brevitentoria, the group of Integripalpia that includes the biggest family -Limnephilidae-, presented the most unresolved terminal nodes, mainly because of unsufficient gene coverage. Other inconsistencies in species labels can be attributed to taxonomic missidentification of specimens sequenced, synonymies and possible cryptic species, which are outside the scope of this study. Unresolved nodes can affect measures of phylogenetic diversity when found in basal lineages of large phylogenies but not in terminal branches (Swenson, 2009). However, in general terms, the PDs recorded using phylogenies with polytomies have been proven to be highly correlated with PDs for the same communities using a fully bifurcating phylogeny (Swenson, 2009). Thus, even if the phylogenetic tree used for estimating PD in this study contained unresolved nodes and missidentified taxa, conclusions regarding patterns found could still be drawn.

The functional space found clearly discriminated the main lineages of Trichoptera along the first and second axes (Fig.2). For instance, the Rhyacophilidae, Polycentropodidae, Molanidae and Ecnomidae seem to be specialized in predation, while Uenidae, Ptilocolepidae, Goeridae, Psychomyiidae, Apataniidae, Glossosomatidae and part of the Hydroptilidae in grazing. Furthermore, the Sericostomatidae, Odontoceridae, Calamoceridae and great part of the Limnephilidae tend to be shredders, bear gills and have long forewings indicating dispersive capacity. On the other hand, there are families that comprise more variability of traits, such as the Brachycentridae and Leptoceridae that spread along all the length of the PcoA2, meaning that they use multiple feeding strategies.

It has been previously shown that local phylogenetic overdispersion is indicative of a local high divergence in traits composition when traits are strongly conserved across the regional pool (Kraft et al., 2007). However, the functional niche of caddisflies was only partly phylogenetically conserved, and therefore only tentative explanations based on phylogenetic niche conservatism could be drawn. However, the decoupled patterns of PD and FD found could still offer useful insights on the evolution and distribution of the species (de Bello et al., 2017).

For instance, the position on the third axis of the functional space, which was not at all conserved, is related to the emergence period being summer or autumn (Fig.2a). This is a plastic character that depends on the climatic conditions of the area in which species live.

For example, it is known that in the Alps many species emerge in September instead of during the summer, which is a common emergence period in other southern latitudes (Wolda, 1988). Forewing length was not conserved either, showing that species with different dispersive capacities have emerged within the same lineage.

Patterns Within Regions (γ-diversity)

According to hypotheses, both TRic and MPD (γ-diversity) declined linearly in parallel to increasing latitude. This is consistent with global patterns of diversity, i.e species richness declining when moving to the poles (Gaston, 2000). It is also consistent with the environmental filtering hypothesis that states that the extreme climatic conditions in the north restrict regional diversity, and with the incomplete recolonization after the LGM hypothesis (Pinkert et al., 2018).

However, contrary to expectations, FRic displayed a unimodal relationship with latitude instead of a negative one (Fig. 4c.i), following more the genus richness-latitude relationship (Fig. 4d.ii). Thus, the high species richness in the Mediterranean would not necessarily imply higher FRic. This decrease of FRic in the Mediterranean regions might be attributed to low precipitation and droughts as well as to long-term geographic isolation from Central Europe likely impeding functional trait enrichment through colonization (Leprieur et al., 2009). Indeed, in isolated areas, where speciation is favoured, species are more similar than in areas where the same number of species is reached through colonization, which implies a small accumulation of FRic (Mittelbach et al., 2007; Reyjol et al., 2007). This result of low FRic in isolated areas (mainly Mediterranean Peninsulas) is also consistent to what has been found for freshwater fish communities of the European drainage basins (Schleuter et al., 2012). In contrast, the observed peak in central Europe should reveal greater habitat heterogeneity and better connectivity to other ERs that favours species migration and functional traits accumulation. ER11 (Hungarian lowlands) forms an exception to the trend of higher FRic in intermediate latitude, probably because of the homogeneity of its habitats (Frost et al., 2007). Finally, the decline in FRic found towards the north could be a consequence of character restriction by cold temperatures and less habitat heterogeneity.

The higher than expected FDis in the Alps could reveal strong competitive exclusion in alpine communities based on the high value of TRic, whereas the low FDis in the ERs 14 and 15 could imply that the harsh environmental filtering starts to be effective at this latitude. In these areas, TRic is still not as low as in the northern ERs 20 and 22, but species seem to be functionally more aggregated. Contrarily, in 20 and 22, FDis is not lower than expected because TRic is very low, too.

The high MPD in the south could have resulted from the higher speciation rates and species accumulation favoured by habitat stability during the Pleistocene and greater habitat heterogeneity. These have led to higher species richness that promotes competitive exclusion and habitat diversification (biotic filter acting stronger than the abiotic). Contrarily, in the north, the lower than expected MPD could be a consequence of the stronger environmental filtering selecting on certain sets of traits that permit survival to extreme climatic conditions and thus leading to phylogenetically clustered communities. What is more, in the south there are more intermittent rivers than in the north, due to the increased seasonality of precipitation, that permit only to specially adapted species to survive. Thus, in the south, there is a coexistence of specialist species and generalist ones at the regional level, which could increase the overall MPD of these ERs.

Patterns of β-diversity within and across regions

At the local scale, all β-TD and β-PD between communities were low and mostly driven by turnover independently of the ER (Fig. 3a-b,ii). In the north, regional species pools were phylogenetically clustered whereas communities displayed high species turnover between them.

Variation partitioning highlighted the importance of elevation in determining the turnover between communities. Elevation is relevant to multiple factors. For instance, in high elevations are found communities of the headwaters including species probably adapted to high stream velocity and colder waters that are not found in communities of lower elevations. What is more, in high elevations, communities are more isolated and less subjected to colonization. Accordingly, changes in caddisflies community composition downstream have been reported to be associated with elevation, stream order and channel width and their related variables such as conductivity, macroinvertebrate community composition, riparian structure and heterogeneity elements, rather than with temperature (Bonada et al., 2005).

Both latitude and the regional species pool were proven to explain differences in local community composition. Latitude is related to temperature differentiation, but, surprisingly, current annual mean temperature was not selected as an explanatory variable for the best model of the dbRDA. Therefore, latitude as an explanatory variable of community differentiation should be related to other factors, such as more to biogeographic processes rather than current climatic conditions.

Maybe it would be more relevant to aggregate communities by ecoregion and conduct the variation partitioning within each ER separately, because different local factors could affect the variability in different ERs. For example, in the south, the ERs are characterized by higher temperature, precipitation and elevation variability than in the north, where a more prominent influence of the regional species pool could account for variability between communities. However, this analysis was not successfully conducted due to the complexity and the amount of the data.

At the regional scale, taxonomic and phylogenetic turnover, but not nestedness, increased with increasing geographic distance (Gaston, 2000). However, this relationship resulted from correlating the Sorensen, turnover and nestedness dissimilarity matrices to the geographic distances among the whole set of the ecoregions (Fig. 4), however when focusing on particular cases of ERs, the results were different (Fig. 5) for both β-TD and PD. For instance, considering the Iberian Peninsula, the Pyrenees, Italy or the Hellenic Western Balkan as the origin point, turnover increased linearly with increasing geographic distance from these areas, while nestedness maintained low values, except for particular cases of closely neighbouring ERs (Fig. 5a and S2). This would imply that species from the mostly isolated Mediterranean regions have not managed to overpass the extrinsic dispersal barriers and thus contradicts the hypothesis that northern regions were recolonized by species of southern refugia (Malicky, 1983). On the contrary, departing from the Alps, the Central or Western Highlands, nestedness increases with geographic distance, while turnover maintains stable low values, suggesting that the recolonization of the north could have originated from there (Fig. 5b and S2). This is furtherly confirmed when ERs 20 and 22 are taken as the origin point. More specifically, results suggest a recolonization route departing from central Europe, reaching the Scandinavian Peninsula (20,22) via the Central Plains (14) and the Baltic Province (15) because, taxonomically, ER14 is a subset of 9, ER15 is a subset of 14 and ERs 20 and 22 are subsets of all the above (Fig. 2c and S2). The same recolonization route has also been suggested for Lepidoptera (Schmitt & Krauss, 2004) and for beetles (Mayer et al., 2014).

Following the same line of thought, another possible pathway of colonization would start from the Western Highlands (ER8) to reach England (ER18) through the Western plains (ER13) (Fig. S2). In the past 500 kya, Britain's geographical status has fluctuated between an island and a peninsula of Europe several times as sea-levels rose and fell in response to global climate change (White & Schreve, 2000). Moreover, the current connection of ER 8 and 13 by the rivers Seine and Loire would favour downstream dispersion of larvae and active adult dispersion across the flow of the rivers.

The mountain chain of the Carpathians (ER10) is also believed to have acted as a refugium during the Pleistocene (Kühne et al., 2017). However, very low nestedness was observed between this and other ERs, possibly due to the isolation given the lack of big rivers connecting the Carpathians to other ERs, and especially the northern ones.

Conclusions

The decoupled use of taxonomic, phylogenetic and functional diversity can reveal patterns and offer insights to community assembly processes that are otherwise difficult to observe in the field. Taxonomic and phylogenetic diversities decrease as latitude increases, following a pattern that characterizes a wide range of terrestrial and freshwater biota. Functional diversity follows a unimodal relationship with latitude, which can be attributed to the long-term historical isolation of the Mediterranean from the rest of Europe, and to the extreme climatic conditions in the south (droughts) and in the north (extremely low temperatures). The Pleistocene glaciations seem to have been a main driver of the current European patterns of TD and PD. The northern areas that were subject to extinctions during the Pleistocene were subsequently recolonized by highly dispersive, cold-tolerant species from central Europe which possess characters that allow them to pass through the harsh environmental filters, as revealed by patterns of regional taxonomic and phylogenetic β-diversity. Therefore, northern species pools were found to be phylogenetically clustered while the southern ones were overdispersed, although this was not reflected in their phenotypic structure, as functional niche was not entirely conserved. These findings contradict the idea that northern Europe was recolonized exclusively by species from Mediterranean refugia and highlight the role of Central European refugia such as the Alps, the Central (Massif central) and Western Highlands, in the recolonization following the Pleistocene extinctions.

Finally, the dominance of species replacement (turnover) over nestedness between the communities within the same ecoregion, would imply that conservation strategies need to include the protection of many different sites and not only of those with the higher diversity.

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Table S3. Life history traits and their categories

Trait	Trait explanation	Category name	Abbrev.	Category explanation	Category Author	Author of the description
feeding type	Feeding preference	grazers and scrapers	gra	feed from endolithic and epilithic algal tissues, biofilm, partially POM, partially tissues of living plants	Moog, 1995	Graf, Schmedtje, AQEM expert Consortium 2002
		xylophagous taxa	xyl	feed from woody debris		
		shredders	shr	feed from fallen leaves, plant tissue, CPOM		
		gatherers/collectors	gat	feed from sedimented FPOM		
		passive filter feeders	pff	feed from suspended FPOM, CPOM, prey; food is filtered from running water, e.g., by nets or specialised mouthparts		
		predators	pre	feed from prey		
		other feeding types	oth	use other food sources not meeting the above categories		
respiration	Strategies to overcome oxygen limitation	tegument	teg	respiration through the body surface	Graf	Graf
		gill	gil	respiration using special respiration organs		
forewing length	Might serve as surrogate information for the dispersal ability of the species.	max. length of male anterior wing	male max	length of the forewing in males; maximum value	Malicky, 2004	Malicky, 2004
		max. length of female anterior wing	female max	length of the forewing in females; maximum value		
emergence-flight period	The timing of emergence (following the four seasons) reflects the requirements of both the aquatic and terrestrial life stages.	winter	win	emerging mainly in winter	Graf	Graf
		spring	spr	emerging mainly in spring		
		summer	sum	emerging mainly in summer		
		autumn	aut	emerging mainly in autumn		

Table S4. Pearson correlations of each trait category to the five PCoA axes and p-values of the correlations. Strong correlations and their respective significant p-values are presented in bold.

	Correlation					*p*-value of the correlation				
Trait category	A1	A2	A3	A4	A5	A1	A2	A3	A4	A5
win	0.080	0.152	0.123	0.220	-0.137	0.224	0.020	0.060	0.001	0.035
spr	-0.046	-0.147	-0.122	0.013	-0.113	0.481	0.023	0.062	0.841	0.083
sum	-0.393	0.074	**-0.589**	-0.520	0.142	0.000	0.259	**0.000**	0.000	0.029
aut	0.422	0.001	**0.670**	0.477	-0.032	0.000	0.982	**0.000**	0.000	0.621
gra	-0.443	**-0.679**	-0.116	0.168	-0.450	0.000	**0.000**	0.077	0.010	0.000
xyl	-0.018	-0.074	-0.024	0.062	0.043	0.780	0.259	0.719	0.341	0.508
shr	**0.687**	-0.414	0.103	-0.266	0.388	**0.000**	0.000	0.115	0.000	0.000
gat	-0.182	-0.212	0.163	0.065	0.282	0.005	0.001	0.012	0.319	0.000
pff	-0.315	0.249	-0.177	0.274	0.050	0.000	0.000	0.006	0.000	0.441
pre	0.189	**0.882**	0.079	-0.082	-0.231	0.004	**0.000**	0.224	0.208	0.000
oth	-0.135	0.010	-0.038	-0.114	0.263	0.038	0.880	0.560	0.082	0.000
teg	-0.059	-0.094	-0.043	-0.047	0.103	0.370	0.151	0.515	0.475	0.113
gil	**0.771**	-0.045	-0.526	0.331	-0.019	**0.000**	0.494	0.000	0.000	0.768
male.max	0.455	-0.045	-0.047	-0.218	-0.261	0.000	0.494	0.469	0.001	0.000
female.max	**0.580**	-0.132	0.075	-0.510	-0.508	**0.000**	0.042	0.254	0.000	0.000

Table S5. SES.MPD for the 18 ERs. The standardized effect size of the MPD is in the column 'mpd.obs.z' and its respective p-value in the column 'mpd.obs.p'. SES.MPD>0 and p>0.95 imply that the observed MPD is significantly greater than the expected (phylogenetic overdispersion), whereas SES.MPD<0 and p<0.05 imply the contrary (clustering). Those values are marked in bold.

	ntaxa	mpd.obs	mpd.rand. mean	mpd.rand. sd	mpd.obs .rank	**mpd.obs.z**	**mpd.obs.p**	runs
ER1	161	1.403	1.306	0.029	9999	**3.305**	**1.000**	9999
ER2	166	1.366	1.306	0.029	9902	**2.089**	**0.990**	9999
ER3	187	1.380	1.306	0.026	9992	**2.807**	**0.999**	9999
ER4	297	1.283	1.306	0.017	904	-1.374	0.090	9999
ER5	196	1.338	1.306	0.025	8963	1.242	0.896	9999
ER6	150	1.397	1.306	0.031	9993	**2.920**	**0.999**	9999
ER7	172	1.368	1.306	0.028	9894	**2.170**	**0.989**	9999
ER8	233	1.303	1.306	0.022	4378	-0.127	0.438	9999
ER9	252	1.308	1.306	0.020	5376	0.116	0.538	9999
ER10	213	1.305	1.306	0.024	4592	-0.053	0.459	9999
ER11	168	1.309	1.306	0.029	5248	0.100	0.525	9999
ER13	211	1.340	1.306	0.024	9320	1.433	0.932	9999
ER14	221	1.302	1.306	0.023	4156	-0.172	0.416	9999
ER15	195	1.269	1.306	0.026	747	-1.462	0.075	9999
ER16	182	1.275	1.306	0.027	1237	-1.161	0.124	9999
ER18	182	1.343	1.306	0.027	9195	1.372	0.920	9999
ER20	193	1.205	1.306	0.026	6	**-3.894**	**0.001**	9999
ER22	197	1.219	1.306	0.026	11	**-3.418**	**0.001**	9999

Table S6. SES.FRic for the 18 ERs. The standardized effect size of the FRic is in the column 'FRic.obs.z' and its respective p-value in the column 'FRic.obs.p'. SES.FRic>0 and p>0.95 imply that the observed FRic is significantly greater than the expected, whereas SES.FRic<0 and p<0.05 imply the contrary. These values are marked in bold.

	FRic.obs	FRic.rand.mean	FRic.obs.z	FRic.obs.p
ER1	0.732	0.740	-0.123	0.590
ER2	0.788	0.766	0.367	0.636
ER3	0.848	0.796	0.808	0.732
ER4	0.977	0.954	0.595	0.684
ER5	0.752	0.816	-0.968	0.241
ER6	0.679	0.731	-0.871	0.184
ER7	0.716	0.775	-0.964	0.193
ER8	0.889	0.911	-0.435	0.190
ER9	0.942	0.944	-0.040	0.457
ER10	0.808	0.872	-1.065	0.245
ER11	0.702	0.790	**-1.406**	**0.039**
ER13	0.817	0.850	-0.519	0.366
ER14	0.835	0.836	-0.021	0.439
ER15	0.692	0.784	**-1.449**	**0.029**
ER16	0.744	0.764	-0.322	0.505
ER18	0.773	0.804	-0.482	0.429
ER20	0.672	0.745	**-1.243**	**0.039**
ER22	0.651	0.741	**-1.563**	**0.002**

Table S7. SES.FDis for the 18 ERs. The standardized effect size of the FDis is in the column 'FDis.obs.z' and its respective p-value in the column 'FDis.obs.p'. SES.FDis>0 and p>0.95 imply that the observed FDis is significantly greater than the expected, whereas SES.FDis<0 and p<0.05 imply the contrary. These values are marked in bold.

	FDis.obs	FDis.rand.mean	FDis.obs.z	FDis.obs.p	runs
ER1	0.212	0.214	-0.313	0.386	9999
ER2	0.214	0.214	0.038	0.515	9999
ER3	0.220	0.215	1.437	0.93	9999
ER4	0.219	0.216	**1.824**	**0.964**	9999
ER5	0.218	0.215	0.809	0.787	9999
ER6	0.211	0.213	-0.308	0.387	9999
ER7	0.215	0.214	0.170	0.558	9999
ER8	0.217	0.215	0.451	0.67	9999
ER9	0.217	0.215	0.704	0.756	9999
ER10	0.220	0.215	1.558	0.939	9999
ER11	0.211	0.214	-0.930	0.181	9999
ER13	0.218	0.215	0.883	0.814	9999
ER14	0.208	0.215	**-1.855**	**0.036**	9999
ER15	0.207	0.214	**-1.625**	**0.047**	9999
ER16	0.208	0.214	-1.341	0.09	9999
ER18	0.218	0.215	0.830	0.799	9999
ER20	0.210	0.213	-0.577	0.282	9999
ER22	0.208	0.213	-1.179	0.123	9999

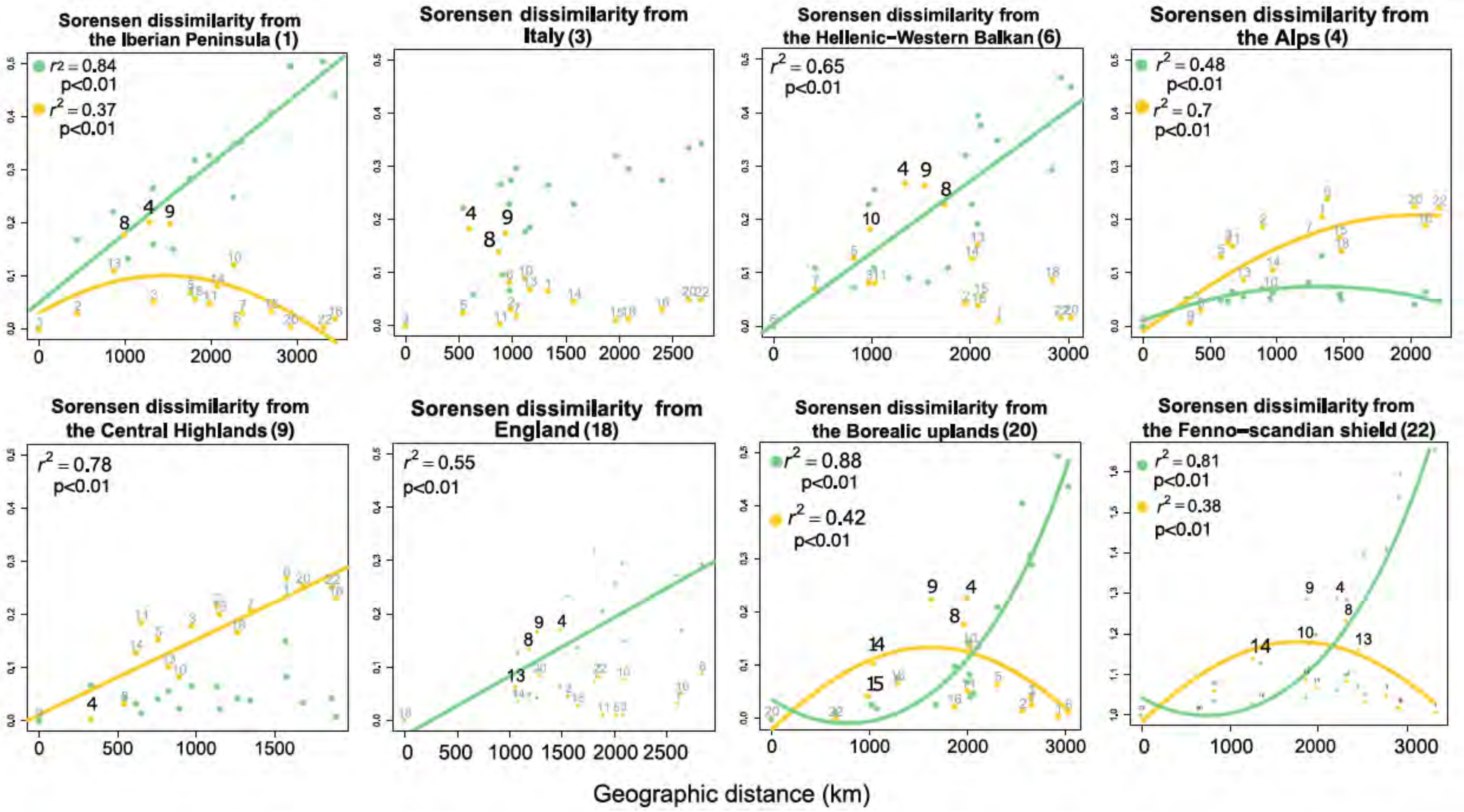

Figure S2. Sorensen β-TD discomposed into the turnover (green) and nestedness (yellow) components as a function of geographic distance, starting from different ERs. Results were the same for β-PD.