On the origin and assembly of communities:

Diversity patterns of Trichoptera across a latitudinal gradient in Europe

Caddisfly larva coloured pencil by Gina Mikel
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Master in Ecology, Environmental Management and Restoration

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ABSTRACT

A fundamental question in macroecology is how current and historical environment, species interactions, physiological constraints and evolutionary processes merge to determine community structure and shape the current distribution of taxa. To elucidate the role that historical and current factors have been playing in establishing patterns of regional diversity, we assessed how species, functional traits and phylogenetic composition of Trichoptera changed among 10 communities within 6 regions located along a latitudinal gradient from Morocco to Sweden. We found a unimodal distribution of α- and γ-diversity being the highest diversity at the Carpathians but also North Iberian Peninsula, which is against the main expectation of decrease of diversity as latitude increases. Despite this and the fact that compositional β-diversity showed little differences across the gradient, phylogenetic relatedness among genera within regions differs from low to high latitudes. The phylogenetic structure of northern communities is clustered, i.e., communities are more phylogenetically related than in the null model, whereas southern communities are overdispersed, i.e., communities are less related than in the null model. Furthermore, traits were found phylogenetically conserved and widespread distributed across the latitudinal gradient. Hence, these results are consistent with the hypothesis that colonization following glaciations in the Pleistocene originated northern communities from southern but also from central Europe refuges, which contrasts to what is widely accepted. Herein, the integration of ecology and evolution at the community level explain how Trichoptera lineages have been originally assembled in Europe. Overall the latitudinal pattern of phylogenetic diversity and structure of communities can be explained by historical environmental constraints that together with phylogenetic niche conservatism are the key factors that promote current diversity distribution of Trichoptera across Europe.

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INTRODUCTION

Why different species are distributed the way they are and how we can explain the global patterns of biodiversity is a recurring debate in evolutionary biology that many ecologists are trying to answer since a long time ago (Gentry, 1982). To examine macroecological patterns of diversity for understanding the evolutionary processes underlying current patterns of species diversity, three approaches must be integrated at the community level: phylogeny, trait information, and species assemblage at both local and regional scales (Webb et al., 2002).

Resolving contingencies in community ecology requires comparative studies of local communities along broad-scale environmental gradients. Composition of local-scale communities depends upon the regional species pool (Lessard et al., 2012), and the composition of the regional species pool is, in turn, determined by large-scale biogeographical processes (Ricklefs & Schluter, 1993; McPeek & Brown, 2000). Some species have certain biological traits and ecological preferences to inhabit a specific region and use a specific habitat, whereas others present in the same region may fail handling the local ecological requirements and they are filtered out. Species may persist within a community if minimize intra- and interspecific competition for resources ("habitat partitioning"; Wisheu, 1998) because species that have similar biological traits and habitat preferences lead to high overlapped ecological niche promoting competitive exclusion (Hutchinson, 1959). Phylogenetically close related species are more likely to share traits, a pattern known as phylogenetic niche conservatism (Harvey & Pagel, 1991), whereas phylogenetic divergence increases in parallel trait differentiation (Darwin, 1859). Niche conservatism may be due to active, stabilizing selection (Lord et al., 1995) or to a reduction in the potential range of ecological character evolution caused by the fixation of ancestral traits (Westboy et al., 1995), but trait disparification occurs as the lineage diversifies over time, and it can be reconstructed on a phylogeny (Pagel, 1999). Phylogenies linked together with the distribution patterns of communities and traits disparity are used to infer the historical patterns of movement and distribution in the taxa (Webb et al., 2002; Múrria et al., 2012). For instance, the phylogenetic community structure is phylogenetically clustered when species co-occurring in a local community are phylogenetically more closely related than one would expect from a random sampling of species. Alternatively, over-dispersed is expected when species co-occurring in a local community are phylogenetically more distantly related than one would expect from a random sampling of species from the larger regional pool (Pyron et al., 2014).

The high habitat heterogeneity across the Globe offers a huge variety of climates, ecosystems and habitats leading to an enormous variability in biota lifestyles that occupy these areas depending on acquired traits and adaptability. However, similar habitats in different regions do not share the same species composition and abundances because of the differing evolutionary history and biogeography. For instance, areas differ in the length of time they have been occupied and therefore lineages have variable time constraints, or singular local ecological processes, connectivity and geographic barriers (Blondel & Vigne, 1993; Latham & Ricklefs, 1993) for diversification and disparification (Brown et al., 2000; Ricklefs et al., 2005). Compositional differences among communities are quantified using β-diversity, which increases when species assemblages highly vary between two regions due to changes in habitat conditions or biogeographical history (Anderson et al., 2011). Phylogenetic distances between individuals can also change in relation to the time of divergence, geographical range or ecological heterogeneity, and therefore, phylogenetic β-diversity varies depending on the phylogenetic distance of co-occurring species among communities in the same way as compositional β-diversity varies when local communities are assembled by different taxonomical composition. These dissimilarity measures are used to infer current patterns due to historical factors and processes (Kraft et al., 2011; De Cáceres et al., 2012; Myers et al., 2013). Here, we want to test evolutionary hypotheses linking together biogeography, phylogeny, character evolution and niche structure in order to understand how macroevolutionary and macroecological processes shape assemblages at large geographical scales, in particular, across a latitudinal gradient in Europe.

There is a well-established pattern shared between a wide range of taxa to find high diversity levels in south regions whereas this diversity decreases in the northern areas (Gaston, 2000; Hillebrand, 2004; Jablonski et al., 2006; Willig et al., 2003). At global scale, the latitudinal gradient in species richness must be a consequence of a greater period of diversification in the tropics than temperate regions, with higher and more stable temperatures leading to high metabolic rates, and hence shorter generation times and higher mutation rates (Gaston, 2007). The accumulation of species at higher latitudes may have been limited by the historical adaptation of most clades to tropical environment that reduces northward expansion (Kerkhoff et al., 2014). In Europe, current geographical patterns are intimately associated to cyclic glaciations and ice retreat during the Pleistocene (approximately 2–0.01 Ma ago) that resulted in exchanges and mixing of organisms between northern and southern European regions (Hewitt, 1999; Hewitt, 2000; Hewitt, 2004). The latter acted as a refuge and origin for successive northern colonization following the glacial periods, yielding speciation and species accumulation (Ribera & Vogler, 2004). However, post-glacial recolonization of northern regions can be incomplete due to environmental filtering, dispersal limitation of organisms and the limited time since the last glacial maximum (LGM, 21000 years ago), so the richness and composition of previously glaciated areas may be affected (Svenning & Skov, 2007; Hortal et al., 2011). These historical events should contribute to shaping current patterns of diversity in Europe (Bonada et al., 2009; Mathieu & Davies, 2014; Ricklefs, 2007).

The aim of the study is to investigate patterns of Trichoptera diversity across a latitudinal gradient in Europe and, secondly, to elucidate the underlying mechanism that have originated the distinct communities. Trichoptera is a well-studied freshwater aquatic insects group in terms of phylogenetic relatedness, distribution, ecological niche preferences and life-history traits; for this reason, caddisflies are a model organism for studying patterns of phylogenetic structure, functional traits disparity and community composition associated to evolutionary history and biogeography. All in all, we note three points to achieve this objective: (1) to discover patterns of genus composition and distribution within and among regions across a latitudinal gradient in Europe. Concretely, we want to know if there is a gradient of diversity at the taxonomical, functional or phylogenetic levels and how these levels relate among them. Secondly, to infer what ecological or evolutionary processes are the responsible of the current distribution of diversity across Europe and how the origin of these communities at both large spatial and temporal scale can be explained. (2) To test niche conservatism and phylogenetic signal in order to explain latitudinal gradients in diversity, answering if ancestral traits have been retained or not and how traits evolved across the latitudinal gradient. (3) To assess the phylogenetic distances among species within and across communities and which ones are more phylogenetically overdispersed or clustered to test niche filtering from South to North following recolonization after the cyclic Pleistocene glaciations. For all three objectives, the building of a phylogenetic tree at genus level of all European Trichoptera is a fundamental tool which will be useful for future research in testing eco-evolutionary questions on Trichoptera. To test these hypotheses on the origins of communities and how co-occurrence or biologic traits could influence it, two possible explanations are proposed. One refers to the neutral theory of ecology, which considers that current patterns of diversity are explained by geographic distance between species (Hubbell, 2001). Alternatively, the second considers that species functional traits pass environmental filters and species interact with other individuals (Wiens & Graham, 2005; Pearman et al., 2008). This two contrasting processes underlie community assembly and have phylogenetic consequences. Under the assumption of ecological non-equivalence and that niche is a phylogenetically conserved trait, phylogenetic clustering is explained by environmental filtering at the local scale whereas local overdispersion is due to competitive interactions (Emerson & Gillespie, 2008). Basically, we will know if the processes underlying the present distribution of species are associated with species-neutral or niche-based assembly model. Figure 1 is a scheme to clearly explain the hypothesis.

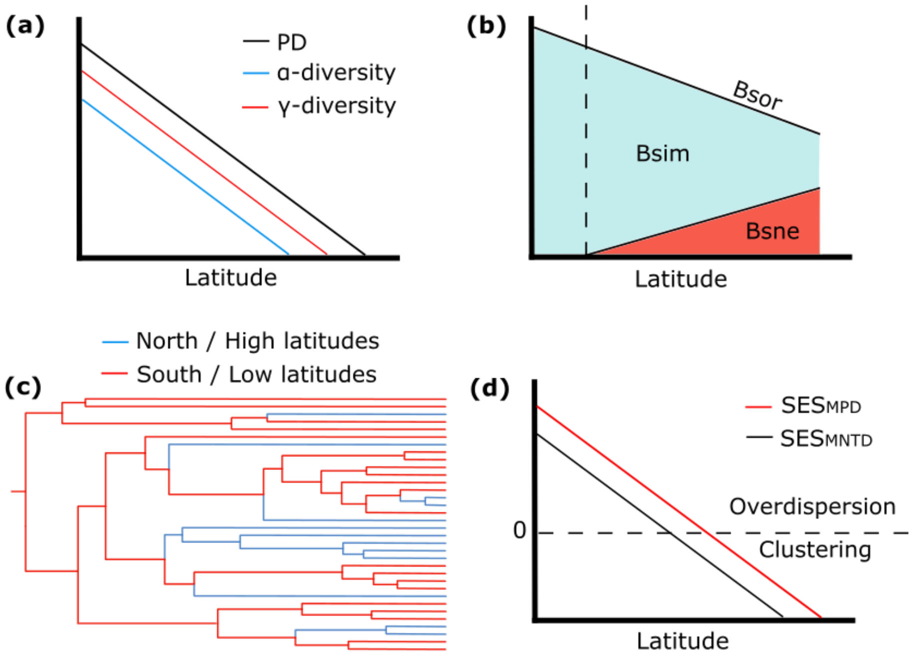

Fig. 1: Scheme of the main hypothesis of the study. (a) The phylogenetic distance (PD), α- and γ-diversities are expected to decrease towards northern latitudes. (b) Total β-diversity (Bsor) is also expected to decrease due to species turnover (Bsim) in lower latitudes and nestedness (Bsne) in the highest latitudes. (c) Only few linages of the phylogeny would have the effective functional traits to colonize and cope harshness in north latitudes, and therefore (d) SES$_{MPD}$ and SES$_{MNTD}$ would have a negative slope in relation to latitude. Values upper 0 indicate overdispersion whereas values lower 0 show clustering processes.

Assuming a diversity gradient across latitude, it is expected relatively continuous increase in richness towards lower latitudes (fig. 1a). α- (local diversity), γ-(regional diversity), habitat, trait, functional and phylogenetic diversity is expected to be higher in regions at southern European latitudes, such as the Iberian Peninsula, than in higher latitudes because southern regions are typified by their physiographical heterogeneity, its geographical situation in south-western Europe near Africa, and the fact that it served as a refuge during the Quaternary Glacial Age (Vargas et al., 1998). Also, at southern latitudes is expected high number of distant related endemic species by allopatric speciation (stable habitat) and long-term accumulated speciation. In contrast, in northern Europe will inhabit species phylogenetically nearby, originated in southern Europe but recolonized and diversified in northern latitudes following GLM surpassing environmental and dispersion filters. In relation to partition of β-diversity, Simpson index and nestedness component are supposed to be inverses. Baselga (2010) suggests that dissimilarity among communities is partitioned by two indexes that indicate different causes of β-diversity: Simpson index refers to dissimilarity due to species turnover, whereas the other component explains nestedness because the number of species falls with increasing latitude, without species replacement. The sum of both gives us the Sørensen β-diversity, which decreases as latitude increases due to less habitat richness (fig. 1b). Assuming niche conservatism, the phylogenetic distance is also lower in northern areas because it is expected that individuals living in these habitat conditions will have similar traits acquired in a few particular lineages of the phylogeny (fig.1c). If traits are phylogenetically conserved, a clustering phenomenon (coexistence of close relatives) is expected in north communities by environmental filtering that should favour close related species with high ecological niche overlapping of suitable traits such as cold tolerance (fig. 1d). At southern latitudes, the communities should be phylogenetically overdispersed (coexistence of distant relatives) because the expected long-term diversity accumulation in multiple lineages in the phylogeny should result in an increase of taxonomical variability across the whole phylogenetic tree (Hortal et al., 2011).

MATERIAL AND METHODS

Study area

The study targeted a total of 62 sites included in six biogeographical regions with comparable altitude distributed across Europe following a latitudinal gradient (Múrria et al., in preparation). These locations are, from South to North: Rif (Morocco), Betic Region (SE-Iberian Peninsula), Picos de Europa (N-Spain), Jura (France-Switzerland), Carpathian Mountains (Slovakia) and Jämtland (Sweden) (fig. 2). Geographic distances between sampling locations were dated to enable examining geographic-scale population genetic structure. The samples were conducted in 2008 during two different seasons in order to achieve the greatest annual diversity of freshwater macroinvertebrates possible: spring and autumn. All habitats were sampled, including riffles and pools, using a 250 μm mesh kick net along 150 m reach. Furthermore, three quantitative samples were randomly collected using a cylindrical Hess Sampler of 500 μm mesh size net. Back to laboratory, taxonomical composition abundances of the six regions were determined. All Trichoptera individuals were identified to genus level (Vieira et al., 2006; Tachet et al., 2010) and abundances of each taxon were recorded following a certain rank code: 1 from one to three individuals, 2 from four to 10 individuals, 3 from

11 to 100 individuals, and 4 for more than 100 individuals. For molecular analysis, samples were preserved in absolute ethanol whereas formalin was used for quantitative samples.

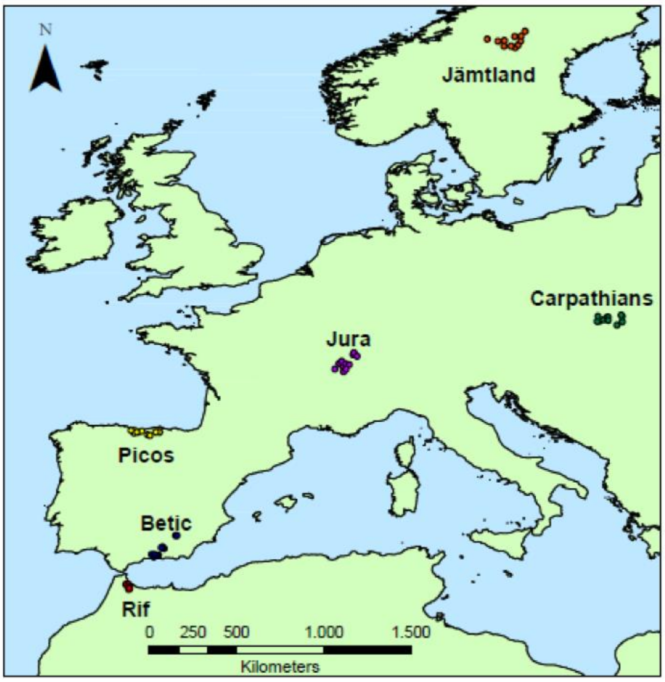

Fig. 2: Map showing the 62 sampling sites distributed across the 6 regions across the latitudinal gradient in Europe.

Community composition

To explore community assemblage composition, α- and γ-diversities were calculated as the local and regional species richness, respectively. Rarefied α-diversity was used to compare region with different abundance. Observed β-diversity measures how species composition changes across communities, and this value is compared to a null-model to account for the statistical dependence on γ-diversity and determine if observed β-diversity deviates from the expectations of a random (stochastic) assembly process (Chase & Myers, 2011) and whether the magnitude of environmental and spatial processes varies among regions along latitudinal gradients (Kraft et al., 2011). Two indexes were used to measure the dissimilarity among sites within a region: (1) Hellinger distance and its total variance within a region were used as the average dissimilarity between sampling units (De Cáceres et al., 2012; Legendre et al., 2013). Because the rank of abundance data at the community level, the null model used ran 1000 randomizations of the presence of species in a region based on the total richness for the region that had the lowest diversity (Jura), while keeping the number and abundances distribution of each species in that region. This method is independent of the value of α- and γ-diversity for each region. (2) The other index measures β-diversity as the multiplicative β-partition ($\beta=1-\bar{\alpha}/\gamma$) (Whittaker, 1960) and a null model that randomly shuffles individuals among sites while preserving γ-diversity and the number of individuals per site for 1000 iterations was run (Kraft et al., 2011; Myers et al., 2013).

Finally, in order to reflect the two different phenomena of variation of the species composition among assemblages, "beta partition" package in R (Baselga & Orme, 2012) was executed to partitioning β diversity into turnover (β_{SIM}) and nestedness (β_{NES}) components, which are additive fractions of β_{SOR} (Sørensen index) (Baselga, 2010). Correlations between diversity and latitude were calculated using Spearman's rank correlation coefficient because of the continuous variation of the diversity across the latitudinal gradient. Pearson's product-moment correlation was used as a measure of the degree of linear dependence between diversity variables. In order to determine possible indicator species for any specific region, the function multipatt included in R package "indicspecies" (De Cáceres, 2013) was conducted to study community assemblages from another perspective because considers endemism and homogeneity within composition. The criteria that determines which species are chosen as indicators is when (1) reflect the biotic or abiotic state of the environment, (2) provide evidence for the impacts of environmental change, and (3) predict the diversity of other species, taxa or communities within a region.

Phylogenetic analyses and DNA sequencing

The list of Trichoptera genera in Europe was built using the information at the checklist of European caddisflies from Freshwater Ecology website (http://www.freshwaterecology.info/index.php). The final dataset included a total of 129 genus spread over 23 families.

DNA sequences of several European species per genus were compiled from GenBank, and all available genes were downloaded to get as much information as possible for further gene and species selection. The most frequent sequenced genes collected in the genetic database were selected resulting in the mitochondrial cytochrome *c* oxidase subunit I gene (COI*;* 657 bp), the 18S ribosomal RNA (541 bp), and five nuclear genes: wingless (*wg;* 512 bp), cadherin (CAD*;* 850 bp), isocitrate dehydrogenase (IDH*;* 720 bp), polymerase II (POLII*;* 772 bp) and elongation factor-1 alpha (EF-1α*;* 1099 bp). Adding more genes increases phylogenetic accuracy and robustness because the degree of genetic conservation and variation in the substitution rates differs among these genes, and this is informative on the different lengths of the tree branches (San Mauro & Agorreta, 2010). Additionally, authors that collaborate in the DNA-barcode project

“Trichoptera barcode of Life” were request for unpublished COI sequences to cover as much genera missing in GenBank.

To cover an even larger part of the phylogeny, one specimen per each genera of *Wormaldia* (Location: R4. Rif; Date of sampling: 23/11/2008), *Lithax niger* (Location: C7. Carpathians; Date of sampling: 06/06/2008), *Glossosoma* (Location: C5. Carpathians; Date of sampling: 05/06/2008), *Allogamus mortoni* (Location: Río Prado Negro, Betic; Date of sampling: 30/10/2007), and *Lasiocephala* (Location: B09. Betic; Date of sampling: 07/04/2008) were sequenced. DNA was extracted from the abdominal segments II-V of individuals collected. The cycling profile of polymerase chain reaction (PCR) to amplify loci was performed under the following conditions: an initial denaturation step for 5 min at 94ºC; 35 cycles of 30 s at 94 ºC, 30 s at 50 ºC, 45 s at 72 ºC; and a final extension of 10 min at 72 ºC (modified from Múrria et al., 2012). Each 20 µL reaction contained 5 µL of Red Buffer, 0.2 µL of Taq-Polymerase, 1 µL of primer (x2) and 11.8 µL of water. PCR products were visualized by ultraviolet light on a 1% agarose gel checking for positive bands before DNA sequencing. Forward and reverse primers were used to sequence each region in both directions. Primers LCO1490 (5’ GGT CAA CAA ATC ATA AAG ATA TTG G 3’) and HCO2198 (5’ TAA ACT TCA GGG TGA CCA AAA AAT CA 3’) (Folmer et al., 1994) were used for amplifying COI. *Wg* was amplified using LepWG1 (5’- GAR TGY AAR TGY CAY GGY ATG TCT GG -3’) and LepWG2 (5’- ACY ICG CAR CAC CAR TGR AAT GTR CW -3’) (Silva-Brandão et al., 2008), EF1α was amplified using Kjer1F (5’-ATC GAD AAG TTC GAG AAG GAR GC-3’) and Kjer1R (5’-CCA HCC YTT RAA CCA NGG CAT-3’) (modified from Kjer et al., 2001). Forward and reverse strands were sequenced using Big Dye Terminator version 3.1 Cycle Sequencing Kit Protocol (Applied Biosystems). Sequences were assembled and edited using Geneious (Biomatters, Auckland, New Zealand). Primer sequences were removed from the beginning and end of each sequence and sequence data were checked for accuracy by matching forward and reverse sequences for each gene region. Only unambiguous sequences without double peaks were included in the study, also the generated COI for *Glossosoma* was removed because the shorter length than the downloaded sequence. DNA sequenced were aligned in MAFFT 7 (Katoh & Standley, 2013) using the FFT-NS-2 strategy (Fast; progressive method).

To select best-fit partitioning schemes and models of molecular evolution for phylogenetic analyses, Partition Finder (Lanfear et al., 2012) was used. The models of sequence evolution were implemented for different partitions of it, such as the different codon positions of a protein-coding gene. The analyses of the single genes as well as the concatenated data set were performed with each codon position of each gene sequence treated as one partition ("fullcodon" code), in exception of 18S because it is ribosomal. Individual models of nucleotide substitution were specified for each gene partition and the best model of substitution for each partition was determined using the AIC (Akaike Information Criterion). Gene partitions were combined in a single data supermatrix using MEGA 5.0 (Tamura et al., 2011). Two methods of phylogenetic inference were used to reconstruct phylogenetic relationships: maximum likelihood with RAxML (Randomized Axelerated Maximum Likelihood) (Stamatakis et al., 2008) and Bayesian was conducted using MrBayes 3.2.5 (Ronquist & Huelsenbeck, 2003). The likelihood of a phylogenetic tree is the probability of observing the data given the tree and the used Gamma model of evolution. Like maximum likelihood, Bayesian analysis allows complex models of sequence evolution to be implemented for the whole sequence dataset, and for different partitions of it. However, Bayesian approaches rely on Markov chain Monte Carlo (MCMC) procedures for searching the "best set of trees" in the landscape of possible trees (Hastings, 1970), whereas the maximum likelihood searches for a single most likely tree. Bootstrapping was used to know how strongly the data supports each of the relationships depicted in the maximum likelihood tree. As conservative measures of node support, a value of 80% or greater might indicate substantial confidence for a group. In the Bayesian inference, posterior probabilities should only be considered reliable if greater than 0.95.

Analyses of trait variation

Functional trait data and ecological preferences of European caddisflies were extracted from Tachet et al. (2010). Uninformative categories of traits were removed which resulted in a matrix including 11 biological traits that comprise 50 categories. A fuzzy coding approach was used to quantify the affinity of each genus for each trait category according to specific requirements of available information of as much species as possible within the genus and their geographical variance to take into account potential trait plasticity (Chevenet et al., 1994). All scores were standardised so that the sums for a given genus and a given trait were 1. Trait categories variance for each genus was measured conducting Fuzzy Principal Component Analysis (FPCA, Chevenet et al., 1994) in order to visualize how traits and families were distributed based on their trait composition.

Evolutionary niche conservatism

To explore the phylogenetic basis of community niche structure (character evolution) and to establish if related taxa are more similar than expected by chance and also if niche preferences are affected by the convergence of trait evolution, the correlation of ecological trait variation with the phylogenetic tree was tested. Trait variation associated to phylogenetic distance (niche conservatism) was measured using Pagel’s λ parameter (Pagel, 1999; Freckleton et al., 2002) based on maximum likelihood phylogenetic tree. If the value of λ goes towards 1, it means that the internal branches retain their original length indicating that there is a strong correlation between trait and the tree, so niche is preserved. Similarly, when the estimate of λ is close to 0 means trait evolution has not followed the tree topology. For comparison, Blomberg’s K-statistic (Blomberg et al., 2003) was also measured. This method tests phylogenetic signal, a quantitative measure of the degree to which phylogeny predicts the ecological similarity of species or, in other words, the tendency of related species to resemble each other. The higher the K statistic, the more phylogenetic signal in a trait. In the same way as Pagel’s λ, K values of 1 indicates that trait disparification follows the topology of the tree, which implies some degree of phylogenetic signal or conservatism of traits, whereas a little K means trait variation is independent to the phylogeny, which corresponds to a random or convergent pattern of evolution. The principal difference between the two methods is that Pagel’s λ compare all branches while Blomberg’s K compare pairs of branches. The test of significance also differs: Pagel’s λ uses likelihood ratio tests against simpler models but Blomberg’s K make randomizations of the original trait data, comparing signal in a trait to the signal under a Brownian motion model of trait evolution on a phylogeny, that is to say, with stochastically equal rates along each branch. The associated test statistics compares the original data to a null distribution obtained by randomly assigning the trait data to the tips of the phylogenetic tree. These analyses were carried out using two libraries of the R package: “geiger” (Harmon et al., 2008) and “picante” (Kembel et al., 2010).

Community phylogenetic structure

In order to elucidate if the distribution of species among habitats or samples in a community is non-random with respect to the phylogeny, the phylogenetic distance (PD) in each region was calculated as the sum of the lengths of those branches spanned by the tree that include all taxa in a local community. Another way of thinking about the phylogenetic relatedness of genera within a community is to relate the patterns observed to what is expected under various null models of evolution during community assembly. This approach provides evidences on the phylogenetic distance of each pair of species that co-occur within a community using two different measures of phylogenetic distance: the net relatedness index (NRI) and nearest taxon index (NTI). These indexes were estimated using the measures of standardized effect sizes (SES) of the mean pairwise distance (MPD), and the mean nearest taxon distance (MNTD), respectively. MPD is the observed phylogenetic relatedness between all genera in each community whereas

MNTD is the mean minimum phylogenetic distance separating each species in the community from its closest relative. The SES_{MPD} and SES_{MNTD} are equivalent to -1 times NRI and NTI respectively (Webb et al. 2002). These SES functions were weighted by its abundance, and the observed MPD and MNTD were compared to the null distribution of MPD and MNTD generated by 9999 randomizations under both the “sample.pool” and the “independentswap” null models for comparison. The first one randomizes community data matrix by drawing species from pool of species occurring in at least one community (sample pool) with equal probability, and the second randomizes community data matrix with the independent swap algorithm (Gotelli, 2000) maintaining species occurrence frequency and sample species richness (Kembel, 2009). Positive SES values indicate phylogenetic overdispersion (species more distantly related than expected), whereas negative values indicate clustering or that co-occurring genera are more closely related than expected by change (Webb et al., 2002). Maximum conservatism in traits yields both low SES_{MPD} and SES_{MNTD}. Conservatism at more terminal levels in the phylogeny causes SES_{MNTD} to increase in significance relative to SES_{MPD} (Webb, 2000; Kembel & Hubbell, 2006; Swenson et al., 2006).

Finally, a measure of phylogenetic β-diversity was calculated using MPD and MNTD between pairs of taxa from two distinct communities. Phylogenetic β-diversity measures the amount of no shared phylogenetic history between two communities in the same way that taxonomical β-diversity measures the dissimilarity in species composition across sites. The observed phylogenetic β-diversity can be high when the shared history between two communities is small relative to average shared history among random communities; in essence when species in the two communities are distantly related. This may occur along a strong environmental gradient if traits that influence existence along it are conserved, so competition influences the local composition. Alternatively, if phylogenetic β-diversity is smaller than that expected based on compositional β-diversity then communities compared are composed mostly of closely related species. This should occur along environmental gradients or biogeographic barriers that promoted speciation events for a subset of the regional phylogeny (Graham et al., 2009). These analyses were carried out using “picante” package in R (Kembel et al., 2010).

RESULTS

Assemblage composition

α- and γ-diversities are correlated ($R^2 = 0.73$; p-value = 0.03) but do not follow a lineal negative pattern as it was hypothesized. Instead, local and regional diversities are continuous along the latitude, without showing a marked slope (rho = 0.2, p-value = 0.714 and rho = -0.2, p-value = 0.714, respectively) (fig. 3a), but the trend is rather unimodal being Picos de Europa (27 genera) and Carpathians (26 genera) the richest communities. In contrast, Jura has the poorest diversity (21 genera). Overall Trichoptera are well-distributed around Europe and we can find the vast majority of genera in every region (see figure S1). Observed and expected β-diversities are very similar within regions across the gradient (fig. 3b). However, expected β-diversity for the Whittaker indexes in southern areas was lower than observed indicating a highest number of species per area than expected, whereas in northern communities expected β-diversity is higher than observed, which means that there are aggregation factors effective during species assembly. The exception was Jura, where the observed and expected values were identical indicating no aggregation or dispersion events so the distribution of genera in this region is random with little interaction among species. Although not significant, regional β-diversity seems to decrease as latitude increases (rho = -0.714, p-value = 0.1361 for Whittaker's index; rho = -0.543, p-value = 0.2972 for Legendre's index); in other words, northern communities were more similar within a region than those located at lower latitudes. The partition of β-diversity showed low contribution of the nestedness to the total β-diversity (10.91%), whereas Simpson index (turnover component of β-diversity) participates till 89.09% (fig. 3c). Neither total β-diversity nor the nestedness and turnover components correlate with geographic distance (rho = 0.45, -0.375 and 0.436; p-values = 0.09435, 0.1691 and 0.1038, respectively), which does not support the second hypothesis.

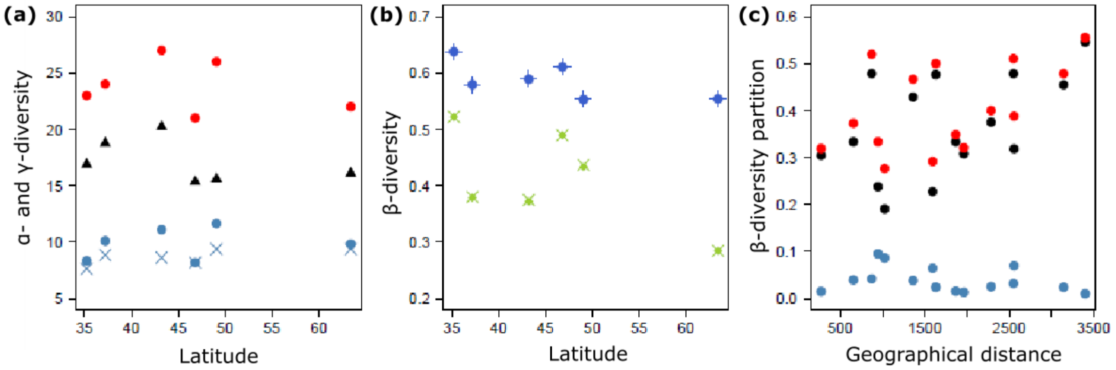

Fig. 3: Community composition. (a) α- and γ-diversity in relation to latitude. Observed γ-diversity (red dots), mean α-diversity (observed: blue dots; expected: blue cross x), phylogenetic distance (black triangles). There is no correlation with latitude. (b) β-diversity values using two indexes by Whittaker (observed: blue dots; expected: blue cross +) and the sums of squares (observed: green rhombus; expected: green cross x) along the latitudinal gradient. There is no correlation with latitude. (c) Partition of the Sørensen β-diversity (red dots) into turnover (black dots) and nestedness (blue dots) components along the latitudinal gradient. Any partition of β-diversity is correlated with latitude.

Indicator species analyses show that Rif was the region with more endemism species (A=1) followed by Carpathians (table 1). Although not sampled in all region sites, three genera (*Calamoceras*, *Schizopelex* and *Mesophylax*) were found only in the lowest latitude region, while *Lithax* genus inhabits Carpathian region. Betic region had *Athirpsodes* as an indicator genus but was also found in other regions. Other regions had no endemic species, individually, but the combination of two nearby regions can cover the distribution area of a single genus (e.g., *Stenophylax* in Rif and Betic, and *Melampophylax* in Jura and Carpathians).

Group	Genera	A	B	stat	p-value
R	Calamoceras	1.0000	0.6667	0.816	0.001
R	Schizopelex	1.0000	0.6667	0.816	0.001
R	Mesophylax	1.0000	0.4444	0.667	0.001
B	Athripsodes	0.7122	0.4000	0.534	0.019
C	Ecclisopteryx	0.8571	0.6364	0.739	0.001
C	Lithax	1.0000	0.2727	0.522	0.023
C	Halesus	0.8000	0.2727	0.467	0.043
R + B	Stenophylax	1.0000	0.2632	0.513	0.012
J + C	Drusus	0.9138	0.6818	0.789	0.001
J + C	Melampophylax	1.0000	0.5455	0.739	0.001
J + C	Glossosoma	0.8532	0.5909	0.710	0.001

Table 1. Indicator genera of each region or combination of regions based on "Indval.g" index. Group code: R, Rif; B, Betic; J, Jura; C, Carpathians. Component A: probability of the surveyed site belongs to the target site group given the fact that the genus has been found (the specificity or positive predictive value of the species as indicator of the site group). Component B: probability of finding genera in sites belonging to the site group (the fidelity or sensitivity of the species as indicator of the target site group). Only significant p-values are shown.

Phylogeny

Fullcodon RAxML phylogenetic tree was built using 7 genes for a total of 105 genera out of a total of 129 genera recorded in Europe (taxonomic coverage ~ 81.4%), belonging to 22 families (missing only one family, Ptilocolepidae). It was not possible to complete the entire list of European caddisflies and some genera such as *Martynomyia* or *Tricholeiochiton* were not included in further analysis due to a lack of molecular data. On the contrary, for five genera two species were used to verify the position of these taxa in the tree (*Apatania*, *Brachycentrus*, *Crunoecia*, *Mesophylax* and *Oligotrichia*). Hence, the total data set comprises 114 taxa combined into a 5151 bp alignment including four outgroup species that cover the early lineages within Lepidoptera (Malm et al., 2013; Kjer et al., 2001). COI gene covers 97 genera (75.19%), CAD gene covers 42 genera (32.56%), 18S and POLII genes cover 38 genera (29.46%), *wg* covers 37 genera (28.68%), IDH gene covers 36 genera (27.91%) and, finally, EF-1α gene covers 32 genera (24.81%).

The final phylogenetic tree was reconstructed using maximum-likelihood method (fig. 4) because it was better resolved than by Bayesian inference, especially for Limnephilidae, which is the most species-rich Trichoptera family. This is outlined with the three highly supported suborders (Annulipalpia, Spicipalpia and Integripalpia). The four outgroups genera were also in the correct position. Integripalpia can be divided into two infraorders (Plenitentoria and Brevitentoria), both well supported except of the Plenitentoria genus *Odontocerum.* Spicipalpia is not a monophyletic group because it is consisting of two branches as reported before (Holzenthal et al., 2007; Kjer et al., 2001). Note that all genera were well classified within the taxonomical family and also that every repeated genus is coupled together and strongly supported, giving robustness to the topology. All in all, the tree is highly concordant with previous taxonomic classifications (Holzenthal et al., 2007; Johanson et al., 2012; Kjer et al., 2001; Malm et al., 2013).

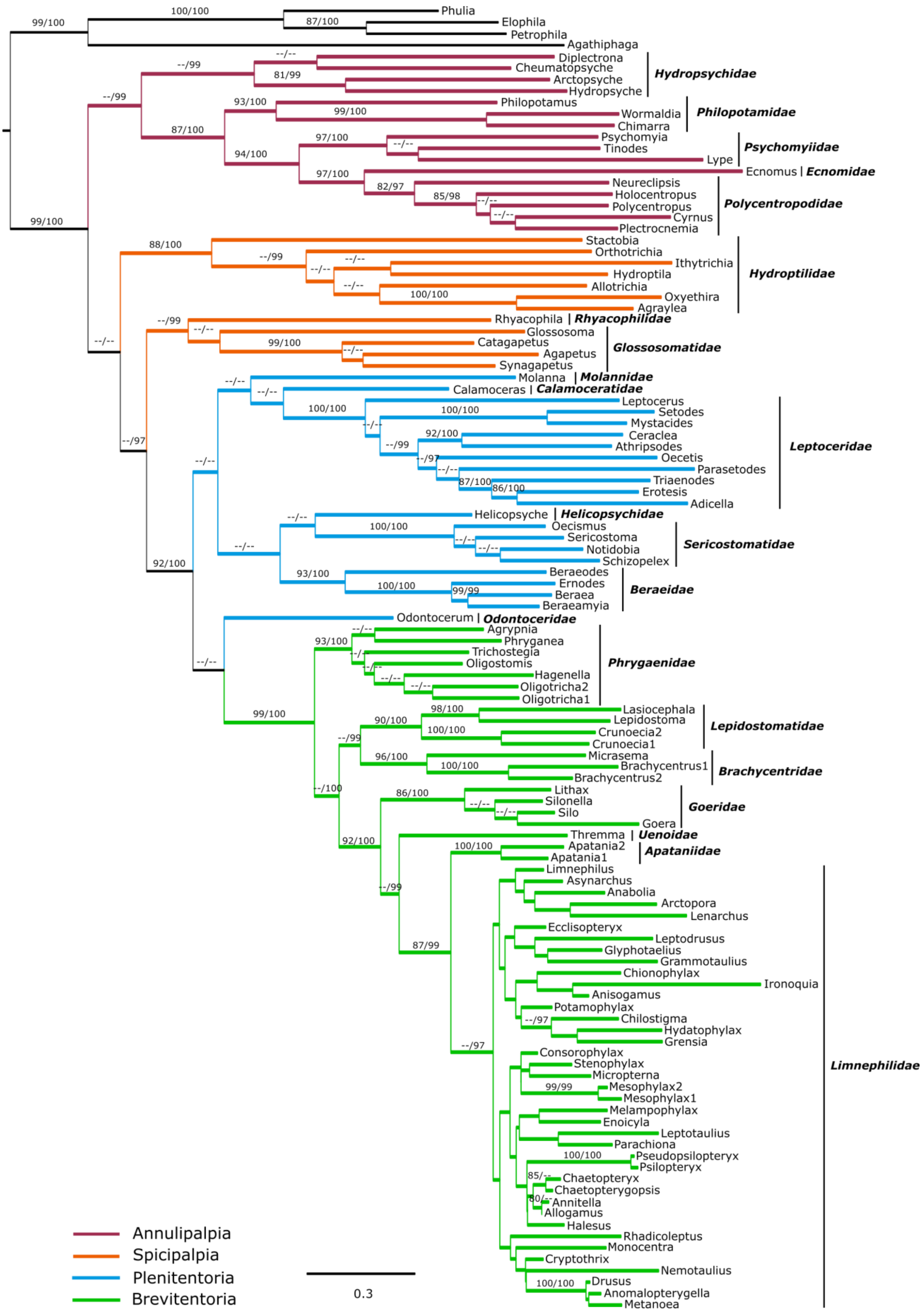

Fig. 4: Maximum-likelihood phylogenetic tree of seven genes of Order Trichoptera including 105 European genera (out of 129) belonging to 22 families. Comprise 114 taxa including 4 outgroup genera of Lepidoptera. Branch support percentage is indicated: maximum likelihood bootstrap (>80) / Bayesian inference (>95), - - refers to clades that appeared in the analysis but with support values below levels indicated above. Suborder classification is given by colored branches: Annulipalpia, Spicipalpia and Integripalpia (Plenitentoria and Brevitentoria).

Ecological niche and trait evolution

The FPCA of traits disparity within genera revealed how ecological characters are distributed among families (fig. 5). The total variability explained by the first two axes is 46.33% (33.08% and 13.25%, respectively) and the eigenvalues are 0.15 and 0.06, respectively. To distinguish the degree of separation among trait categories, and therefore the contribution of each trait to a given axis, the categories of traits that explain greater amount of variance along the axis scores were indicated (fig. 5a). The first axis differentiates small size individuals temporarily attached to substrate from shredders and crawlers, whereas the second axis separates small size individuals with only one potential reproduction cycle per year from the larger predators. The colours gradient of each family across the first axis (fig. 5b) had the same order as the tree topology (fig. 4), which indicates that each family is characterised by a certain biological and ecological preferences and identity, therefore species diversification presumably originates new functional traits with which lineages are able to adapt to habitat changes, linking ecological requirements with cladogenesis. In fact, multivariate analysis of variance indicates significant differences between suborders in relation to traits diversification (p-value = 2.319e-15). Phylogenetically closely related species showed similar and commonly overlapping ecological niche space on the PCA axis 1 (fig. 5). Pagel's λ in both FPCA axes was close to 1 (0.95 and 0.81, respectively), which means that internal branches retain their original length indicating strong trait correlation with the phylogenetic tree. In regards to K statistic, results corroborate this pattern only on the first axis which was greater than 1 (K = 1.66, p-value = 0.001), indicating strong phylogenetic signal on traits conservatism. However, ecological variables on PCA axis 2 did not show a correlation of trait disparity with the phylogenetic tree (K = 0.40; p-value = 0.13), which means that the disparity of traits on the second axis is random. Only the first axis was significant, which means that the three suborders are no randomly dispersed, there is little niche overlap between them and closely related species were more ecologically similar than would be expected based on their phylogenetic relations. Despite this, it is likely that a large community scale the phylogenetic distribution of traits is a complex mix of conservatism and convergence, however clear cases of simple conservatism occur.

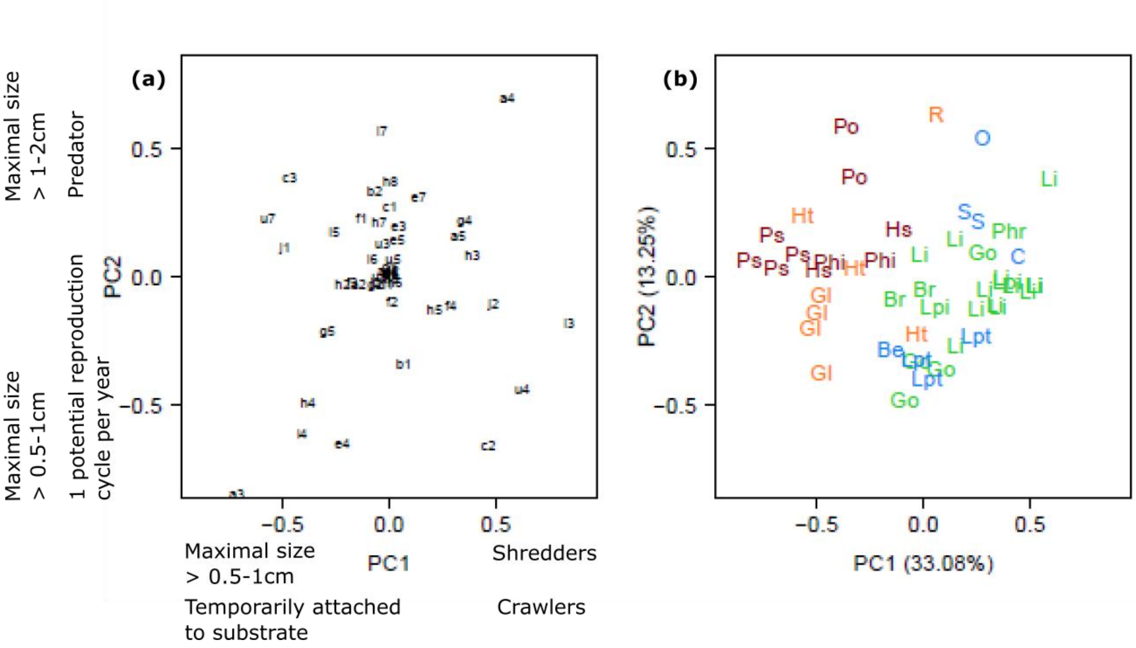

Fig. 5: Results of the Fuzzy Principal Component Analysis (FPCA) on trait data for 17 Trichoptera families: (a) distribution of functional traits on FPCA axes, and (b) distribution of the families on FPCA axes. Axes 1 and 2 explained 33.082% and 13.252% of the total variability, respectively. Family names code is: Be, Beraeidae; Br, Brachycentridae; C, Calamoceridae; Gl, Glossosmatidae; Go, Goeridae; Hs, Hydropsychidae; Ht, Hydroptilidae; Lpi, Lepidostomatidae; Lpt, Leptoceridae; Li, Limnephilidae; O, Odontoceridae; Phi, Philopotamidae; Phr, Phryganeidae; Po, Polycentropodidae; Ps, Psychomyiidae; R, Rhyacophilidae; S, Sericostomatidae. Four suborder groups are represented in the second plot in the same colors as phylogenetic tree in figure 4. See Table S2 for traits labels in (5a).

Phylogenetic structure

PD was constant across the latitudinal gradient. Even though, MPD and MNTD (phylogenetic β-diversity) have a negative significant slope (fig. 6a), which means taxon distance between pairs of species is lower in northernmost communities. So, phylogenetic community structure within regions shift across the latitudinal gradient. Similarly, observed SES_{MPD} was commonly greater than 0 but significant only in Betic region, which indicates phylogenetic overdispersion in this region, except in the Carpathians, where SES_{MPD} and SES_{MNTD} were significatively negative, which indicates phylogenetic clustering in this region (fig. 6b). There is a gradual decrease of SES_{MPD} and SES_{MNTD} in relation to the latitudinal gradient (fig. 6b), although this correlation is not always significant.

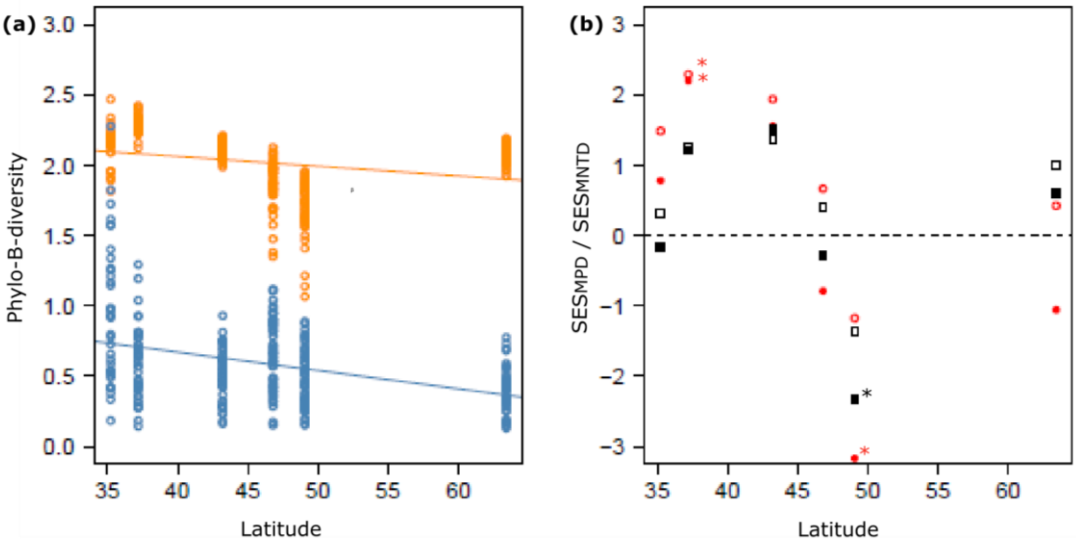

Fig. 6: (a) Phylogenetic beta diversity. Mean phylogenetic distance (MPD, orange dots) and mean nearest taxon distance (MNTD, blue dots). Both distances have a negative slope correlated with latitude (rho=-0.53, p-value<2.2e-16; and rho=-0.37, p-value=2.275e-11, respectively. (b) Measures of standardized effect size of phylogenetic community structure in each region. SES_{MPD} using the two null models: "sample.pool" (red empty circles) and "independentswap" (red dots); and SES_{MNTD} using "sample.pool" (empty squares), and "independentswap" (full squares). * indicates significance. There is no correlation with latitude.

DISCUSSION

Major α- and γ-diversities in central Europe implies a unimodal distribution, which is against the main expectation of decrease of diversity as latitude increases. The highest diversity at the Carpathians but also Picos indicates a possible location of glacial refuges in these regions and, consequently, these areas have had longer time for species accumulation than what it was thought (Stewart et al., 2010). This result contradicts the theories that locate glacial refuges exclusively in southern areas that are expected to act as centres of speciation and sources of recolonization (Hewitt, 1999). For instance, scarab beetles, that are sensitive to cold temperatures revealed a decrease in diversity by nestedness as latitude increase in Europe (Hortal et al., 2011). Similarly, other examples covering a wide range of taxa from plants (Kerkhoff et al., 2014) to vertebrates (Gaston, 2000) support this model of southern refuges and posterior northward recolonization. Alternatively, our results support the hypothesis of Dinodal that predicts that suitable habitat within the periglacial area persisted throughout the Pleistocene giving suitable conditions for survival in central Europe (Malicky, 1983). This hypothesis should explain richness trend for cold tolerant biota (Hawkins, 2010; Theissinger et al., 2011; Theissinger et al., 2013), and particularly Trichoptera (Malicky, 1983). For instance, there are relevant examples for caddisflies species located at the Carpathians (Bálint et al., 2008; Macher et al., 2015) and Alps (Pauls et al., 2006) that showed high genetic diversity and structure as consequence of postglacial range expansion following bottle-neck in glacial periods, which supports the idea that these areas were refuges in the glaciations. Despite the evidences for central Europe refuges, southern regions showed the greatest endemicity followed by Carpathians, which indicates these two regions are both centres of speciation and origins of northward species expansion following glaciation periods. Overall, our results support both biogeographical models because the highest diversity is found in central regions and the greatest number of endemic species is located at South. It is likely that, during interglacial periods, species expanded their geographical range northward, and this would explain that the vast majority of genera are found in all regions across the entire latitudinal gradient. This fact confirms that Trichoptera is a group with high dispersal ability and well-adapted to a wide range of habitats including the cold environments (Previšić et al., 2009), in contrast to the pattern found for cold sensitive species (Hortal et al., 2011; Machac et al., 2011). Nevertheless, species and phylogenetic diversity and endemicity is lower at high latitudes maybe because the limited time for post-glacial recolonization and the environmental filtering (Svenning & Skov, 2007). Notably, Jura is the community with the poorest diversity. The characteristics of this region influence the patterns of diversity because the majority of these samples were collected in water sources instead of running rivers, which should reduce species richness owing to the habitat stability and isolation of sources (Barquín & Death, 2006).

Little differences in local and regional species richness among regions are also reflected in the pattern of β-diversity. Based on differences between observed and expected compositional dissimilarities within regions, European caddisflies communities can be clustered into two zones: southern Europe with communities drove by competitive exclusion ($\beta\text{-diversity}_{obs} > \beta\text{-diversity}_{exp}$) and northern Europe with aggregated communities ($\beta\text{-diversity}_{obs} < \beta\text{-diversity}_{exp}$). Higher habitat heterogeneity in southern than northern areas offer more ecological niches where species may diversify to avoid competition for resources (Slingsby & Verboom, 2006; Davies et al., 2007). Accepting niche conservatism and phylogenetic overdispersion, this statement would explain why communities of genera inhabiting southern areas are such different between them in relation to their taxonomical composition. Further north, communities tend to have similar ecological requirements because the increasing effect of environmental filters due to more inhospitable habitats that determine the adaptation of species in cold habitats (Kraft et al., 2007). Consequently, the influence of aggregation mechanisms and phylogenetic clustering is stronger in temperate communities. Morphological aggregation is commonly correlated with environmental filtering, which reflects the assemblage of species with shared ecological tolerances resulting in clustered phenotypes in trait space (Ackerly &

Cornwell, 2007). In other words, competition within a local community leads to ecological differentiation of coexisting species as found in southern region, while habitat filtering reduces the spread of trait values, reflecting shared ecological tolerances (Cornwell et al., 2006). Hence, the pattern of β-diversity across the latitudinal gradient is clearly driven by turnover than the weak effect of the nestedness, which confirms this model because spatial turnover implies the replacement of some genus by others with little loss as a consequence of environmental sorting or spatial and historical constraints (Qian et al., 2005).

On the systematics of the Trichoptera, the phylogenetic tree evidences the molecular differences between two genera *Lasiocephala* and *Lepidostoma* (Lepidostomatidae) currently synonymized (Malicky, 2005; González & Menéndez, 2011), and therefore questioned if these genera are really two distinct taxa or the same genus with high genetic variability. On the other hand, the genera *Micropterna* and *Stenophylax* (Limnephilidae) have no differences so that supports its synonymy.

It is needed to highlight the importance of an integrative multi-disciplinary approach as used here to understand the entire evolutionary processes that have driven the current pattern of diversity of communities of Trichoptera across the latitudinal gradient in Europe. The tree revealed that ecological traits are conservative in the phylogeny and therefore each family occupies a differentiated ecological niche and therefore close related species share functional life-history traits. It means that species have evolved associated to their habitat preferences but also interspecific competition; in consequence each family is characterized by certain specific biological functions (Holzenthal et al., 2007). In fact, the results on the first axis of the FPCA definitely separate the three suborders of Trichoptera. Besides, traits are closely linked to evolutionary history and biogeography because different climatic conditions require appropriate ecological features (Garcia-Barros & Benito, 2010). Traits that have been preserved are not identified because the position of each category of traits on the FPCA axes were used for the analysis instead of dealing each trait separately, so it can be that ecological adaptation along latitudinal regions may be coincident with lineage diversification of Trichoptera, while other ecological traits are changing largely independently of lineage diversification. Despite this, our results show that overall traits are strongly conserved along the phylogeny, meaning that closely related species share more trait diversification and evolutionary history than distantly related species, tending to have similar niche-related traits. Assuming this traits conservatism, the fourth hypothesis raised is confirmed. Phylogenetic distances are little shorter in north communities probably because communities inhabiting northern areas have had shorter term for accumulating genetic divergence than southern communities where individuals were settled over longer time. Also, phylogenetic clustering processes (lower phylo-β-diversity than expected by null models) found in northern regions are likely due to environmental filters, that prevent the colonization of high latitudes by non-adapted individuals as a result of retaining ancestral traits. Nevertheless, phylogenetic overdispersion can result either when closely related taxa with the most similar niche-use are being locally excluded (phenotypically repulsed), such that there is minimum niche overlap of coexisting species, or when distantly related taxa have converged on similar niche-use and are phenotypically attracted (Webb et al, 2002). The results of the integrative exploration of taxonomical composition, phylogenetic distances and life-history traits, allow us to understand how northern European communities were originated as a consequence of recolonization from known (South) and cryptic (Central Europe) refuges after the cyclic glaciations period.

The ecological and evolutionary processes generating the regional pool of European caddisflies and the assembly of communities over time have led to differences in the structure of northern and southern assemblages. However, these differences are not as critical as found in other taxonomical groups, particularly terrestrial relatives, because did not follow the decrease of diversity. Similarly, clustering at high elevations or latitudes and overdispersion at low ones is not only detected in Trichoptera but also in other organisms as hummingbirds (Graham et al., 2009), Scarabaeinae (Hortal et al., 2011), ants (Silva & Brandão 2014; Machac et al., 2011) and plants (Kerkhoff et al., 2014), which seems to be a generalizable pattern. Taking this into account, further analysis on diversity patterns associated to ecological preferences and phylogenetic structure are needed in other taxa and/or in other geographical location in order to discover new evolutionary histories and confirm that phylogenetic relatedness within regions change across the latitude.

CONCLUSIONS

Overall, we can say that the proposed goals are achieved and, in brief, the main conclusions reached can be sum up on the following four points:

It is found non-evidence of a decrease in diversity along the latitudinal gradient in Europe, which contrasts with what has been observed in other taxa. Instead, the communities of caddisflies showed a unimodal pattern of diversity being higher mainly in Carpathians but also Picos (Central-temperate Europe). This finding suggests that refuges during the cycle Pleistocene glaciations were found not only at southern regions as it was hypothesized but also in Central Europe, supporting Dinodal's theory.

The phylogenetic tree and the integrative approach used are critical for understanding evolutionary processes and explaining current macroecological diversity patterns of caddisflies across Europe. There is a phylogenetic niche conservatism, which reveals that each family occupies a differentiated ecological niche and close related species share functional characteristics. As traits are preserved, it explains why we found evidences of clustering or aggregation in northern localities and phylogenetic overdispersion at lower latitudes. The reason of these differences is the cyclical period of glaciations that occurred around the world thousands of years ago and that, definitely, influenced the communities structure we found today. Hence, this study provides evidence that historical environmental constraints together with phylogenetic niche conservatism are the key factors that promote current species distribution of Trichoptera across Europe. We conclude that a multidisciplinary approach integrating phylogenies, taxonomical composition and functional traits are critical for understanding the eco-evolutionary mechanisms underlying global patterns of diversity, which is essential for biodiversity conservation under future scenarios of climate change.

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Table S2. Biological traits used and categories from Tachet et al. (2010) with their codes.

MAXIMAL SIZE	**a1**	≤ 0.25 cm
	a2	> 0.25 - 0.5 cm
	a3	> 0.5 - 1 cm
	a4	> 1 - 2 cm
	a5	> 2 - 4 cm
	a6	> 4 - 8 cm
	a7	> 8 cm
LIFE CYCLE DURATION	**b1**	≤ 1 year
	b2	> 1 year
POTENTIAL NUMBER OF REPRODUCTION CYCLES PER YEAR	**c1**	< 1
	c2	1
	c3	> 1
AQUATIC STAGES	**d1**	Egg
	d2	Larva
	d3	Nymph
REPRODUCTION	**e3**	Isolated eggs, cemented
	e4	Clutches, cemented or fixed
	e5	Clutches, free
	e7	Clutches, terrestrial
DISSEMINATION	**f1**	Aquatic passive
	f2	Aquatic active
	f3	Aerial passive
	f4	Aerial active
RESISTANCE FORM	**g1**	Eggs, statoblasts, gemmules
	g2	Cocoons
	g4	Diapause or dormancy
	g5	None
RESPIRATION	**j1**	Tegument
	j2	Gill
LOCOMOTION AND SUBSTRATE RELATION	**u3**	Swimmer
	u4	Crawler
	u5	Burrower (epibenthic)
	u6	Interstitial (endobenthic)
	u7	Temporarily attached
	u8	Permanently attached
FOOD	**h1**	Fine sediment + microorganisms
	h2	Detritus < 1 mm
	h3	Plant detritus ≥ 1 mm
	h4	Living microphytes
	h5	Living macrophytes
	h6	Dead animal > 1 mm
	h7	Living microinvertebrates
	h8	Living macroinvertebrates
	h9	Vertebrates
FEEDING HABITS	**i2**	Deposit feeder
	i3	Shredder
	i4	Scraper
	i5	Filter-feeder
	i6	Piercer (plant or animal)
	i7	Predator (carver/engulfer/swallower)

Figure 1S. Presence of genera in each of the six communities indicated with a black dot at the end of the branches of the phylogenetic tree. (a) R, Rif. (b) B, Betic. (c) P, Picos de Europa. (d) J, Jura. (e) C, Carpathians. (f) S, Jämtland (Sweden).

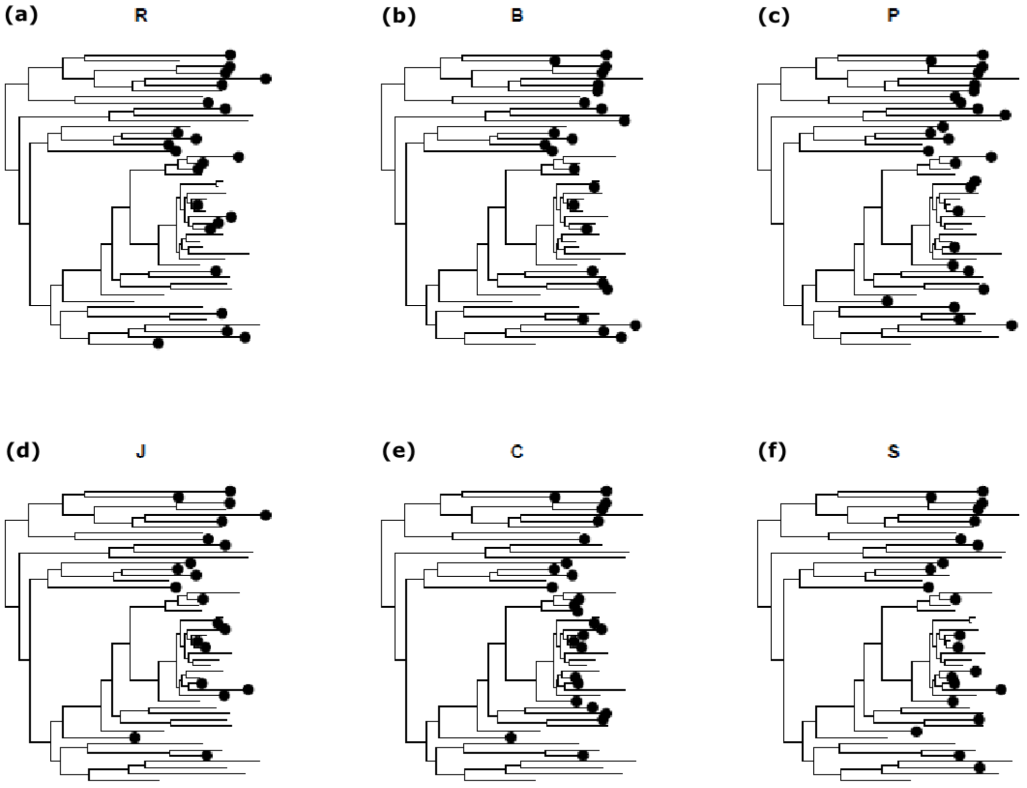

Figure 2S. Bayesian phylogenetic tree of seven genes of Order Trichoptera including 105 European genera (out of 129) belonging to 22 families. Comprise 114 taxa including 4 outgroup genera of Lepidoptera. Branch support percentage is indicated.