Pleistocene climate change and the formation of regional species pools

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Abstract

Despite the description of bioregions dates back from the origin of biogeography, the processes originating their associated species pools have been seldom studied. Ancient historical events are thought to play a fundamental role in configuring bioregions, but the effects of more recent events on these regional biotas are largely unknown. We use a network approach to identify regional and sub-regional faunas of European *Carabus* beetles, and analyse the effects of dispersal barriers, niche similarities and phylogenetic history on their configuration. We identify a transition zone matching the limit of the ice sheets at Last Glacial Maximum. While southern species pools are mostly separated by dispersal barriers, in the north species are mainly sorted by their environmental niches. Strikingly, most phylogenetic structuration of *Carabus* faunas occurred since the beginning of the Pleistocene. Our results show how extreme recent historical events –such as Pleistocene climate cooling, rather than just deep-time evolutionary processes, can profoundly modify the composition and structure of geographic species pools.

Introduction

Naturalists have long been captivated by the geographic distribution of world biotas. Rooted in the seminal ideas by Alexandre von Humbolt, this fascination has promoted a long-term research agenda aiming to delineate biogeographic regions according to

their integrating faunas and floras (e.g. Wallace 1876, Holt et al. 2013, Rueda et al. 2013). Regional biotas are known to determine ecological and evolutionary dynamics taking place at finer scales (Ricklefs 2008, 2015). For instance, regional species pools can modulate local diversity patterns (Ricklefs 2011, Medina et al. 2014, Ricklefs and He 2016), the structure and functioning of ecosystems (Naeslund and Norberg 2006), or co-evolutionary processes (Calatayud et al. 2016a). However, despite their fundamental importance, the processes that have configured regional biotas have been seldom studied (and particularly the historical ones), and most explanations on their origin and dynamics remain largely narrative (Crisp et al. 2011).

Perhaps the earliest speculations about the formation of regional species pools took place during the flourishment of bioregionalizations in the mid-19th century (reviewed by Ebach 2015). During that time, and beyond referring to physical factors (climate, soils, and physical barriers), some authors already started to emphasize historical influences as key elements determining the configuration of plant and animal regions. For instance, when Wallace (1876) proposed his famous zoogeographic regions, he argued that while the distribution of ancient linages such as genera and families would more likely reflect major geological and climatic changes spanning the early and mid-Cenozoic, that of species would be more influenced by recent events such as Pleistocene glaciations (see Rueda et al. 2013). Indeed, these recent events could have fostered many additions and subtractions of species to regional faunas through dispersal and diversification processes. Increasing evidence suggests that Pleistocene glacial-interglacial dynamics may have driven population extinction (e.g. Barnes et al. 2002), allopatric speciation in glacial refugia (e.g. Johnson et al. 2004) and post-glacial recolonization events (e.g. Hewitt 1999; Theissinger et al. 2013). As a consequence, Pleistocene glaciations are known to influence current diversity patterns for many taxa, particularly in the Holarctic (e.g. Svenning and Skov 2007; Hortal et al. 2011; Calatayud et al. 2016b). However, the role of Pleistocene glaciations in shaping regional species pools remains largely unknown.

Besides historical contingencies, niche-based processes may also determine the composition of regional species pools (Mittelbach and Schemske 2015), mainly throughout their effects on species distribution ranges (Soberon 2007, Hortal et al.

2010, Hortal et al. 2012). These processes integrate responses to abiotic conditions along geographical gradients and to local and regional biotic environment (Colwell et al. 2009). Furthermore, they involve a complex interplay between evolutionary, ecological and biogeographical factors. For instance, species with similar climatic tolerances can coexist in regions of similar climate, but their arrival (and *in situ* evolution) may be constrained by geographical barriers, which may also lead to climatic-tolerance divergent species pools (Fig.1a). Further, if species' resemblance in climatic tolerance is phylogenetically constrained, climate-driven regional species pools will be also composed of evolutionary related species (i.e. phylogenetically clustered species pools), although this effect is again filtered by biogeographical processes (Gouveia et al. 2014). Indeed, diversification of lineages within regions separated by strong dispersal barriers (e.g. mountain ranges) may also lead to phylogenetically clustered species pools (Warren et al. 2014; Fig.1a). Over the mid-term, historical contingencies may contribute to erase the signature of such geographically-linked diversification. For example, differential diversification rates may be the predominant driver of regional species pools in climatically stable regions (Cardillo 2011), yet regions where Pleistocene glaciations exerted a greater influence may harbour pools of species shaped by the joint effects of current climate and post-glacial dispersion (Svenning et al. 2015).

In this study we aim to disentangle the relative importance of the processes that may contribute to the formation of regional species pools, using European *Carabus* (Coleoptera: Carabidae) as a model lineage. *Carabus* is a species-rich ground beetle genus of great popularity due to the beautiful jewel-like appearance of some species (Turin et al. 2003). In general, *Carabus* species are flightless nocturnal predators of snails, earthworms and caterpillars. They hold hydrophilic adaptations and are typically associated to deciduous forests (Deuve et al. 2012). Previous evidence suggests that the richness of species from this genus in Europe is determined to a large extent by both current environmental conditions (i.e. climate and habitat) and glacial-interglacial dynamics (Calatayud et al. 2016b). This makes European *Carabus* an ideal case study to evaluate the joint effects of evolutionary, ecological and historical contingency processes as drivers of regional species pools.

Specifically, we use data on the distribution and evolutionary relationships of *Carabus* species, and network and phylogenetic analyses, to evaluate six hypotheses: First, given the presumed low dispersal capacity of the species from this genus, we hypothesize that (H1) European *Carabus* species pools are mainly shaped by the main orographic barriers of the continent, but also, that (H2) glacial-interglacial dynamics have led to strong differentiation between northern and southern regional species pools. If this differentiation is true, (H3) northern European *Carabus* faunas will be composed of species adapted to cold climatic conditions that colonized newly vacant habitats after the withdrawal of the ice sheet, and hence their regional distribution will be mostly determined by current climate. In contrast, (H4) southern faunas will be mainly shaped by the joint influence of diversification events and dispersal limitations, due to the combined effect of higher climatic stability towards southern latitudes (e.g. climatic refugia) and a more complex orography (Alps, Pyrenees, Carpathians). Therefore, (H5) species forming northern regional pools will exhibit comparatively lower levels of regional endemicity, whereas those forming southern regional pools will show comparatively higher levels of regional fidelity. Finally, according to Wallace (1876), the advance and retreat of the ice sheets during the Pleistocene should have determined the spatial distribution of lineages, eroding the effects of the former configuration of the distribution of the main *Carabus* lineages. Therefore, (H6) we expect a temporal signal coincident with the Pleistocene in the phylogenetic structure of *Carabus* faunas, and no effect of deep-time events on the current geographical distribution these lineages.

Material and methods

Data origin

Data on the European distribution of *Carabus* species comes from a recent analysis on the determinants of diversity of this genus in the continent (Calatayud et al. 2016b). Briefly, expert-based range maps of all *Carabus* species inhabiting Europe (n = 131) delineated by Turing et al. (2003) were digitized and overlaid into a 100-km equal-area

grid using ARCGIS (grid available at http://dataservice.eea.europa.eu). The resulting gridded distribution maps were reviewed by several experts, eventually correcting cells wrongly classified as either presences or absences (see Calatayud et al. 2016b for further details). The resulting presence-absence matrix was used to represent the distribution of *Carabus* species in all analyses.

We used environmental and physical geography GIS data from several commonly used public domain digital repositories. Bioclimatic variables were extracted from Worldclim (v1.4 Hijmans et al. 2005; available at http://www.worldclim.org/). Altitudinal data were derived from the 30-arcsecond digital elevation model GTOPO30 provided by the U. S. Geological Survey (available at https://lta.cr.usgs.gov/GTOPO30), and the location of water bodies was extracted from vector information coming from Natural Earth (available at http://www.naturalearthdata.com/). Finally, data for the geographical distribution of forest habitats come from MODIS Land Cover (Channan et al. 2014, available at http://glcf.umd.edu/data/lc/).

Statistical analyses

Rationale and structure of the analyses

Exploring the determinants of regional faunas requires analysing ecological, evolutionary and historical factors jointly. We did so through three consecutive steps (Fig.1b). First, we identified distinct regional species pools within Europe by using a network community detection algorithm. From this analysis we derive a species pairwise similarity matrix of occurrence into different modules that represent different regions. Second, we assessed the relative importance of the environmental, spatial and evolutionary determinants of such similarity. We constructed four pairwise matrices to describe the relationships among species; namely, *i*) a matrix of climatic niche similarity, *ii*) a matrix of habitat similarity, *iii*) a matrix of spatial connectivity among their ranges, and *iv*) a phylogenetic distance matrix. Then, we used generalized partial matrix regression to model the similarity in species occurrences as a function of these four matrices (Fig.1b). We used this workflow to explore the factors involved in the configuration of *Carabus* faunas either at the European scale (i.e. co-occurrence patterns of European *Carabus* species across the delimited regions, hereafter “regional

scale") and independently (i.e. within regions co-occurrence patterns of *Carabus* species within each delimited region, "hereafter sub-regional scale"). Finally, we also applied ancestral range estimation analysis in order to identify the time period from which ancestral areas are estimated with less uncertainty. By doing so, we aimed to detect important historical periods contributing to the regional organization of *Carabus* linages.

The interpretation of the joint and independent effects of explanatory matrices can shed light on the different processes configuring regional faunas (see Fig.1a). In that way, if niche similarities (i.e. represented by the climatic and habitat similarity matrices) and phylogenetic distances altogether explained the regional co-occurrence of species, then this could be interpreted as indicative of constrained niche evolution (or a tendency to resemble ancestral niches) in shaping regional faunas (Fig.1a.i). However, if spatial connectivity also accounted for part of this co-occurrence, this would indicate that this niche conservatism pattern can be caused by geographical constrains (Fig.1a.ii). Further, the independent effects of niche similarities together with spatial connectivity can be more likely the consequence of a convergence of climatic niches due to geographical isolation (Fig.1a.iii). Whereas the effects of connectivity and phylogeny would be indicative of a primacy of intra-regional speciation driven by geographical barriers. Niche similarities alone would point to an unconstrained niche evolution shaping regional faunas, while phylogeny alone would indicate a primacy of geographically unconstrained intra-regional speciation events. Finally, either a *cul-de-sac* effect (i.e. the accumulation of species in past climatic refugia) or a primacy of vicariant speciation events could lead to the existence of independent effects of connectivity and regional co-occurrence (Fig.1a.iv).

Identification of regional species pools

We took advantage of community detection analysis —borrowed from network theory— to identify *Carabus* regional species pools in Europe. To do so, we derived a bipartite network from the presence-absence matrix, where species and grid cells constitute two disjoint sets of nodes that are mutually connected according to the presence of species in grid cells (e.g. Calatayud et al. 2016a). Then, we conducted a

192 modularity analysis using the index proposed by Barber (2007) and the Louvain
193 algorithm (Blondel et al. 2008, as implemented in the Matlab function "Gen Louvain",
194 available at http://netwiki.amath.unc.edu; Mucha et al. 2010) to identify the optimal
195 modular structure of the bipartite network. That is, the optimal groups of grid cells
196 that show similar *Carabus* species composition together with their associated species
197 (i.e. network modules). We performed a heuristic search for an optimal solution,
198 where this analysis was repeated iteratively to obtain 100 different modular solutions,
199 retaining the one that showed the highest modularity value. This optimal solution was
200 used to conduct all subsequent analyses, although all the solutions were qualitatively
201 similar. We evaluated the statistical significance of the modules by comparing its
202 associated modularity value to a null distribution of values (n = 100) where the original
203 presence absence matrix was randomized using the independent swap algorithm (a
204 fixed-fixed null model implemented in the R package "picante", Kembel et al. 2010). In
205 order to detect potential sub-modules nested within modules (i.e. sub-regional species
206 pools within regional species pools), we derived a new bipartite network from each of
207 the modules previously identified in the optimal solution, and applied the procedure
208 described above in each case.
209
210 It is important to note that despite species and grid cells were assigned to just one
211 module, they could participate with different degrees in other modules. For example,
212 despite most species in a grid cell will belong to the same module the cell does, this
213 cell could also hold species of other modules. Similarly, although a species will mostly
214 be present in cells assigned to its module, it may also occur in cells from other
215 modules. Thus, we calculated the degree of module affinity for each node of the
216 bipartite network as the proportion of links shown by a given node within its module
217 divided by the total number of its links (note that this index is related to the inter-
218 modular participation index of Guimera and Amaral 2005). Species with low module
219 affinity will tend to be widespread throughout Europe, belonging to different regional
220 species pools. While species with high module affinity will be mainly distributed within
221 their corresponding modules (highly endemic species). On the other hand, grid cells
222 with low module affinity may represent transition zones between regions.
223

Assessing the determinants of regional species pools

In order to disentangle the determinants of the current configuration of *Carabus* faunas in Europe, we first generated a species-per-module matrix, where each inentry of the matrix represents the percentage of the distributional range of a certain species that lies in a given module. Then we derived a co-occurrence pairwise similarity matrix from the former matrix using the Schoener index (Schoener 1970). This index describes the degree of overlap between species pairs according to their distributions throughout the modules (see Krasnov et al. 2012) for a previous application to similar purposes). It ranges from 0 (i.e. complete lack of overlap) to 1 (i.e. identical pattern of module distribution). Note that this similarity matrix reflects the co-occurrence similarities at regional scale, ignoring lower-scaled distributional patterns (i.e. two species may have identical regional distribution but differ in the grid cell they are present). The resultant co-occurrence pairwise similarity matrix was used as dependent variable. We generated four different pairwise similarity matrices to be used as explanatory variables. Two of them to account for environmental factors, namely (i) a climatic-niche similarity matrix, and (ii) a habitat similarity matrix; and the other two for geographical and evolutionary factors, respectively: (iii) a spatial-connectivity matrix, and (iv) a phylogenetic distance matrix.

i) Climatic-niche similarity matrix. We characterized the climatic niche of each *Carabus* species in the dataset following the approach proposed by Broennimann et al. (2012). We selected six bioclimatic variables to account for the main water and energy aspects of climate –namely mean annual temperature, temperature of the warmest quarter, temperature of the driest quarter, total annual precipitation, total precipitation of the warmest quarter and total precipitation of the driest quarter– and altitudinal range to account for the effects of mesoclimatic gradients within each grid cell. These variables may be among the main determinants of the distribution of *Carabus* species diversity within Europe (see Calatayud et al. 2016b). We conducted a principal components analysis (PCA) on these variables to obtain a bidimensional climatic space defined by the two main axes (81.4% of the variance, Fig.S2). Finally, we divided this climatic space into 100 grid cells, and applied kernel smoothers to the species occurrence

densities in the gridded climatic space to calculate niche overlap between species using the Schoener index (see above). Note that the kernel density function requires at least five occurrences of the species, hence species occurring in less than five grid cells were excluded from subsequent analysis (n = 12).

ii) Habitat similarity matrix. The distribution of *Carabus* species may also be shaped by their forest preferences (Turin et al. 2003). Thus, we made use of information relative to the type of vegetation where each species tends to occur in order to characterize their environmental niche. We calculated the fraction of each species' distributional range laying within each vegetation category, according to 10 vegetation categories derived from MODIS dataset (Evergreen broadleaf forest, deciduous needle leaf forest, deciduous broadleaf forest, mixed forest, closed shrub lands, open shrub lands, woody savannas, savannas and grasslands). Then, we computed pairwise similarities in the preference to different vegetation types using the Schoener index (see above).

iii) Spatial connectivity matrix. To evaluate the potential influence of dispersal barriers on the current distribution of *Carabus* species we first created a dispersal-cost surface by weighting each pixel in the study area according to both its topography (in this case, slope) and the presence of water bodies. Slope values ranging from 0 to 100% at each pixel and were determined from GTOPO30 altitudinal data using the GRASS tool r.slope (GRASS Development Team 2017). Water bodies from Nature Earth were assigned arbitrary values of friction to the dispersal of *Carabus* species, namely 30% for pixels containing rivers and lakes and 99% for pixels that lay on sea water masses (note that *Carabus* species show hydrophilic adaptations). Then, the connectivity between all pairs of cells was calculated as least-cost path over the dispersal-cost surface that connect both cells, using the "gdistance" R package (van Etten 2015). Finally, the spatial connectivity between two species' distributional ranges was estimated as the average distance among all grid cells within the range of each species. Average distances were preferred over absolute least-cost distances to avoid disproportionate differences in spatial connectivity between overlapping and non-overlapping distributional ranges.

iv) Phylogenetic distance matrix. To unravel the evolutionary history of *Carabus* lineage and the importance of evolutionary processes in determining the formation of *Carabus* species pool, we reconstructed a species-level time-calibrated molecular phylogeny including 89 species. We used a maximum-likelihood inference based on ten DNA markers (see Appendix S3 for full details). We used taxonomic information and phylogenetic uncertainty methods (Rangel et al. 2015) to place species lacking molecular information into the phylogeny (see Appendix S3). Thus, we derived 100 different phylogenetic hypotheses from the maximum-likelihood phylogeny, by randomly inserting missing species within their most derived consensus clade based on taxonomic knowledge.

We used multiple regression on distance matrices and variance partitioning to disentangle the relative importance of climatic niche, habitat preferences, dispersal barriers and evolutionary history in determining *Carabus* species pools in Europe. First, we conducted single regressions between the co-occurrence pairwise similarity matrix and each of the four explanatory matrices described above to seek for significant associations between the variables. Given that the distribution of co-occurrence pairwise values was rather bimodal with modes at 0 and 1, respectively, we set a binomial family for error distribution and "logit" as the link function (see Ferrier et al. 2007 and Calatayud et al. 2016a for a similar approach). To assess for significance, we randomized the original species per module matrix using the independent swap algorithm (see above) to derive 999 null occurrence pairwise similarity matrices. Then, we fitted single regressions between the null occurrence pairwise similarity matrices and each of the explanatory similarity matrices, and considered a variable to have a significant effect when the regression based on observed data explained a higher deviance than 99% of the null regressions. In the case of phylogenetic pairwise distances we repeated this procedure for each phylogenetic hypothesis to take into account phylogenetic uncertainties, applying the same criterion for significance. Finally, we retained those variables that showed significant associations with the co-occurrence pairwise similarity matrix, and conducted partial multiple regression (Legendre and Legendre 2012) on distance matrices to explore patterns of covariation among niche similarities (i.e. climatic and habitat similarity matrices) dispersal barriers

and phylogenetic history. We repeated this whole procedure for each module independently, to further explore the determinants of *Carabus* species co-occurrence into submodules.

Ancestral range estimation

Probabilistic models of geographic range evolution have proven to be of great utility to deal with historical biogeograhical questions (see Ronquist and Sanmartín 2011 and references therein). Here we used these models to try to unravel whether, as predicted by Wallace (1876), deep historical signals were eroded by Pleistocene glaciations in the configuration of European *Carabus* faunas. We used a maximum likelihood approach for inferring geographical range evolution implemented in the R package BioGeoBears (Matzke 2014), using independently each one of the 100 phylogenetic hypotheses created before. BioGeoBears fits a variety of models that differ in their rates and/or types of dispersal, extinction, sympatric speciation, vicariance and founder event speciation. We fitted the six available models (DEC, DEC+J, DIVALIKE, DIVALIKE+J, BAYAREALIKE, BAYAREALIKE+J, see Matzke 2013) comparing their informative capacity through the Akaike information criterion (AIC). Species ranges were here coded as present/absent in each region detected in the former network analyses.

The estimation of ancestral ranges usually tends to be more ambiguous in deeper nodes of the phylogeny, as the potential lability of geographical ranges would tend to blur deep time signals (Losos and Glor 2003). Yet, if the Pleistocene glacial periods had important effects in the distribution of species it could be expected that ancestral range estimations will begin to be more accurate around the Pleistocene (that is, pre-Pleistocene signals on distributional range evolution will be eroded). To explore this we attended to potential changes in the relationship between node age and the marginal probability of the single most-probable ancestral state at each internal node. We obtained these probabilities from the best model among the six geographical range evolution models in BioGeoBears (see above) and in each of the phylogenetic hypotheses used. Then, general additive mixed models (GAMMs) were fitted to the node marginal probability as a function of node age, including the phylogenetic

hypothesis as a random factor. Further, in case we found different best models in different phylogenetic hypotheses we also included a fixed variable with the model type. Finally, we also carried out generalized linear mixed models (GLMMs) using a piecewise regression procedure to assess for a shift in the relationship between marginal probability and node age. The breakpoint in the piecewise regression was assessed by including the breakpoint as a new parameter in the GLMM and then minimizing the deviance of the fitted model using the function "optimize" in the R package lme4 (Bates et al. 2014). Finally, we used AICc to compare the models with and without breakpoint. In both cases we used a binomial family and a loglink function, since the marginal probabilities of the nodes vary between zero and one. All analyses were carried out in R (R core team 2015),: GAMM with the package gamm4 (Wood and Scheipl 2014), GLMM with Lme4 (Bates et al. 2014) and AICc with AICcmodavg (Mazerolle 2011).

Results

Identification of regional faunas

The *Carabus* occurrence network was significantly modular (M=0.385, p=0.01), dividing Europe in seven modules that group zoogeographically distinct regions with their associated faunas; that is, different regional species pools (Figs. 2 and S1). Further, all modules but module 2 showed a significant sub-modular structure, presenting a decrease in modularity with latitude (mean M=0.316, ranging from 0.154 to 0.468; all p-values < 0.05, see Table S1). Module 1 holds 21 species mainly living in Southwestern Palearctic (Iberian Peninsula, North of Africa, Balearic Islands, Corsica, Sardinia and the western half of Sicilia). This module was subdivided into four submodules. Module 2 only groups two species which are endemic of Crete. Module 3 identifies an East Mediterranean region including the Italic Peninsula, part of Greece and Turkey. This module holds 18 species and was subdivided into five submodules. Module 4 depicts a Central European region embracing the Alps and Carpathian Mountains, as well as Central European plains. This module is the richest one, with forty-nine *Carabus* species, and was split into 4 submodules. Module 5 and module 6 correspond with northern regions and are the poorest ones, holding ten species each. The former expands from Iceland and the British Isles almost to the Ural Mountains. The latter

includes The Ural Mountains and expands to the easternmost zone of the study area. Both modules were divided into 3 submodules. Finally, module 7 includes 21 species and embraces a south-eastern central European region expanding from the Carpathian Mountains to the south Ural Mountains. This module was split into 3 submodules.

The transition zones between regions appear to be associated with geographical barriers such as the Pyrenees, Alps, Carpathian and Ural Mountains, as well as the Bosphorus strait (Fig. 2c). Further, we also found a transition zone running through Central Europe in a west-to-east belt, separating southern and northern regions. Interestingly, this transition zone closely follows the southern limits of the ice sheet at the Last Glacial Maximum (LGM), suggesting a link between the configuration of *Carabus* regional faunas and Pleistocene glacial conditions.

Correlates of regional co-occurrence

Matrix regressions showed that both environmental niche similarity (i.e. climate and habitat) and spatial connectivity were significantly related to species co-occurrence in regions and sub-regions ($p<0.01$). In contrast, evolutionary relatedness was not related with regional co-occurrence, as phylogenetic distances did not show a significant relationship at $p>0.01$ for any single phylogenetic hypothesis. Environmental niche similarity was the best predictor of co-occurrence in both regions and sub-regions, although its effects were stronger when predicting the co-occurrence into sub-regions (Fig. 3). Spatial connectivity had stronger effects when predicting the co-occurrence into regions relative to sub-regions, where its effect mostly overlapped with that of environmental niche. The relative importance of these two sets of factors showed a clear latitudinal trend. The effects of connectivity were stronger in southern regions (i.e. modules 1 and 3), which is not surprising given the more complex orography of these regions. But importantly, although niche similarity was the best predictor of co-occurrence in all cases, its effects became more evident towards northern regions. Indeed, niche similarities and spatial connectivity were significant predictors of the co-occurrence into sub-regions in all regional pools except the two northern ones (Table S2). Only niche similarities (mainly climate) showed significant effects in these two

415 modules (i.e. 5 and 6), as expected if the species in these regional pools colonized the
416 northern regions only recently, and were geographically sorted according to current
417 climate.
418
419 **Ancestral range estimation**
420 BAYAREALIKE+J model was the best supported by data in 59% of the phylogenetic
421 hypotheses, followed by DEC+J and DEC, which were the best in 23 and 18% of the
422 hypotheses, respectively (Table 1). Interestingly, the BAYAREALIKE+J model does not
423 account for vicariant cladogenetic events. Rather, it considers founder effect
424 speciation, which could be seen as a signal of Pleistocene glaciations promoting
425 speciation in glacial refugia (i.e. acting as islands) and eroding the ancestors' ranges.
426 Indeed, according to this model the range contraction parameter had the highest
427 estimate (Table 1). This parameter was however of little importance in the other two
428 models, which accounted for vicariant events in both cases. Regardless the
429 biogeographical model used, GAMM results showed that node marginal probability of
430 the most probable state increased as expected towards younger nodes ($P<0.01$, R^2
431 =0.34). However, this increase became abrupt coinciding with the beginning of the
432 Pleistocene – which started around 2.59 Mya.– (Fig.4). Indeed, we found that the
433 GLMM including the breakpoint was better in terms of AICc (AICc weight for the model
434 including the breakpoint = 1), and that the relationship between state probability and
435 node age changed at 2.47 Mya. (confidence interval at 95% = 2.23, 2.73). This points to
436 the signal left by the Pleistocene on the configuration of European *Carabus* faunas.
437 These results were confirmed using different approaches (see Appendix S4).
438
439
440 **Discussion**
441 More than 140 years ago, Wallace (1876) foresaw that the influence of Pleistocene
442 glaciations on the distribution of diversity had been strong enough so as to erode the
443 imprint of previous events. Our results confirm Wallace's thoughts, showing a
444 remarkable coincidence between the distribution of the ice sheet at the Last Glacial
445 Maximum and the current configuration and evolutionary structure of European
446 *Carabus* Faunas. There is a close spatial relationship between the southern limits of the

447 area glaciated at LGM, and the transition zone separating the southern and northern
448 regions identified by our analyses. Indeed, this border also coincides with the line
449 identified by Calatayud et al. (2016b), where the relationship between *Carabus* species
450 richness and current climate changes in shape and strength (Fig. 2). Thus, it seems that
451 the climate changes underwent during the Pleistocene not only shape local diversity
452 patterns (e.g. Svenning and Skov 2007, Araujo et al. 2008, Hortal et al. 2011), but they
453 have also left a strong imprint on the geographical structure of species composition at
454 a regional scale. Accordingly, the species from the northernmost region (module 5)
455 show the lowest level of endemism (Fig. S2, as expected for regional faunas composed
456 of species that have recently colonized the north of Europe from southern glacial
457 refugia; Araujo et al. 2008, Calatayud et al. 2016b). In fact, although these species are
458 widely distributed across southern Europe, all of their ranges only overlap near to the
459 northern Carpathian Mountains, an area that has been shown to be a glacial refugia
460 for some *Carabus* species (Homburg et al. 2013, Fig. S3). Additionally, the decrease in
461 modularity values with latitude also points to a lesser geographical structure of
462 northern assemblages, which can be interpreted as the result of a post-glacial
463 colonization.
464
465 Besides the Pleistocene effects in the definition and geographical structure of regional
466 pools of species, we also found evidence of the imprint of this geological period on the
467 processes configuring the distribution of *Carabus* faunas. The generally strong
468 relationship between regional patterns of co-occurrence and both niche similarities
469 and spatial connectivity shows that co-occurring species tend to have similar realized
470 environmental niches and that also tend to be geographically constrained by the same
471 dispersal barriers. This latter result was expected, given the low dispersal capacity of
472 *Carabus* species (see Turin et al. 2003), which is likely to be behind the spatial
473 coincidence of the zones of transition between regional faunas and geographical
474 barriers. Perhaps more unexpected is the relationship of regional co-occurrence and
475 niche similarities without any significant phylogenetic effect. This implies that the
476 geographical configuration of barriers to dispersal has restricted species within regions
477 of similar climate, rather than climatic-niche conservatism constraining their co-
478 occurrences. These results point to that *Carabus* niche evolution is, to some extent,

479 evolutionary unconstrained, which is congruent with the high adaptation capacity of
480 insects in general (e.g. Overgaard and Sørensen 2008). Going further, this questions to
481 what extent the observed species' regional occurrence is the consequence of their
482 environmental niche and not the other way around (see Hortal et al. 2012, Wüest et al.
483 2015).
484
485 Whatever the origin of the relationship between species occurrence and
486 environmental conditions, which is certainly true is that its strength changes among
487 regions. These changes follow a latitudinal gradient in the importance of
488 environmental niche similarities. In northern regions, the similarity in the realized
489 niche seems to be stronger related to the occurrence into sub-regions than in the
490 south. This might be a direct consequence of the processes that determine regional
491 pools being dependent on the particular history of each region (Ricklefs 2015). These
492 findings are also congruent with the effects of post-glacial colonization, where
493 formerly glaciated areas show a clear sorting of species northwards due to low time for
494 dispersal (Svenning and Skov 2007). This contrasts with southern regions, where
495 climate has been milder and more stable and the species have had more time to
496 diversify. This suggests that species' geographical arrangement in southern latitudes is
497 more likely the result of historical contingency rather than environmental preferences
498 (Hortal et al. 2011, Calatayud et al. 2016b).
499
500 The lack of relationship between the phylogenetic distances among *Carabus* species
501 and their regional co-occurrence can be the outcome of either a generalized
502 preponderance of vicariant events and/or a "cul-de-sac effect" (O'Regan 2008). The
503 former will more likely imply that the generation of the dispersal barriers that shaped
504 the regions will also promote the geographical split of many lineages and subsequent
505 allopatric speciation events (Weeks et al. 2016). Yet, the formation of the geographical
506 accidents associated to the delimitation of *Carabus* regions largely predates the origin
507 of the genus (see Beccaluva et al. 1998, Deuve et al. 2012). On the other hand, a
508 generalized dispersion into climatic refugia, together with a subsequent stagnancy
509 within them (i.e. a "cul-de-sac" effect) may also produce the observed mixing of
510 unrelated linages into regions. Although it is difficult to distinguish between both

processes, we believe it is more plausible to think that southern regions have accumulated unrelated species while acting as glacial refugia, whereas northern regions were recolonized by unrelated species with similar environmental niches ---or simply with higher dispersal capacity (see above).

In agreement with this idea, the model of ancestral range estimation that is best supported by the data does not include vicariant events. On the contrary, this model takes into account founder speciation events, a process typical from islands (Provine 1989). This could be interpreted on the light of southern regions acting as islands during the different Pleistocene glacial maxima. Should this be true, a temporal signal of this period on the spatial organization of *Carabus* lineages should be evident in their phylogeny. We found such signal on the relationship between node maximum probability state and node age. Indeed, the striking coincidence between the breakpoint where this relationship becomes steeper and the beginning of the Pleistocene argues in favour of the imprint of this epoch on the configuration of *Carabus* regional faunas. We obtained similar results using several other approaches (Appendix S4), supporting the notion that the current regional organization of *Carabus* species and linages has its very roots at the beginning of the Pleistocene. This contrasts with ancestral range estimations for clades inhabiting areas that were never glaciated, where more ancient signals were found in the spatial sort of lineages (Condamine et al. 2015, Economo et al. 2015, Tänzler et al. 2016, Toussaint and Balke 2016). It thus seems that the repeated advances and retreats of ice sheets during this geological period produced the repeated cycles of retreat to southern regions and advance towards northern regions of *Carabus* species, a hustle-and-bustle process that ultimately led to the observed mixing of unrelated lineages.

In sum, our results provide solid arguments in favour of the importance of Pleistocene glaciations along with geographical barriers in structuring the regional faunas of this group. On the one hand, European *Carabus* faunas are primarily delimited by the location of the southern limit of the ice sheet at LGM, which separates two large regions that differ not only in species composition, but also in the processes underlying the spatial organization of these species. On the other hand, the phylogenetic

structure of these faunas coincides with the beginning of the Pleistocene. This not only implies that the geographical distribution of species and lineages is deeply shaped by past climates, but also that the ecological processes (Naeslund and Norberg 2006, Madrigal et al. 2016) and evolutionary mechanisms (Wüest et al. 2015, Calatayud et al. 2016a) that are dependent on regional species pools may be profoundly affected by the history of Earth climates. It is therefore essential to take into account past historical events while trying to understand, not only current diversity patterns and the processes behind them, but also processes and patterns occurring at local scales.

References

1. Araújo, M. B., D. Nogués-Bravo, J. A. F. Diniz-Filho, A. M. Haywood, P. J. Valdes, and C. Rahbek. 2008. Quaternary climate changes explain diversity among reptiles and amphibians. Ecography **31**:8–15.
2. Barber, M. J. 2007. Modularity and community detection in bipartite networks. Physical Review E **76**:066102.
3. Barnes, I., P. Matheus, B. Shapiro, D. Jensen, and A. Cooper. 2002. Dynamics of Pleistocene population extinctions in Beringian brown bears. Science **295**:2267–2270.
4. Bates, D., M. Maechler, B. Bolker, S. Walker, et al. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version **1**.
5. Beccaluva, L., M. Shallo, M. Coltorti, I. Premti, and F. Siena. 1998. Encyclopedia of European and Asian Regional Geology. Springer.
6. Blondel, V. D., J.-L. Guillaume, R. Lambiotte, and E. Lefebvre. 2008. Fast unfolding of communities in large networks. Journal of Statistical Mechanics **2008**:P10008.
7. Broennimann, O., M. C. Fitzpatrick, P. B. Pearman, B. Petitpierre, L. Pellissier, N. G. Yoccoz, W. Thuiller, M.-J. Fortin, C. Randin, N. E. Zimmermann, et al. 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. Global Ecology and Biogeography **21**:481–497.
8. Calatayud, J., J. L. Hórreo, J. Madrigal-González, A. Migeon, M. Á. Rodríguez, S. Magalhães, and J. Hortal. 2016*a*. Geography and major host evolutionary transitions shape the resource use of plant parasites. Proceedings of the National Academy of Sciences USA **113**:9840–9845.
9. Calatayud, J., J. Hortal, N. G. Medina, H. Turin, R. Bernard, A. Casale, V. M. Ortuño, L. Penev, and M. Á. Rodríguez. 2016*b*. Glaciations, deciduous forests, water availability and current geographical patterns in the diversity of European *Carabus* species. Journal of Biogeography **43**:2343–2353.
10. Cardillo, M. 2011. Phylogenetic structure of mammal assemblages at large geographical scales: linking phylogenetic community ecology with macroecology. Philosophical Transactions of the Royal Society of London B **366**:2545–2553.
11. Channan, S., K. Collins, and W. Emanuel. 2014. Global mosaics of the standard MODIS land cover type data. University of Maryland and the Pacific Northwest National Laboratory, College Park, Maryland, USA .

12. Colwell, R. K., and T. F. Rangel. 2009. Hutchinson's duality: the once and future niche. Proceedings of the National Academy of Sciences USA **106**:19651–19658.
13. Condamine, F. L., E. F. Toussaint, A.-L. Clamens, G. Genson, F. A. Sperling, and G. J. Kergoat. 2015. Deciphering the evolution of birdwing butterflies 150 years after Alfred Russel Wallace. Scientific Reports **5**:11860.
14. Crisp, M. D., S. A. Trewick, and L. G. Cook. 2011. Hypothesis testing in biogeography. Trends in Ecology and Evolution **26**:66–72.
15. Deuve, T., A. Cruaud, G. Genson, and J.-Y. Rasplus. 2012. Molecular systematics and evolutionary history of the genus *Carabus* (Col. Carabidae). Molecular Phylogenetics and Evolution **65**:259–275.
16. Ebach, M.C. 2015. Origins of Biogeography. Springer, New York.
17. Economo, E. P., E. M. Sarnat, M. Janda, R. Clouse, P. B. Klimov, G. Fischer, B. D. Blanchard, L. N. Ramirez, A. N. Andersen, M. Berman, et al. 2015. Breaking out of biogeographical modules: range expansion and taxon cycles in the hyperdiverse ant genus *Pheidole*. Journal of Biogeography **42**:2289–2301.
18. Ehlers, J., and P. L. Gibbard. 2004. Quaternary glaciations-extent and chronology: part I: Europe. Elsevier.
19. Ferrier, S., G. Manion, J. Elith, and K. Richardson. 2007. Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. Diversity and Distributions **13**:252–264.
20. Gouveia, S. F., J. Hortal, M. Tejedo, H. Duarte, F. A. Cassemiro, C. A. Navas, and J. A. F. Diniz-Filho. 2014. Climatic niche at physiological and macroecological scales: the thermal tolerance–geographical range interface and niche dimensionality. Global Ecology and Biogeography **23**:446–456.
21. GRASS Development Team, 2017. Geographic Resources Analysis Support System (GRASS GIS) Software, Version 7.2. Open Source Geospatial Foundation. http://grass.osgeo.org.
22. Guimerà, R., and L. A. N. Amaral. 2005. Functional cartography of complex metabolic networks. Nature **433**:895–900.
23. Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society **68**:87–112.
24. Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology **25**:1965–1978.
25. Holt, B. G., J.-P. Lessard, M. K. Borregaard, S. A. Fritz, M. B. Araújo, D. Dimitrov, P.-H. Fabre, C. H. Graham, G. R. Graves, K. A. Jønsson, et al. 2013. An update of Wallace's zoogeographic regions of the world. Science **339**:74–78.
26. Homburg, K., C. Drees, M. M. Gossner, L. Rakosy, A. Vrezec, and T. Assmann. 2013. Multiple glacial refugia of the low-dispersal ground beetle *Carabus irregularis*: molecular data support predictions of species distribution models. PloS One **8**:e61185.
27. Hortal, J., P. De Marco Jr, A. Santos, and J. A. F. Diniz-Filho. 2012. Integrating biogeographical processes and local community assembly. Journal of Biogeography **39**:627–628.
28. Hortal, J., J. A. F. Diniz-Filho, L. M. Bini, M. Á. Rodríguez, A. Baselga, D. Nogués-Bravo, T. F. Rangel, B. A. Hawkins, and J. M. Lobo. 2011. Ice age climate,

631 evolutionary constraints and diversity patterns of European dung beetles. Ecology
632 Letters **14**:741–748.
633 29. Hortal, J., J. M. Lobo, and A. Jiménez-Valverde. 2012. Basic questions in
634 biogeography and the (lack of) simplicity of species distributions: putting species
635 distribution models in the right place. Natureza & Conservação **10**:108–118.
636 30. Hortal, J., N. Roura-Pascual, N. Sanders, and C. Rahbek. 2010. Understanding
637 (insect) species distributions across spatial scales. Ecography **33**:51.
638 31. Johnson, N. K., C. Cicero, and K. Shaw. 2004. New mitochondrial DNA data affirm
639 the importance of Pleistocene speciation in North American birds. Evolution
640 **58**:1122–1130.
641 32. Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D.
642 Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating
643 phylogenies and ecology. Bioinformatics **26**:1463–1464.
644 33. Krasnov, B. R., M. A. Fortuna, D. Mouillot, I. S. Khokhlova, G. I. Shenbrot, and
645 R. Poulin. 2012. Phylogenetic signal in module composition and species
646 connectivity in compartmentalized host-parasite networks. The American
647 Naturalist **179**:501–511.
648 34. Legendre, P., and L. F. Legendre. 2012. Numerical ecology. Elsevier.
649 35. Losos, J. B., and R. E. Glor. 2003. Phylogenetic comparative methods and the
650 geography of speciation. Trends in Ecology and Evolution **18**:220–227.
651 36. Madrigal-González, J., P. Ruiz-Benito, S. Ratcliffe, J. Calatayud, G. Kändler,
652 A. Lehtonen, J. Dahlgren, C. Wirth, and M. A. Zavala. 2016. Complementarity
653 effects on tree growth are contingent on tree size and climatic conditions across
654 Europe. Scientific Reports **6**:32233.
655 37. Matzke, N. J., 2013. BioGeoBEARS: BioGeography with Bayesian (and Likelihood)
656 Evolutionary Analysis in R Scripts. University of California, Berkeley, Berkeley, CA.
657 http://CRAN.R-project.org/package=BioGeoBEARS.
658 38. Matzke, N. J. 2014. Model selection in historical biogeography reveals that
659 founder-event speciation is a crucial process in island clades. Systematic Biology
660 **63**:51–70.
661 39. Mazerolle, M. J. 2011. AICcmodavg: model selection and multimodel inference
662 based on (Q) AIC (c). R package version **1**.
663 40. Medina, N. G., B. Albertos, F. Lara, V. Mazimpaka, R. Garilleti, D. Draper, and
664 J. Hortal. 2014. Species richness of epiphytic bryophytes: drivers across scales on
665 the edge of the Mediterranean. Ecography **37**:80–93.
666 41. Mittelbach, G. G., and D. W. Schemske. 2015. Ecological and evolutionary
667 perspectives on community assembly. Trends in Ecology and Evolution **30**:241–
668 247.
669 42. Mucha, P. J., T. Richardson, K. Macon, M. A. Porter, and J.-P. Onnela. 2010.
670 Community structure in time-dependent, multiscale, and multiplex networks.
671 Science **328**:876–878.
672 43. Naeslund, B., and Norberg, J. 2006. Ecosystem consequences of the regional
673 species pool. *Oikos*, **115***:*504-512.
674 44. O'Regan, H. J. 2008. The Iberian Peninsula–corridor or cul-de-sac? Mammalian
675 faunal change and possible routes of dispersal in the last 2 million years.
676 Quaternary Science Reviews **27**:2136–2144.

677 45. Overgaard, J., and J. G. Sørensen. 2008. Rapid thermal adaptation during field
678 temperature variations in *Drosophila melanogaster*. Cryobiology **56**:159–162.
679 46. Provine, W. B., 1989. Founder effects and genetic revolutions in microevolution
680 and speciation: an historical perspective. Pp. 43–76 *in* L. Val Giddings and K. Y.
681 Kaneshiro, editors. Genetics, speciation, and the founder principle. Oxford
682 University Press.
683 47. R Core Team, 2015. R: A Language and Environment for Statistical Computing. R
684 Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
685 48. Rangel, T. F., R. K. Colwell, G. R. Graves, K. Fučíková, C. Rahbek, and J. A. F. Diniz-
686 Filho. 2015. Phylogenetic uncertainty revisited: Implications for ecological
687 analyses. Evolution **69**:1301–1312.
688 49. Ricklefs, R. E. 2008. Disintegration of the ecological community. The American
689 Naturalist **172**:741–750.
690 50. Ricklefs, R. E. 2011. Applying a regional community concept to forest birds of
691 eastern North America. Proceedings of the National Academy of Sciences USA
692 **108**:2300–2305.
693 51. Ricklefs, R. E. 2015. Intrinsic dynamics of the regional community. Ecology Letters
694 **18**:497–503.
695 52. Ricklefs, R. E., and F. He. 2016. Region effects influence local tree species diversity.
696 Proceedings of the National Academy of Sciences USA **113**:674–679.
697 53. Ronquist, F., and I. Sanmartín. 2011. Phylogenetic methods in biogeography.
698 Annual Review of Ecology, Evolution and Systematics **42**:441–464.
699 54. Rueda, M., M. Á. Rodríguez, and B. A. Hawkins. 2013. Identifying global
700 zoogeographical regions: lessons from Wallace. Journal of Biogeography **40**:2215–
701 2225.
702 55. Schoener, T. W. 1970. Nonsynchronous spatial overlap of lizards in patchy
703 habitats. Ecology **51**:408–418.
704 56. Soberón, J. 2007. Grinnellian and Eltonian niches and geographic distributions of
705 species. Ecology Letters **10**:1115–1123.
706 57. Svenning, J.-C., W. L. Eiserhardt, S. Normand, A. Ordonez, and B. Sandel. 2015. The
707 influence of paleoclimate on present-day patterns in biodiversity and ecosystems.
708 Annual Review of Ecology, Evolution and Systematics **46**:551–572.
709 58. Svenning, J.-C., and F. Skov. 2007. Could the tree diversity pattern in Europe be
710 generated by postglacial dispersal limitation? Ecology Letters **10**:453–460.
711 59. Tänzler, R., M. H. Van Dam, E. F. Toussaint, Y. R. Suhardjono, M. Balke, and
712 A. Riedel. 2016. Macroevolution of hyperdiverse flightless beetles reflects the
713 complex geological history of the Sunda Arc. Scientific Reports **6**:18793.
714 60. Theissinger, K., M. Bálint, K. A. Feldheim, P. Haase, J. Johannesen, I. Laube, and
715 S. U. Pauls. 2013. Glacial survival and post-glacial recolonization of an arctic–alpine
716 freshwater insect (*Arcynopteryx dichroa, Plecoptera, Perlodidae*) in Europe.
717 Journal of Biogeography **40**:236–248.
718 61. Toussaint, E. F., and M. Balke. 2016. Historical biogeography of Polyura butterflies
719 in the oriental Palaeotropics: trans-archipelagic routes and South Pacific island
720 hopping. Journal of Biogeography **43**:1560–1572.
721 62. Turin, H., L. Penev, A. Casale, E. Arndt, T. Assmann, K. Makarov, D. Mossakowski,
722 G. Szél, F. Weber, H. Turin, et al., 2003. Species accounts. Pp. 151–284 *in* H. Turin,

723 L. Penev, and A. Casale, editors. The Genus *Carabus* in Europe: A Synthesis.
724 Pensoft Publishers.
725 63. van Etten, J., 2015. gdistance: Distances and Routes on Geographical Grids.
726 http://CRAN.R-project.org/package=gdistance.
727 64. Wallace, A. R. 1876. The geographical distribution of animals: with a study of the
728 relations of living and extinct faunas as elucidating the past changes of the earth's
729 surface. Cambridge University Press.
730 65. Warren, D. L., M. Cardillo, D. F. Rosauer, and D. I. Bolnick. 2014. Mistaking
731 geography for biology: inferring processes from species distributions. Trends in
732 Ecology and Evolution **29**:572–580.
733 66. Weeks, B. C., S. Claramunt, and J. Cracraft. 2016. Integrating systematics and
734 biogeography to disentangle the roles of history and ecology in biotic assembly.
735 Journal of Biogeography **43**:1546–1559.
736 67. Wood, S., and F. Scheipl, 2014. gamm4: Generalized additive mixed models using
737 mgcv and lme4. http://CRAN.R-project.org/package=gamm4.
738 68. Wüest, R. O., A. Antonelli, N. E. Zimmermann, and H. P. Linder. 2015. Available
739 climate regimes drive niche diversification during range expansion. The American
740 Naturalist **185**:640–652.
741

742 **Table 1.** Results of the best ancestral range estimation models. d= dispersion, e=
743 extinction; j= founder speciation. Prop.best = proportion of phylogenetic hypotheses
744 where a model was the best in terms of AICc. Mean values among different
745 phylogenetic hypotheses (incl. standard deviation within brackets), are shown for each
746 parameter.

Model	N° param.	d	e	j	Prop. Best
BAYAREALIKE+J	3	0.015(0.001)	0.063(0.017)	0.015(0.005)	0.59
DEC+J	3	0.025(0.001)	<0.001(<0.001)	0.008(0.003)	0.23
DEC	2	0.026(0.001)	0.003(0.001)	0	0.18
BAYAREALIKE	2	0.019(0.001)	0.111(0.006)	0	0
DIVALIKE	2	0.031(0.001)	0.002(0.001)	0	0
DIVALIKE+J	3	0.03(0.001)	0(0)	0.006(0.003)	0

747

Figure 1. Hypothetical examples of the factors configuring regional faunas and work flow. a) Figure showing four hypothetical processes configuring regional faunas. Dotted lines depict different regions while colours correspond with different climates. In each case, the tips of the phylogeny point to regional distribution of the species. b) Workflow and potential results: 1) Hypothetical results of modularity analysis over the occurrence network; 2) similarity matrix of occurrence into modules; 3) pairwise matrix of environmental niche similarities; phylogenetic distances and topographical connectivity; and 4) hypothetical results and interpretations of a partial matrix regression on species occurrence similarities as a function of niche similarities, phylogenetic distances and connectivity.

Figure 2. European *Carabus* regions found by the network community detection analysis. a) Geographical location of modules (i.e. regions) and submodules. b) Module simplification of the occurrence network. Circles represent a module, being their size proportional to the species group within them. Links depict the species shared among regions, being its width proportional to the number of species. c) Values of module affinity per grid cell; green colours (i.e. cells with low affinity) identify transition zones. The dotted black line corresponds with the southern limit of the ice sheet at LGM (extracted from Ehlers and Gibbard 2004). The blue line depicts the breakpoint where the temperature--*Carabus* richness relationship changes, as found in Calatayud et al. (2016b).

Figure 3. Results of the partial generalized matrix regression of similarity in regional co-occurrence, as a function of environmental niche similarity (climate and habitat), topographical connectivity and phylogenetic distances. The first and second bars correspond with the models including all regions and subregions, respectively. The remaining bars correspond with the models where the similarities in subregional occurrence were analysed independently for the species of each region. Con.= spatial connectivity; Envir. = environmental niche similarities; Overl.= Overlap.

779 **Figure 4.** Results of the ancestral range estimation. a) Marginal probability of the most
780 probable state at each internal node for a phylogenetic hypothesis where the
781 BAREALIKE+J model was the best supported by the data. The size of the circles depicts
782 the value of probability, while the colours represent the combinations of regions
783 forming the state. The phylogeny is labelled at subgenera level. b) GAMM predictions
784 of the marginal probability as a function of node age for the BAREALIKE+J model. The
785 shaded area corresponds with the interval confidence at 95%. The dotted black line
786 represent the breakpoint found by picewise GLMM regression. The interval confidence
787 at 95% for the breakpoint is also provided. See Fig. S5 for the predictions of the other
788 models.
789

790 **Figure 1.**

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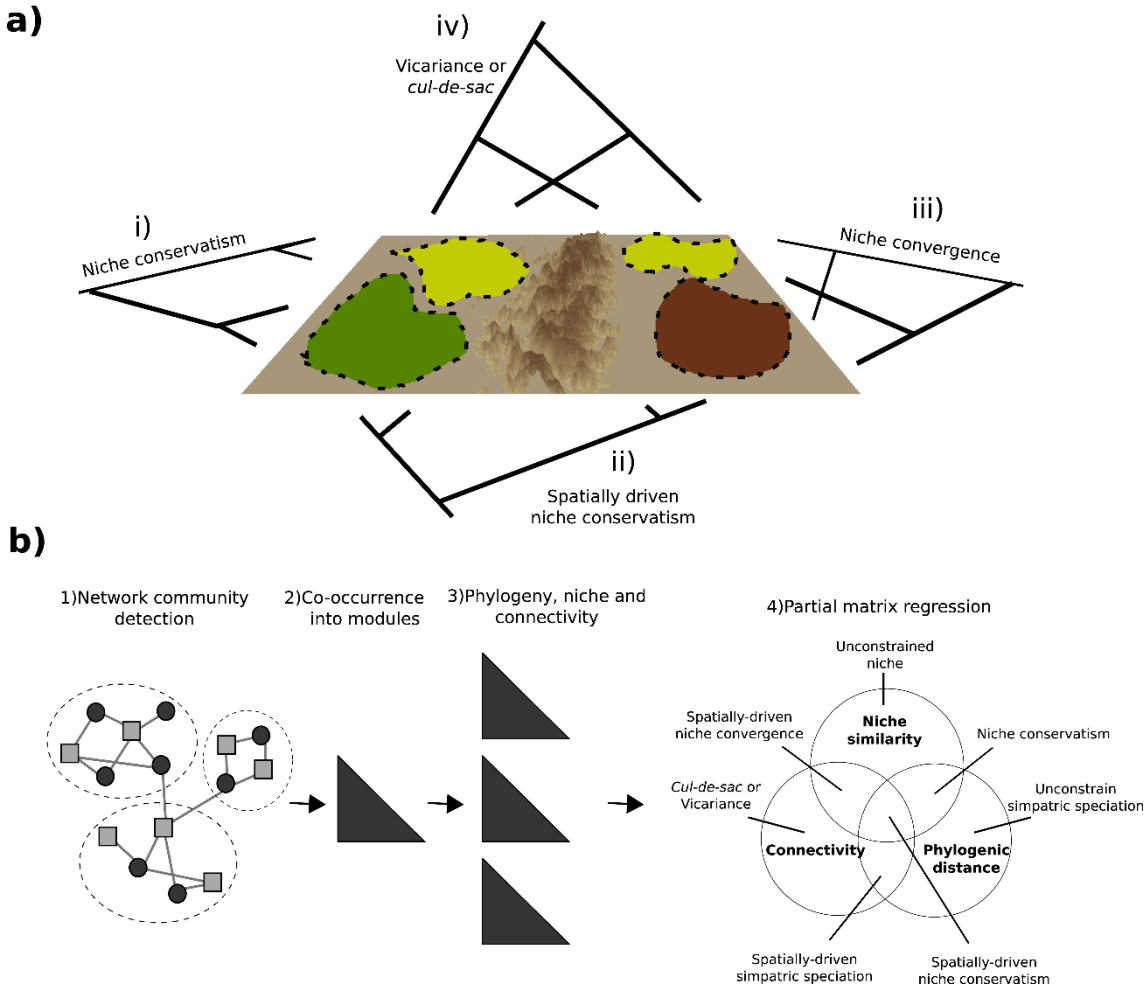
792



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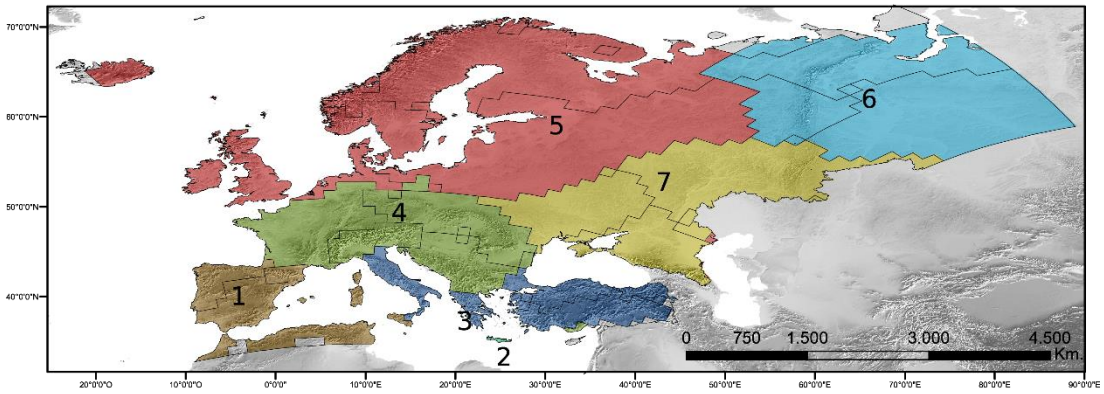
794 **Figure 2.**

a)



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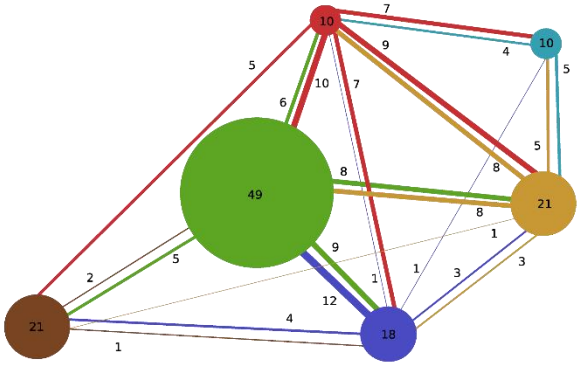

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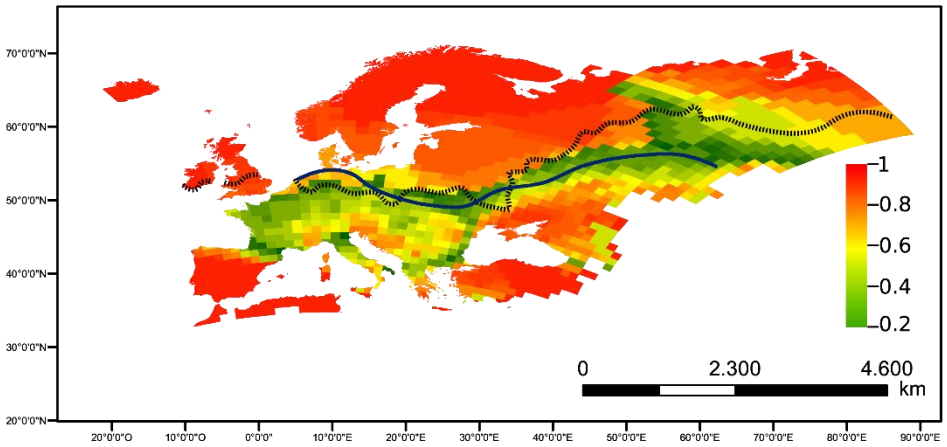

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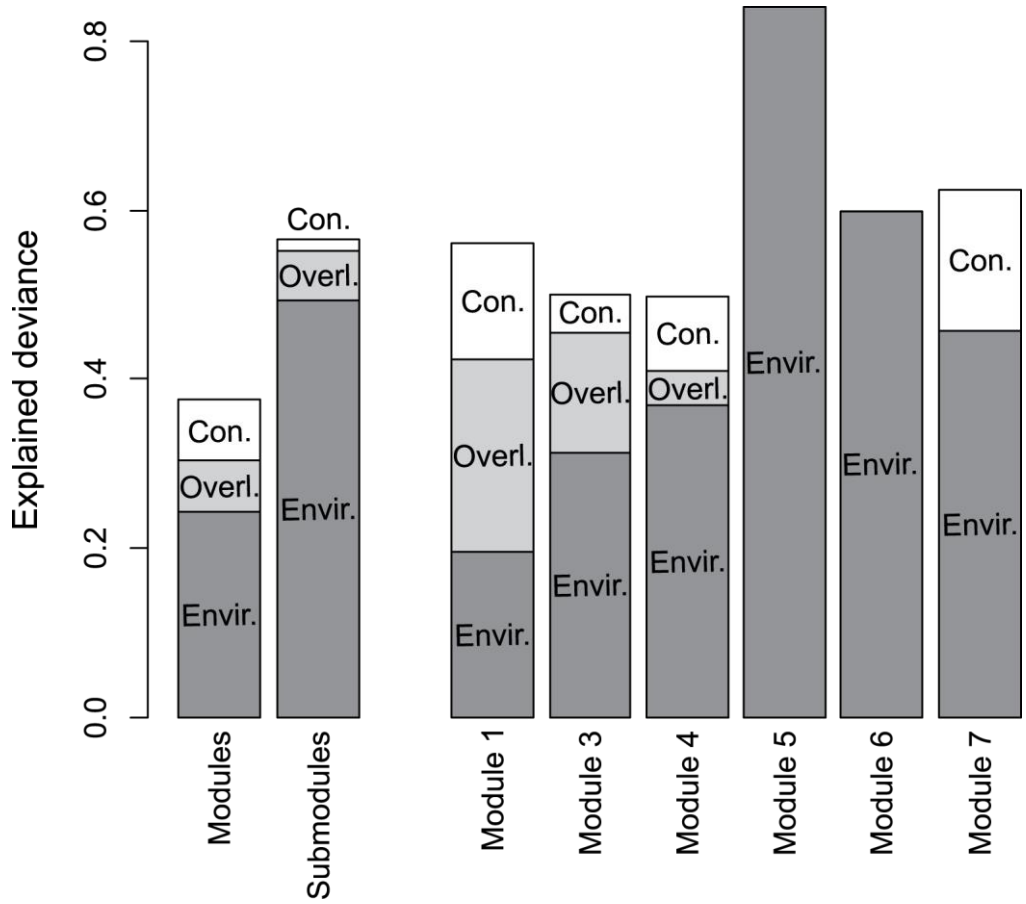
797 **Figure 3**



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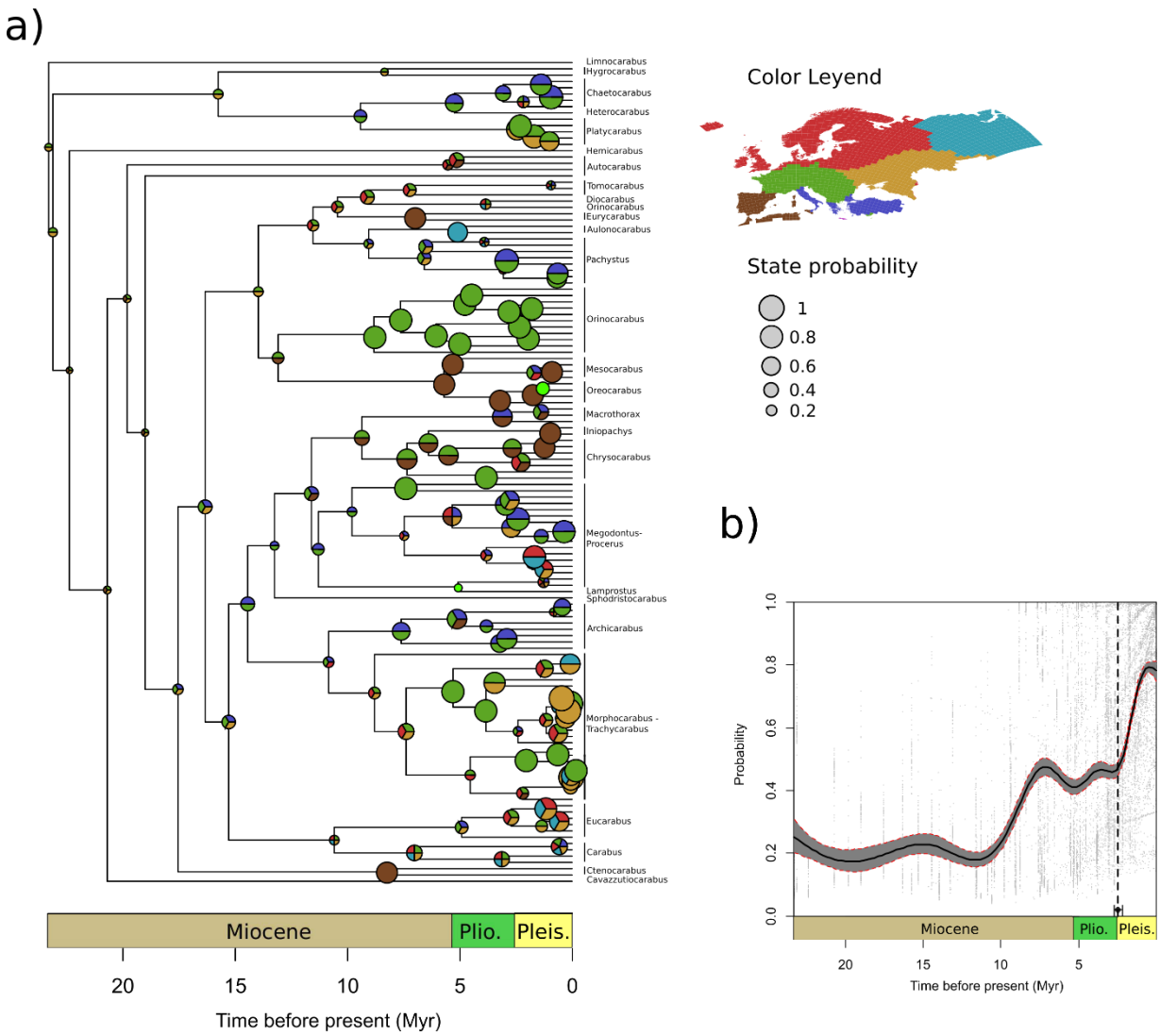
800 **Figure** 4.



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