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Research

Jumping into the grids: mapping biodiversity hotspots in groundwater habitat types across Europe

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Biodiversity hotspots are routinely identified by grid-based analyses, despite grids encompassing different habitats, thus hindering the potential to assess which habitat type accounts for the conservation priority assigned to a grid. In this study, we aimed at identifying the main hotspots for the conservation of the European stygobitic Crustacea Copepoda Harpacticoida at the groundwater habitat scale. A multi-metric approach was used, based on six biodiversity indicators: species richness, endemicity, evolutionary origin, phylogenetic rarity, taxonomic distinctness, habitat specificity. The Hot Spot Analysis, based on the statistics Getis-Ord Gi*, was used to compare the local to the global average values of each indicator to identify hotspots of conservation. The operational units used to perform the analyses were the groundwater habitat types, in order to gather all the possible patterns of spatial occupancy in terms of habitat variability. Eight biodiversity hotspots of stygobitic Crustacea Harpacticoida were highlighted: 1) the Pyrenees (Spain and France), 2) the Jura Massif (France), 3) the Alpine arc (France, Switzerland and Italy) embracing southward the River Po alluvial plain and the Slovenian External Dinarides, 4) the Central Apennines (Italy), 5) the Carpathian and Balkan mountains in Romania and at the boundary between western Bulgaria and north-west Macedonia, 6) the Dinaric Alps (from Croatia to Albania), 7) the Sardinia Island, 8) an area in central-northern Europe embracing Denmark, the Netherlands and Germany. The hotspots showed a clear spatial distribution in southern Europe where they were distributed predominantly south to the 45th parallel, in line to what reiteratively observed in previous studies. Many hotspots embraced more than one habitat type. The adoption of discrete groundwater habitat types as working spatial units rather than grids provided a higher resolution of where the stygobitic harpacticoid species effectively live, with the possibility of intervening more precisely to preserve them and their habitats.

Keywords: aquifers, crustaceans, Gi*, harpacticoids, stygobites

Introduction

As the world's largest liquid freshwater reservoir, ground water plays a significant role in maintaining the ecological structure and function of many terrestrial and aquatic ecosystems. Aquatic groundwater-dependent ecosystems are at risk under climate change scenarios (Taylor et al. 2013), thus losing biodiversity and related ecosystems services (Mammola et al. 2019a). Groundwater biodiversity has long been underestimated due to difficult sampling (Ficetola et al. 2019), and its conservation has never been a priority, especially with respect to the many regulations issued for protecting ground water as primary source of water supply for drinking purposes (Fouché et al. 2019). This perception has gradually changed and a manifesto to intervene on the conservation of the most precious water resource and the intimately cross-linked biodiversity was signed by many scientists (Mammola et al. 2019b). Ground water hosts a variety of living forms (bacteria, protists, invertebrates, amphibians and fish) that complete their life cycle in the darkness; they are called stygobites and provide key groundwater ecosystem services (Griebler et al. 2019).

The stygofauna has a high conservation value due to the high number of narrowly endemic species (Culver and Sket 2000, Culver and Pipan 2014, Mammola et al. 2019b, Boulton 2020) and evolutionary isolated taxa, sometimes called 'living fossils' because they represent the only remnants of ancient lineages disappeared elsewhere. Groundwater communities consist of distantly related taxa, thus they are characterized by high taxonomic disparity. High endemicity and high taxonomic disparity would determine several phylogenetic lineages to become extinct (Galassi et al. 2009, Cantonati et al. 2020), sometimes before being discovered (Mammola et al. 2019b), due to anthropogenic disturbance. In spite of this, records are still scanty and the groundwater biodiversity remains underestimated (Racovitzan shortfall; Ficetola et al. 2019), despite some taxonomic groups are even richest in the underground. In Europe, the number of stygobitic crustacean species exceeds the number of surface-dwelling crustacean species (1174 versus 1111 species, respectively; Stoch and Galassi 2010). Around 11–15% of the 17 000 freshwater animal species in Europe are stygobites, some crustacean orders, families and genera being composed only by obligate groundwater-dwellers.

The distribution patterns of stygobites have been analysed at different spatial scales (Rouch 1988, Marmonier et al. 1993, Gibert et al. 2000, Stoch and Galassi 2010, Fiasca et al. 2014, Stoch et al. 2016, Galassi et al. 2017), the most exhaustive and global approach being in Botosaneanu (1986). The choice of the spatial unit (Gigante et al. 2016, Pipan et al. 2018), as well as the metrics used (Deharveng et al. 2009, Michel et al. 2009) for assessing groundwater biodiversity hotspots, are crucial for the identification of areas of conservation priority. On this regard, grid-based analyses of biodiversity patterns have been widely used especially at the broad spatial scales (Ferreira et al. 2007, Deharveng et al. 2009,

Michel et al. 2009, Zagmajster et al. 2014). Grids are geometric units very practical to use, but at the same time they encompass different habitat types, thus hindering the potential to assess which habitat type accounts for a certain conservation score assigned to a grid, as well as to identify which habitat types within the grids deserve the highest conservation priority (Gibert and Culver 2009, Gigante et al. 2016, Pipan et al. 2018, 2020).

Hotspot mapping techniques are diverse, from point maps, thematic maps of geographic administrative units, grid thematic maps and many others. Despite their scientific accuracy, all these approaches often lack a measure of statistical significance, i.e. the identification of a coldspot or hotspot which significantly differs from its surroundings, based on zonal-statistics (De Giglio et al. 2019, Iannella et al. 2019a, Sánchez-Martín et al. 2019). In this study, we aim at identifying the main hotspots for the conservation of the European stygobitic Crustacea Copepoda Harpacticoida at the groundwater habitat scale addressing our study to available species records. We select this crustacean order for two reasons. First, because stygobitic harpacticoids have the primacy in groundwater communities in terms of both species richness and abundances. This implies that conserving the biodiversity hotspots of this crustacean order means preserving most of the groundwater biodiversity worldwide. Secondly, stygobitic harpacticoids have a low potential for dispersal because they live tightly attached to the sediments of their native habitats, thus representing good biogeographic indicators.

A multi-metric approach is used, based on six biodiversity indicators (species richness, endemicity, evolutionary origin, phylogenetic rarity, taxonomic distinctness, habitat specificity), so as to identify areas featuring the highest values of each indicator. To this end, we perform the Hot Spot Analysis (HSA) based on the statistics Getis-Ord Gi* (Getis and Ord 1992, ESRI 2010), that compares the local to the global average values of each indicator to identify hotspots of conservation. The HSA allows to determine whether a local biodiversity pattern is statistically different from the general trend found in the whole study area, and also offers information about the 'strength' of the biodiversity hotspots detected, in terms of standard deviations. Despite some modelling techniques have proved to increase the general understanding of a variety of topics concerning subterranean ecosystems and their communities (as reviewed by Mammola and Leroy 2018 and references therein), we apply here an alternative approach using, as operational spatial units, the three groundwater habitat types defined by Cornu et al. (2013): 'aquifers in consolidated rocks', 'aquifers in unconsolidated sediments' and 'practically non-aquiferous rocks'. This classification fits, in part, the one given by Hahn (2009).

Our approach allows jumping 'into the grids'. In the context of the broad spatial scale analysed, the rationale is that we have to face with environmental gradients, organisms, communities and ecological systems arrayed in space to form distinct and peculiar patterns or configurations, i.e. 'specific arrangement of spatial elements' (Turner et al. 2015) into a

cell. Cells are replaced here by patches, each of different shape and size and each representing one out of the three groundwater habitat types according to Cornu et al. (2013). The use of an approach not based on grids was preferred because it avoids any possible undesired truncation (or inclusion) of different groundwater habitats within (or among) the cells (Gigante et al. 2016). The main advantage of this approach is that the prescriptions made by the International Union for Conservation of Nature (IUCN 2012) in terms of scale choice for conservation purposes are followed as much as possible, because a taxon will not usually occur throughout the area of its extent of occurrence, which may contain unsuitable or unoccupied habitats. In our study all the habitat components are summarized into a single unit, i.e. the groundwater habitat, that is an environmental 'patch' defined by unequivocal geological and hydrogeological features (Cornu et al. 2013).

Material and methods

Study area

The study covered the European continent, main islands included (longitude min = −31.3, longitude max = 65.2; latitude min = 27.6, latitude max = 69.2; decimal degrees). Collectively, the study area consisted of 61 275 patches (Fig. 1), each belonging to one out of the three groundwater habitat types identified upon the criteria of the groundwater flow type after Cornu et al. (2013). In detail, 'aquifers in consolidated rocks', 'aquifers in unconsolidated sediments' and 'practically non-aquiferous rocks' were considered

three distinct groundwater habitat types providing subhabitats that can be colonized by different species assemblages (Malard et al. 2009). For instance, the habitat type 'aquifers in consolidated rocks' includes subhabitats such as caves and karstic springs, while the habitat type 'aquifers in unconsolidated sediments' includes saturated porous aquifers, hyporheic zones and alluvial springs. The fractures in igneous rocks represent the main subhabitat of the habitat type 'practically non-aquiferous rocks' (Cornu et al. 2013).

Database of occurrences

Crustacea Copepoda are among the most abundant and species-rich group in ground water, rivalling only with Crustacea Amphipoda (Galassi et al. 2009). Cyclopoida and Harpacticoida are the groundwater copepods par excellence, the former being represented by over 350 and the latter by more than 700 stygobitic species. Copepoda Harpacticoida are ubiquitous and highly diversified in any groundwater habitat type (Galassi et al. 2009).

A total of 3248 occurrence records of the 408 stygobitic harpacticoid species and subspecies present in Europe was collected and stored in a database. Occurrence data were retrieved from: the European PASCALIS database (Deharveng et al. 2009); the Hypogean Crustacea Recording Scheme (Knight 2012); the Checklist of the Italian fauna (Ruffo and Stoch 2005); bibliographic collections and unpublished data (DMPG). For a few species, distribution maps from the literature were scanned and georectified and the coordinates of occurrence points were computed in ArcMap ver. 10.0 software (ESRI 2010). Synonymies and incorrect species/
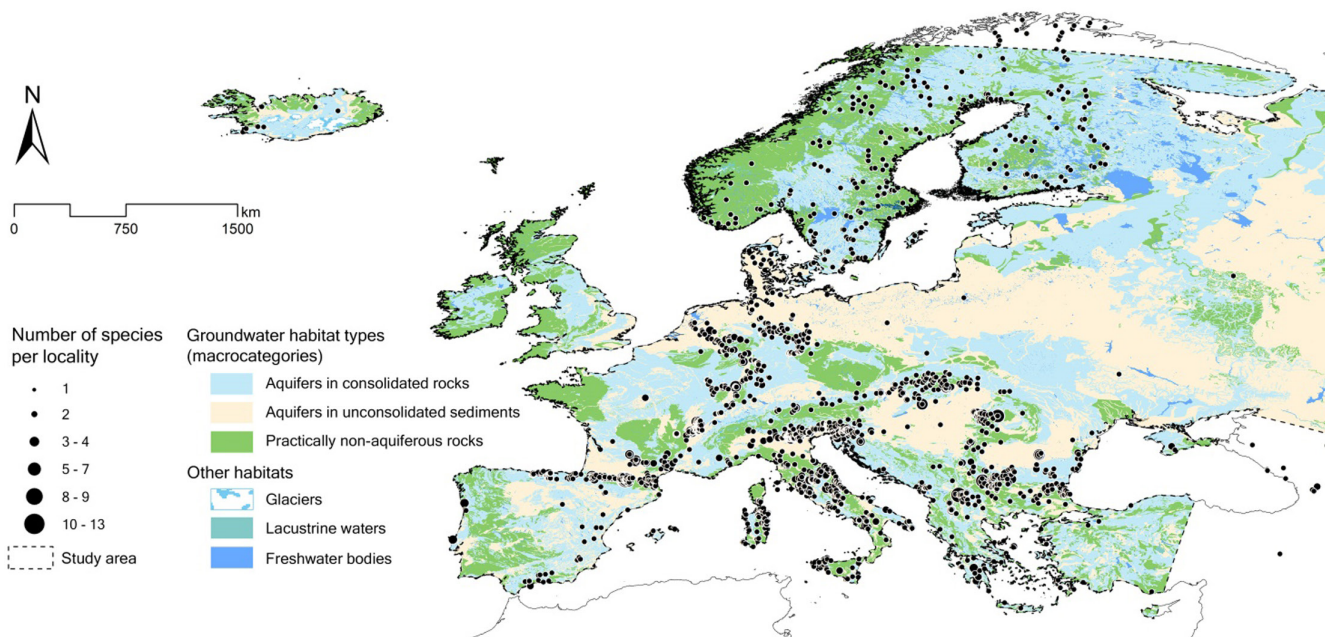

Figure 1. Patterns of species richness of stygobitic harpacticoids per locality of occurrence across Europe. Dots represent 3248 records of occurrence. Dot diameters represent the Jenks natural breaks of species richness. Three groundwater habitat types are used as operational spatial units: 'aquifers in consolidated rocks', 'aquifers in unconsolidated sediments' and 'practically non-aquiferous rocks' (Cornu et al. 2013). Features such as 'glaciers', 'lacustrine waters' and 'freshwater bodies' were represented to enhance the map readability.

name spelling were improved and only accepted names were included in the database (Supplementary material Data 1). Undescribed species which were recognized as new to science by taxonomists were included in the database. The list of the stygobitic harpacticoid species and the species-traits referred to in the next paragraph are provided in the Supplementary material Data 1.

Biodiversity indicators

Six different indicators, featuring either species or assemblage traits, were selected. The conservation value of each patch was determined as the value of each indicator in the patch, based on the species composition of the harpacticoid assemblages occurring in it.

1) Species richness. It is an assemblage-trait indicator and features the total number of species occurring in a patch (= groundwater habitat type).

2) Endemicity. It is a species-trait indicator and was scored in five classes: holoendemic – species whose distribution is limited by ecological and physiological tolerance (score = 1); euryendemic – species with broad, more or less continuous or contiguous distribution, limited by biogeographical barriers (score = 2); stenoendemic – species with restricted, more or less continuous or contiguous distribution, limited by biogeographical barriers (score = 3); rhoendemic – species with widely disjunct distribution due to either vicariance or jump dispersal (score = 4); spot endemic (or microendemic) – species occurring in one locality only (score = 5) (Supplementary material Data 1). Scores were attributed following Myers and De Grave (2000) with a few adjustments allowing a better resolution of the degrees of endemicity observed in stygobitic species (Fattorini et al. 2020). Species constrained by geographical barriers were considered less able to cope with environmental changes as so requiring priority in conservation. In order to minimize the effect of species richness, values of the endemicity indicator were computed as the ratio of the sum of the species' endemicity scores to the number of species occurring in a patch.

3) Evolutionary origin. It is a species-trait indicator featuring the evolutionary origin of the stygobitic harpacticoid species of Europe. The rationale is based on associating higher conservation scores to the species that have taken longer evolutionary paths to colonize ground water. A groundwater harpacticoid species was defined of limnicoid origin when the ancestor lived (or its extant relatives still live) in surface fresh waters. The colonization of ground water by freshwater ancestors was, likely, direct and did not require severe adaptations. A groundwater harpacticoid species was defined of thalassoid origin when the ancestor was marine and the colonization of fresh ground water occurred in one or two steps (Notenboom 1991). In the one-step case, the ancestor was a marine/brackish species that was pre-adapted to tolerate wide salinity ranges so as to enter directly ground water from surface marine/brackish environments. In the two-step case, the marine ancestor first entered surface fresh water or coastal wetlands, and only subsequently colonized ground water.

The main difficulty for entering surface fresh water was to cross the 'saline boundary' between the marine and the freshwater compartments. The two-step thalassoid origin was likely based on a shorter evolutionary trajectory than the one-step thalassoid origin, the latter requiring the abrupt transition from a truly marine environment directly to ground water thus being the most challenging evolutionary requirement. The limnicoid origin is likely less restrictive than the two thalassoid origins, overall. Hence, the scores were assigned as following: 1) species of limnicoid origin (score = 1); 2) species of two-steps thalassoid origin (score = 2); 3) species of one-step thalassoid origin (score = 3). This categorization relied upon the knowledge available on the order Harpacticoida (Galassi 2001, Galassi et al. 2009, and references therein). The indicator value for each patch was obtained calculating the ratio between the sum of each species' evolutionary origin score and the number of species recorded from each patch.

4) Phylogenetic rarity. It is a species-trait indicator featuring the taxonomic isolation of a species. The rationale of the indicator consisted in the assumption that the lowest the diversification of a lineage, the highest the conservation value of the species. Scoring was assigned as follows: species belonging to a highly diversified lineage (score = 1); species belonging to a moderately diversified lineage (score = 2); species belonging to a recent lineage including few species (score = 3); species belonging to an ancient lineage including few species (score = 4); taxonomically very isolated species, with no surface-water close relatives (score = 5). This categorization relied upon expert-based evaluations because the degree of diversification and isolation is taxon-specific. The value of the indicator for each patch was obtained calculating the ratio between the sum of each species' phylogenetic rarity score and the number of species recorded from each patch.

5) Taxonomic distinctness. It is an assemblage-trait indicator featuring the taxonomic relatedness of the species in an assemblage. The indicator value was computed using the Linnaean (taxonomic) hierarchy as a proxy for phylogenetic information (Clarke and Warwick 1998). The rationale was that assemblages having the same number of species may have different values for conservation purposes, because the most taxonomically composite assemblages may be considered also the most diverse ones. The protection of evolutionarily significant lineages (Posadas et al. 2001, Faith 2015), which, once lost, cannot be recovered, is a central question in conservation biology, and yet the occurrence of isolated evolutionary units is extreme in groundwater environments, and this index may consistently complement the other biodiversity indicators used herein. The indicator value was based on the concept of taxonomic distinctness of Clarke and Warwick (1998) and calculated as:

$$\Delta^+ = \left[\sum \sum_i <_j \omega_{ij} \right] / \left[s(s-1)/2 \right]$$

where Δ^+ is the average taxonomic distance between two randomly selected species, s = the number of species in a sampled community or assemblage; and ω_{ij} = the taxonomic

path length between the i-th and the j-th species. The weighting assigned to each of the levels in the taxonomic hierarchy was equal to 1. The indicator is independent from species richness.

6) Habitat specificity. It is a species-trait indicator that considers the distribution of the species in the groundwater habitat types across Europe, as well as the number of species colonizing each patch. The habitat specificity (Wagner and Edwards 2001) was used as species' trait to assess the contribution of single patches to the species richness at a landscape scale. This indicator considers both the rarity of species belonging to a certain patch and the area covered by it; considering that some confounding effects could arise when dealing with, for instance, circular versus elongated patches (as in the case of patches analyzed in the present work) according to Wagner and Edwards (2001), we used the habitat specificity index correction by Halvorsen and Edvardsen (2009). In this index, the 'area' contribution can be left out of the calculation, and the final value represents the gamma diversity (i.e. total diversity within a region), thus emphasizing the contribution of rare communities (Halvorsen and Edvardsen 2009). The corrected habitat specificity for a patch (Su_j) is thus calculated as:

$$Su_j = \sum \left(m_j / n_i \right)$$

where m is the number of species occurring in the j-th patch and n is the number of patches in which the i-th species is found in the analyzed landscape.

Gi* statistics for hotspot and coldspot analysis

The prioritization of areas deserving management and/or conservation measures in nowadays approached in different ways (Rabelo et al. 2018, Fattorini et al. 2020), mainly delimiting geographical areas following the outcomes of Species Distribution Modeling and hotspots' identification (Brunetti et al. 2019, Cerasoli et al. 2019, Iannella et al. 2019a, b, c). The Gi* statistics by Getis and Ord (1992) was used to identify hotspots (or coldspots) of stygobitic harpacticoid biodiversity using the six biodiversity indicators (Supplementary material Appendix 1 Note A1). The tool used, implemented in ArcGis 10.0 (Getis and Ord 1992), applies the Gi* statistics to a patch in the context of its neighborhood and to the whole study area. It assesses whether the value of a geometry (in our case, a biodiversity indicator assigned to a single patch) is high or low by chance or it follows a non-random pattern (i.e. an aggregation of patches with high or low values clustering together). For each cluster identified as a hotspot, a p-value (indicating the confidence interval to which a hotspot can be identified as such) and a z-score (reporting the number of standard deviations that a patch belonging to the cluster differs from the global mean) were calculated. When both conditions of high p-value and z-score were satisfied for one patch and its neighborhood, the cluster obtained was not to be considered an outlier but a

statistically significant hot (or cold) spot upon a confidence level range of 90–99 percent, and the so-called 'Complete Spatial Randomness' hypothesis was thus rejected (ESRI 2010). For statistically significant positive z-scores, the larger the z-score, the more intense the clustering of high values (hotspots). For statistically significant negative z-scores, the smaller the z-score, the more intense the clustering of low values (coldspots). The Jenks natural breaks are derived by a clustering method which minimizes the variance within each class and maximizes variance among all the classes inferred (Jenks 1967), and were used to determine the best arrangement of the values of each indicator into different classes. We commented on the 'hottest hotspots' (Myers et al. 2000) that is on the hotspots showing the largest z-scores (i.e. the two highest intervals) with p=99% and p=95% (red and dark orange in the maps). The critical p-values determining confidence levels were reduced to account for multiple testing and spatial dependence.

The False Discovery Rate (FDR) correction was applied to each hotspot analysis, in order to buffer the effect of spatial dependency among the patches close to each other, also by lowering the outcoming p-values considering the number of false positives that FDR detects, thus obtaining more robust results in terms of statistical significance of inferred hotspots (ESRI 2010, Tegegne et al. 2019). The Euclidean distance (shortest straight-line distance) was used among each patch, with the 'Inverse distance' spatial relationship parameter, which proportionally weights the influence of neighboring features with respect to the target feature depending on their distance, e.g. the more distant one patch is from another, the lower their mutual influence will be (ESRI 2010, De Giglio et al. 2019, Iannella et al. 2019a, Sánchez-Martín et al. 2019). Considering all these settings, sampling bias is buffered as much as possible, both because of the low relative influence that the HSA gave to the non-sampled patch (based on the Inverse distance parameter) and because of the false positives' detection and correction.

Results

General patterns of species richness

A total of 3248 records representing 408 species and subspecies (where subspecies are considered different taxonomic units; Agapow et al. 2004) distributed in 7 families and 42 genera of Crustacea Harpacticoida in Europe were assembled. The family Canthocamptidae comprised 14 genera and 193 species/subspecies, the Parastenocarididae 15 genera and 130 species/subspecies and the Ameiridae 7 genera and 68 species/subspecies. The remaining families were Ectinosomatidae (3 genera, 7 species), Chappuisiidae (1 genus, 3 species), Miraciidae (1 genus, 6 species) and Phyllognathopodidae (1 genus, 1 species) (Supplementary material Data 1).

Stygobitic harpacticoid species were distributed throughout Europe, with fewer or no records from most part of the Iberian Peninsula, northern France, British Isles, Turkey and

former Soviet Union countries (Fig. 1). The species richness varied across Europe with the highest values found in southern Europe (Fig. 1). After applying the Jenks natural breaks (Jenks 1967, ESRI 2010) to determine the best arrangement of species richness into different classes, we found out the localities with very high (10–13 species) and high (8–9 species) raw values of species richness. They were located in central and eastern Pyrenees, French Pyrenees Mountains, French Jura Massif, southern Germany, the Alpine arc together with the Slovenian External Dinarides, Central Apennines, Carpathians Mountains (embracing Slovakia, Hungary and Romania), Danube plain (Romania), Dinaric Alps (from Croatia to Albania), Balkan Mountains embracing Bulgaria and Serbia (Fig. 1). The Scandinavian Peninsula showed low values of species richness in contrast to the high number of records of occurrence. The habitat types of the Scandinavian Peninsula were primarily aquifers in consolidated rocks and aquifers in practically non-aquiferous rocks (Fig. 1). Relatively high values of species richness were found in aquifers in unconsolidated sediments of the Netherlands, Denmark and northern Germany (Fig. 1). In central and southern Europe, the highest values of species richness were mainly associated with aquifers in consolidated rocks or at the boundaries between aquifers in consolidated rocks and practically non-aquiferous rocks or alternatively aquifers in unconsolidated sediments (Fig. 1).

Hotspot analysis

The HSA based on the statistics Getis-Ord Gi* after selection of the areas showing the highest z-scores (i.e. the two highest confidence intervals, with p=99% and p=95%, respectively) highlighted eight 'hottest hotspots' of the stygobitic harpacticoids in Europe: 1) the Pyrenees (Spain and France), 2) the Jura Massif (France), 3) the Alpine arc (France, Switzerland and Italy) embracing southward the River Po alluvial plain and the Slovenian External Dinarides, 4) the Central Apennines (Italy), 5) the Carpathian and Balkan mountains in Romania and at the boundary between western Bulgaria and north-west Macedonia, 6) the Dinaric Alps (from Croatia to Albania), 7) the Sardinia Island, 8) an area in central-northern Europe embracing Denmark, the Netherlands and Germany. The Pyrenees hotspot was identified by all six biodiversity indicators (Fig. 2–7). The Alpine arc, Central Apennines, Sardinia, Carpathian and Balkan mountains hotspots were highlighted by all the indicators except habitat specificity. The Jura Massif and the Dinaric Alps hotspots were detected only by habitat specificity. Finally, the central-northern Europe hotspot was highlighted by phylogenetic rarity and a small portion also by taxonomic distinctness (Supplementary material Appendix 1 Note A2). The aquifers in consolidated rocks and unconsolidated sediments occurred in all the hotspots (Fig. 1), except in Sardinia, where the main habitat type was represented by practically non-aquiferous rocks. Significant coldspots with high z-score were not detected by the analysis for any biodiversity indicator.

No correlation was observed between the number of species occurring in hotspots (inferred by species richness and endemicity scores) and their extent (i.e. no area-effect), both considering the hotspots inferred on p-values and the ones defined by z-scores (Supplementary material Appendix 1 Fig. A1a–b).

Distribution of groundwater harpacticoids in the hotspots and groundwater habitat types

The westernmost hotspot was represented by the Pyrenean region, with 53 stygobitic species, at the boundary between aquifers in consolidated rocks and aquifers in unconsolidated sediments, or in the hyporheic zone of streams flowing over practically non-aquiferous rocks. In this area, members of the families Canthocamptidae (31 species), Parastenocarididae (13 species) and Ameiridae (9 species) accounted for high species richness. In the Canthocamptidae, *Antrocamptus* is exclusively known from ground water; its species being primarily linked to aquifers in consolidated rocks (karst), with the sole *Antrocamptus chappuisi* recorded also from the hyporheic zone of the River Nert (France) and from the Lachein brook (France). The genus *Antrocamptus* was considered a biogeographic indicator of this hotspot, its species being spot endemics or stenoendemics (Supplementary material Data 1). *Ceuthonectes chappuisi* and *C. vievilleae* were exclusive to this hotspot, in aquifers in consolidated rocks, with one record only, respectively. Both species are spot endemics, and together with the species of *Antrocamptus*, mainly contributed to high endemicity, phylogenetic rarity, evolutionary origin and habitat specificity of this hotspot (Supplementary material Data 1). *Elaphoidella* (14 species) was the most diversified genus in this hotspot; the spot endemics *E. calypsonis*, *E. longifurcata*, *E. mauro*, *E. garbetensis*, *E. reducta* were all recorded from aquifers in consolidated rocks. *Moraria* is known from this area with the narrow endemic *M. catalana* and the spot endemic *Moraria* sp. S1 (Supplementary material Data 1). The Parastenocarididae were recorded both from aquifers in consolidated rocks and from aquifers in unconsolidated sediments (mainly in hyporheic sites); none of the genera was endemic to this area, species being spot endemics in aquifers in consolidated rocks. The Ameiridae were represented by members of the genera *Nitocrella* and *Parapseudoleptomesochra*. They have a direct marine origin (Supplementary material Data 1); only species of the latter genus accounted for a high endemicity score, with the subspecies *P. subterranea deminuta* known exclusively from the Ariège saturated karst in France. The taxonomic distinctness of this hotspot was significantly high, because stygobites were distributed in a broad taxonomic range (3 families and 14 genera). The two hotspots of the Dinaric Alps and, to a lesser extent, the Jura Massif, which were represented mainly by aquifers in consolidated rocks, were defined by high habitat specificity, with most species belonging to the family Canthocamptidae recorded almost exclusively from these hotspots.
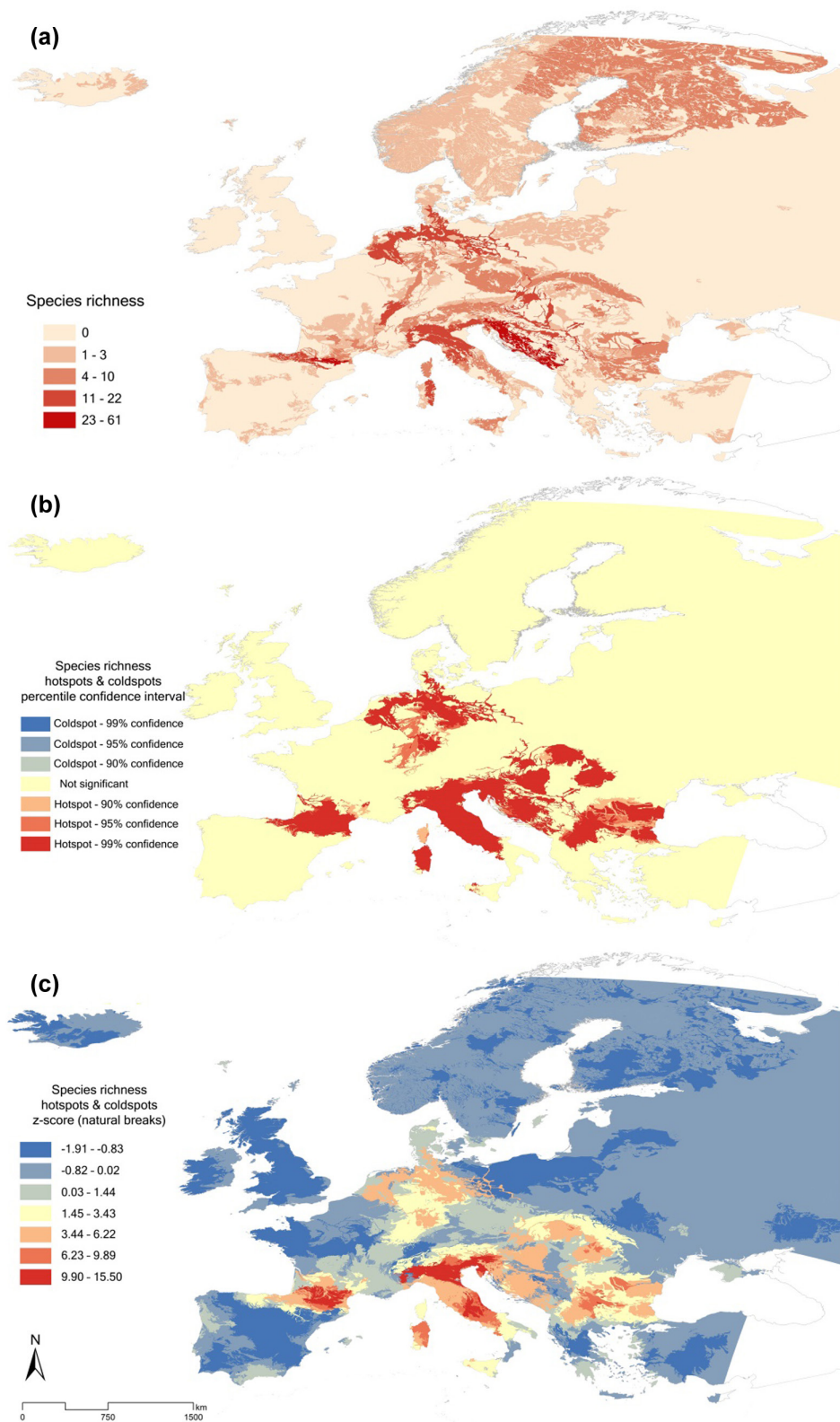
Figure 2. (a) Raw values of species richness, (b) significant hotspots of species richness percentile confidence intervals, (c) natural breaks of z-scores. The red and dark orange areas represent the hottest hotspots.
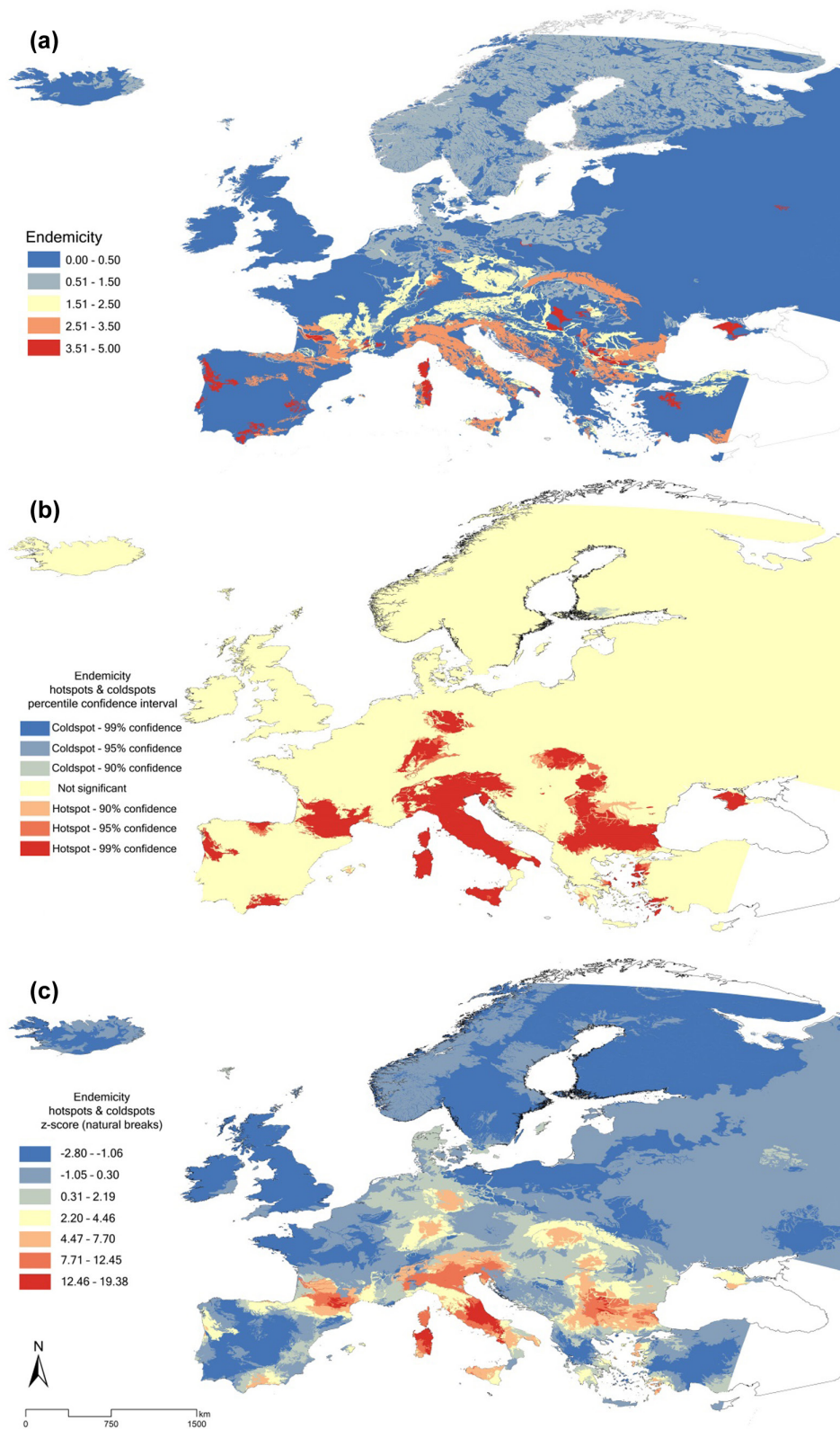
Figure 3 (a) Raw values of endemicity, (b) significant hotspots of endemicity percentile confidence intervals, (c) natural breaks of z-scores. The red and dark orange areas represent the hottest hotspots.
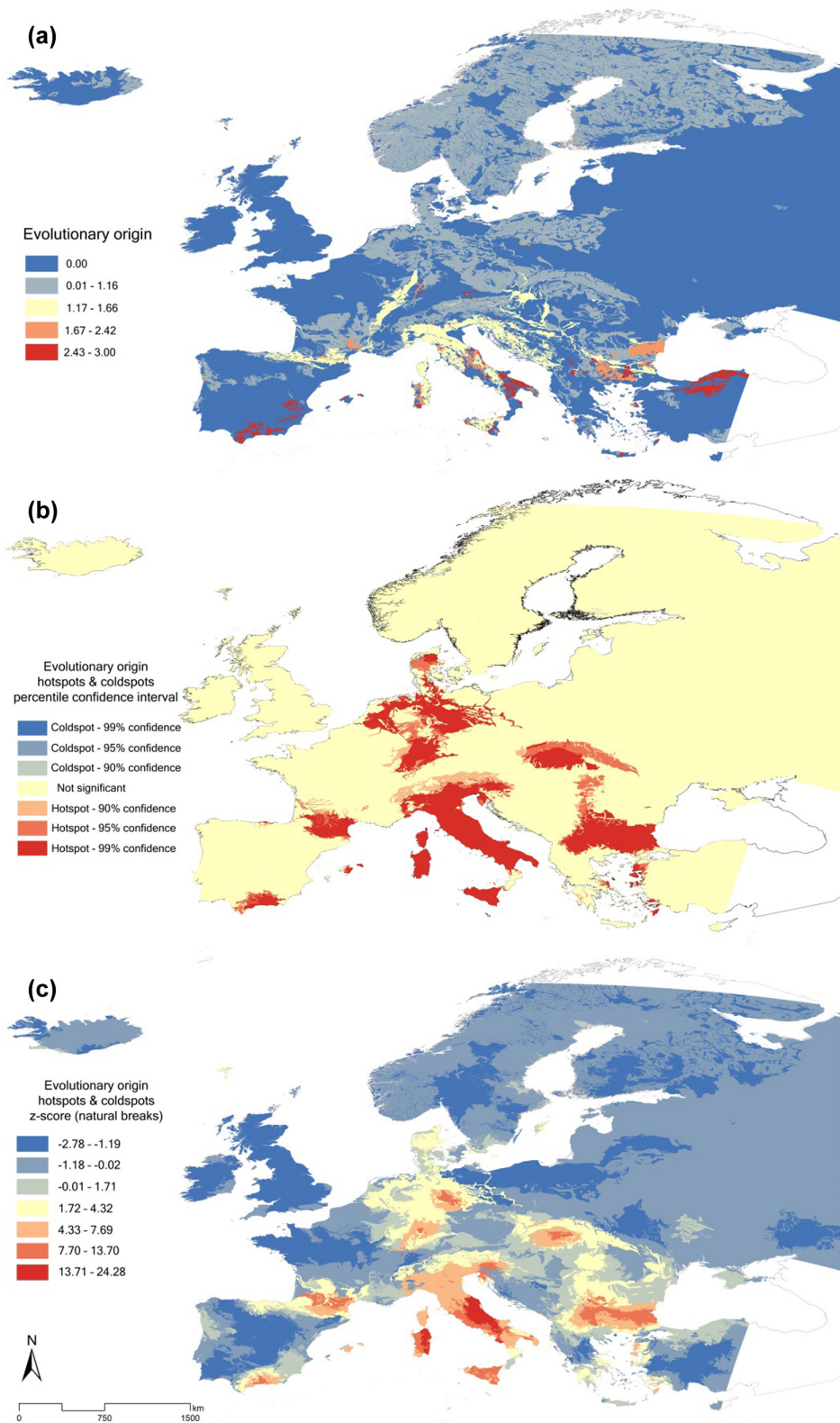
Figure 4. (a) Raw values of evolutionary origin, (b) significant hotspots of evolutionary origin percentile confidence intervals, (c) natural breaks of z-scores. The red and dark orange areas represent the hottest hotspots.
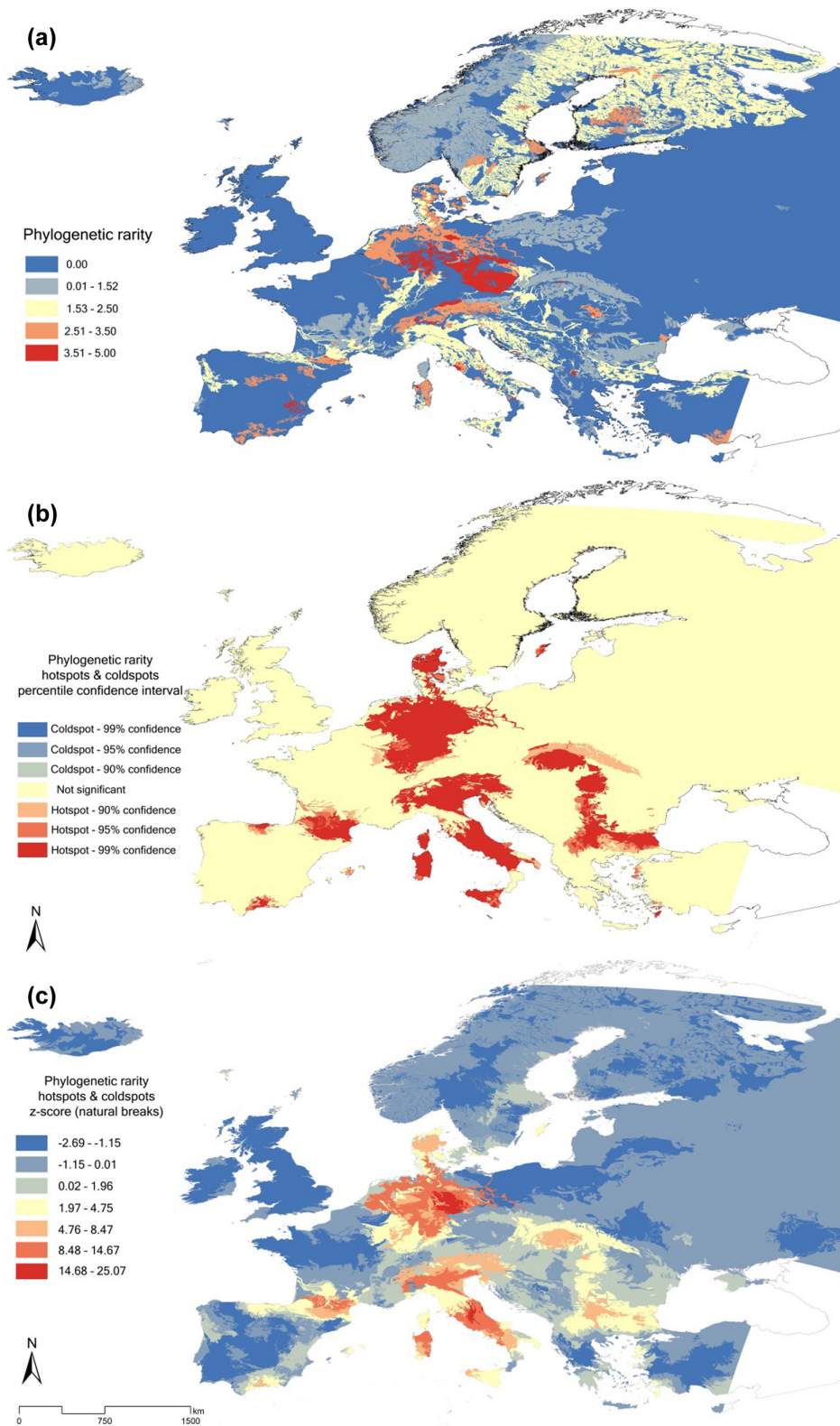
Figure 5. (a) Raw values of phylogenetic rarity, (b) significant hotspots of phylogenetic rarity percentile confidence intervals, (c) natural breaks of z-scores. The red and dark orange areas represent the hottest hotspots.
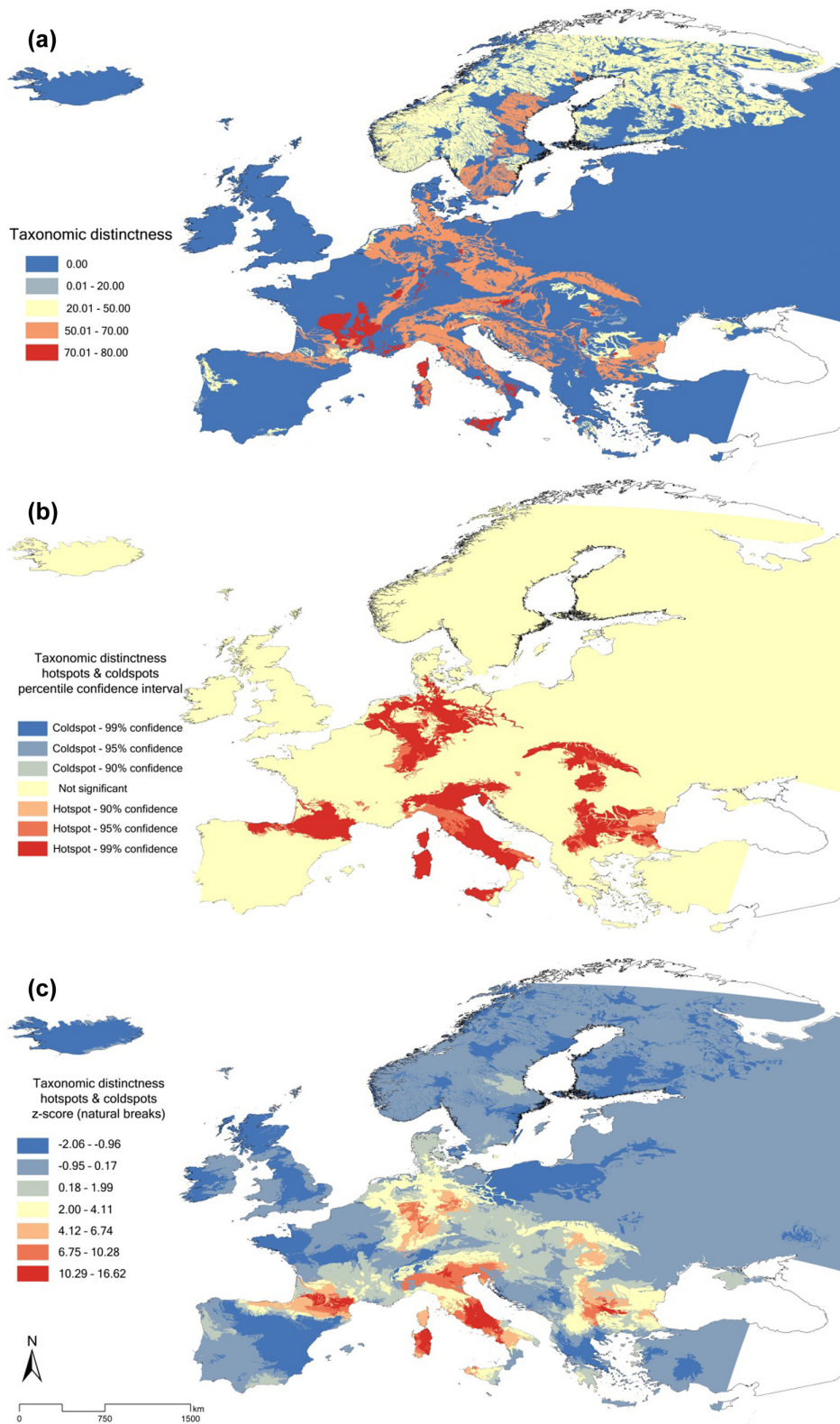
Figure 6. (a) Raw values of taxonomic distinctness, (b) significant hotspots of taxonomic distinctness percentile confidence intervals, (c) natural breaks of z-scores. The red and dark orange areas represent the hottest hotspots.
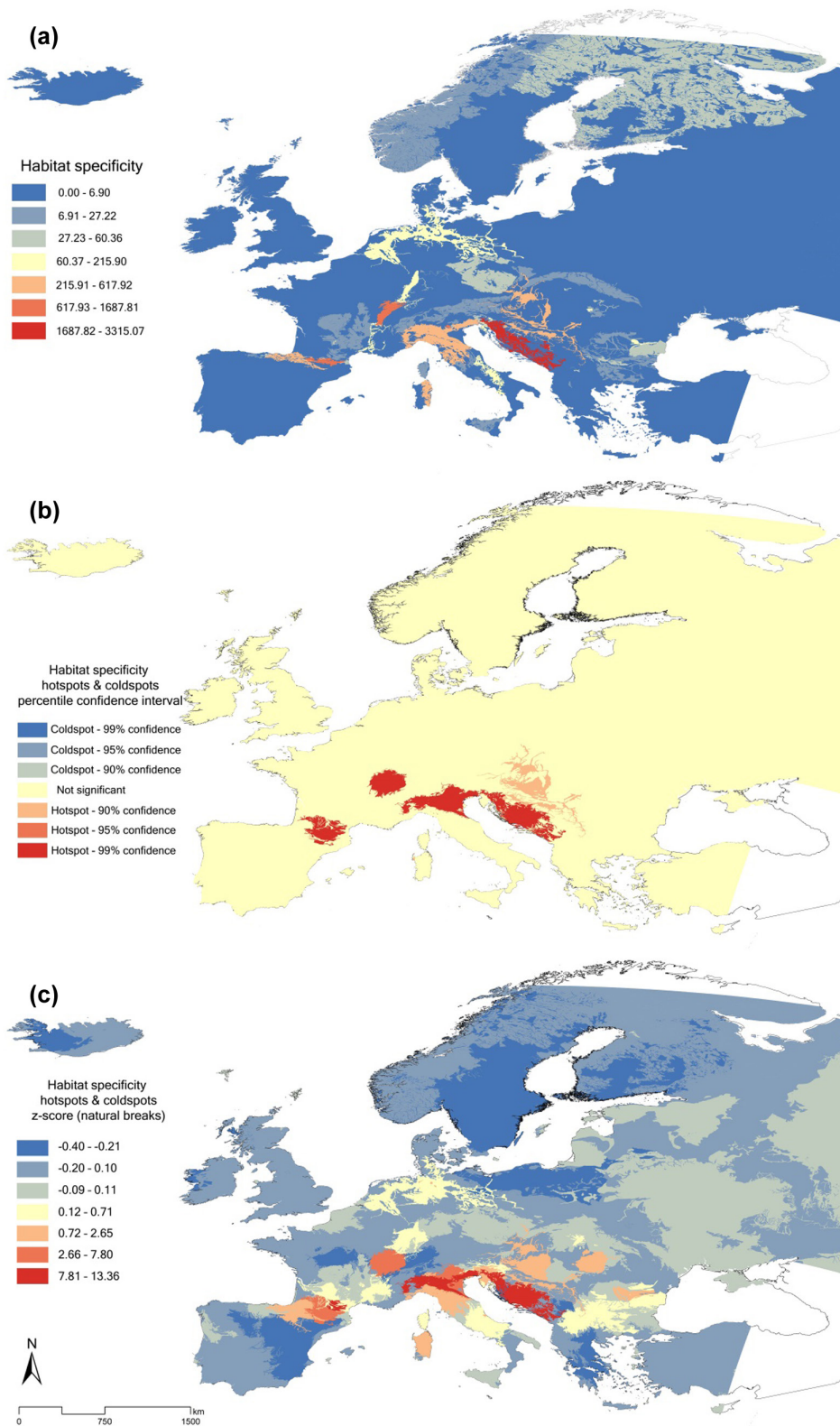
Figure 7. (a) Raw values of habitat specificity, (b) significant hotspots of species habitat specificity percentile confidence intervals, (c) natural breaks of z-scores. The red and dark orange areas represent the hottest hotspots.

The Alpine area represented the main hotspot in central-southern Europe and is located close to the border of the Last Glacial Maximum (LGM, ~21 kyBP; Becker et al. 2015), extending toward the north-eastern part of the External Dinarides (the 'Classical Karst'; Brancelj et al. 2020). This hotspot included southward the vast River Po alluvial plain and hosted 65 stygobitic harpacticoids, 49 of which belonged to the Canthocamptidae with also the highest number of genera (9), followed by the Parastenocarididae (4 genera, 6 species) with lower habitat specificity, the Ameiridae (2 genera, 9 species) and an undescribed Ectinosomatidae. The canthocamptid species of the genera *Morariopsis*, *Paramorariopsis*, *Lessinocamptus* and *Stygepactophanes* mostly contributed to the high endemicity, phylogenetic rarity and habitat specificity of this hotspot, all the species being located in aquifers in consolidated rocks, or at the boundary between aquifers in consolidated rocks and unconsolidated sediments (in the interstitial habitats at the emergences of karstic springs). These genera are also exclusive to ground water, with no surface-water representatives, thus highly increasing phylogenetic rarity and taxonomic distinctness of this hotspot. The Ameiridae were represented by the genus *Nitocrella* (7 species), with the stenoendemic *N. slovenica* recorded from Slovenia to Greece, and the spot endemic *N. stochi*. The mean evolutionary origin and taxonomic distinctness scores were significant, even lower than in the Pyrenean hotspot, especially for the unbalanced distribution of species among families, due to the dominance of the Canthocamptidae.

The Central-Apennines hotspot was mainly driven by the Canthocamptidae (5 genera, 14 species) with a good representativeness of the Ameiridae (2 genera, 11 species), followed by the Parastenocarididae (5 genera, 8 species), the Ectinosomatidae (1 genus, 3 species) and the Phyllognathopodidae, with the sole stygobitic species of the genus *Phyllognathopus* known worldwide so far, *P. inexspectatus* (Galassi et al. 2011). This hotspot hosted a relatively low species richness if compared to the other ones, along with high endemicity score (10 spot endemics), the highest taxonomic distinctness at the family level, high phylogenetic rarity (e.g. the ectinosomatid genus *Pseudectinosoma*; the parastenocaridid *Simplicaris*; Galassi et al. 1999, Galassi and De Laurentiis 2004), and high evolutionary origin score of the species, with high proportion of thalassoid species (Ameiridae and Ectinosomatidae) (Supplementary material Data 1).

Sardinia was the most species-rich island in Europe, with 21 species distributed in 4 families and 9 genera, of which about 50% of the species are spot endemics. All the biodiversity indicators resulted very high, except habitat specificity. Likely, the composite geological history of the island (Cherchi and Montadert 1982) accounts for such high species richness together with the different origin of the taxa, with the limnicoid Canthocamptidae and Parastenocarididae, and the thalassoid Ameiridae and Miraciidae (Supplementary material Data 1).

The Carpathian and Balkan mountains hotspot consisted of several hot areas but the most statistically significant ones were inferred in Romania, and at the boundary between western Bulgaria and north-west Macedonia, despite low species richness was measured in these disjunct hotspots where aquifers in consolidated rocks (karstic areas) sometimes merge in practically non-aquiferous rocks. Canthocamptidae, Parastenocarididae and Ameiridae were the harpacticoid families defining these hotspots, with a total of 10 genera and 32 species. The taxonomic richness was higher at the species level, and rather low at higher taxonomic levels (genus/family). The canthocamptid genus *Spelaeocamptus* was the target genus of this 'hotspot cluster', being endemic to this area, known with two species only, *Spelaeocamptus spelaeus* and *S. incertus*, both restricted to the karst. This genus is only known from ground water. Thirteen spot endemic species were recorded from this area, mostly belonging to the Canthocamptidae.

Finally, the northern-central European hotspot returned a weak signal for many biodiversity indicators. This hotspot was species-poor, with several representatives of the genus *Parastenocaris*, the widely distributed canthocamptid *Elaphoidella elaphoides* and *E. leruthi leruthi*, and the Chappuisiidae predominantly recorded from aquifers in unconsolidated sediments of Germany, Slovakia, Czech Republic and Hungary. The presence of the interstitial Chappuisiidae, with no surface water representatives and exclusively known from this area across Europe and worldwide, mostly accounted for the high taxonomic distinctness and phylogenetic rarity of this hotspot.

Discussion

Mapping groundwater biodiversity for habitat types across Europe

Previous studies on spatial patterns of stygobites at broad spatial scales (Castellarini et al. 2007, Ferreira et al. 2007, Zagmajster et al. 2014) have dealt with spatial analyses by using grids-based mapping. This approach allowed comparison among cells of identical extension and the selection of the more adequate cell size based on the resolution of the information available (Christman and Zagmajster 2012). On the other side, it masked the actual presence of habitats where one or more stygobitic species stably live, and no less relevant, the possible co-presence of different habitats within a single cell. As a rule, quadrat analysis gives results depending on quadrat size and orientation, returning a measure of density-based dispersion, and not a 'relationship' pattern. Thus, clusters are locally highlighted, but they may encompass an internal variability which is lost both in local and global resulting trends.

The application of Gi* statistics (Getis and Ord 1992) allows the identification of a neighborhood distance within which a cluster is expected to occur. In our study, for each habitat type and for each biodiversity indicator, indicator scores were calculated based on neighboring habitat types sharing a border. Since a measure was available for each habitat type, it was mapped indicating how spatial autocorrelation varies over the study area. Moreover, each indicator has an associated statistical test, thus the groundwater habitat

type which returned a statistically significant relationship with the neighboring ones was mapped by assuming the same color in the maps. This analysis displayed biodiversity hotspots as mapped areas of different colors for each indicator used, and with different statistical significances and strengths. For this reason, in this study the groundwater habitat types were adopted as primary working units, on the basic assumption that the abiotic groundwater environment, mainly described by three hydrogeological criteria, has been proved to influence the composition and distribution of groundwater organisms: flow type, void size and permeability. These attributes were used by Cornu et al. (2013) to define the groundwater habitat types that resulted spatially structured in patches, each defined by sometimes sharp or, more commonly, blurred borders on the edges of the patches.

The concurrent roles of historical factors and habitat heterogeneity in shaping groundwater biodiversity hotspots in Europe

Hotspots of stygobitic harpacticoid based on six biodiversity indicators showed a clear spatial distribution in southern Europe where they were dispersed predominantly south to the 45th parallel. This observation is in line to what reiteratively observed in previous studies (Deharveng et al. 2009, Stoch and Galassi 2010, Zagmajster et al. 2014, Eme et al. 2015, 2018). The Quaternary glaciations had strong residual effects on the distribution of groundwater harpacticoids, determining a massive extinction of either surface and groundwater species below the ice cap and in permafrost areas (but see McInerney et al. 2014). Under this assumption, during the LGM the extinction of several species or the retreat of some populations south to the ice cap occurred, thus determining the collapsing of the distribution range of the survivors. After the ice retreat, only the species with high dispersal abilities recolonized northward suitable areas (Stoch and Galassi 2010, Zagmajster et al. 2014).

If the Quaternary glaciations may explain the latitudinal distribution of the hotspots (Castellarini et al. 2007, Stoch and Galassi 2010, Zagmajster et al. 2014), other historical factors, which date back to the Tertiary, should be called upon to explain the disjunct distribution of hotspots south to the Würmian glacial borders. Areas including ancient karstic regions in the Pyrenees or in the Classical Karst (eastern Alps and the Dinaric karst) are hotspots where the stygobitic fauna had long time for speciation by vicariance, favoured also by habitat fragmentation and isolation in the prevailing habitat type (aquifers in consolidated rocks). Multiple invasions have probably further contributed to increase biodiversity by playing in their favour even a longer time available to colonize these ancient areas at different times. Species richness, endemicity and taxonomic distinctness reached high and significant scores in these hotspots. In the Balkan hotspots different paleogeographic events stratifying in the Tertiary may have determined speciation around the fresh- or brackish Pannonian, Dacian, Euxinian basins (Jipa and Olariu

2009) after the fragmentation of the Parathethys, because they became isolated surface water bodies with changing environmental condition up to their desiccation during the Messinian salinity crisis (late Miocene) (~5.5 Mya). In this area the hotpots of endemicity, taxonomic distinctness and, to a lesser extent, species richness are distributed at the borders of these former basins, and on the neighbouring slopes of the Carpathians and the Balkans.

Into the hotspots' boundaries and its significance for groundwater biodiversity conservation

Preserving biodiversity to counteract its loss is becoming a key-issue in conservation management plans across the world and in many environments (Smith et al. 2001, Roberts et al. 2002); in the underground it assumes even greater importance.

Many efforts have been made in the recent past by the scientific community, but awareness among public authorities and citizens of the importance of this biodiversity is still in its infancy, despite its uniqueness, evolutionary peculiarities (e.g. the omnipresence of phylogenetic and distributional relics) and the ecosystem services it performs for human wellbeing (Griebler et al. 2019).

The main shortfalls in groundwater biodiversity assessment and conservation rely on key-impediments: 1) ground water and dependent ecosystems and their ecological services are mostly unknown to the general public (public dilemma); 2) policymakers and stakeholders are mostly unaware of their conservation issues (political dilemma); 3) basic science on groundwater and dependent ecosystems is scarce and underfunded (scientific dilemma). This condition stems from the dramatic decline in faunal inventories of the invertebrates (Eisenhauer et al. 2019), which hold the primacy in ground water. For these reasons, the distribution patterns of stygobitic species largely remain poorly documented in North America, South America, Africa and Asia. Better knowledge is available from ground water of Europe, and in the last decades several new species, genera and families are being discovered in Australia and New Zealand.

Efforts to shed light on patterns and processes shaping groundwater biodiversity on broad spatial scales have been numerous, but it is precisely on the question of scale that we want to convey attention. Indeed, quadrat analyses applied to groundwater biodiversity (Deharveng et al. 2009, Zagmajster et al. 2014) returned results depending on quadrat size and orientation (i.e. modifiable areal unit problem). The result was a measure of dispersion because it is based primarily on the density of points, and not on their arrangement in relation to one another; a single measure for the entire distribution is then returned, thus variations within the quadrat are not recognized (e.g. records could have clustered locally in some areas, but not overall). Despite the usefulness of the above-mentioned approaches, which may offer an overall view of where the main groundwater biodiversity hotspots are located across Europe, the adoption of discrete groundwater habitat types as working spatial units provides a higher resolution of where the species effectively live, with

the possibility of intervening more precisely to preserve them and their habitats.

Many hotspots embraced more than one habitat type, and some of them were somehow unexpected. This is the case of the large hotspot embracing the Alpine arc, the Slovenian Dinarides, together with the alluvial plain of the River Po in northern Italy, because stygobitic species richness was relatively low in the vast plain bordering southward the Alpine arc. In this regard, we would like to draw attention to the fact that larger spatial neighbourhoods are needed to identify spatial patterns associated with broader spatial processes. Moreover, adaptive diversity within species is often well represented along environmental gradients from a groundwater habitat type to another, and these gradients are important for promoting speciation, and increasing species' survival under drastic environmental changes due to anthropogenic disturbance. Biodiversity collapse determines not only species loss, but the loss of the ecological interactions, functions, co-dependencies, structural complexity and mechanisms of resilience that characterize groundwater communities and the ecosystem services they may provide (Valiente-Banuet et al. 2015, Sage 2020). Indeed, the protection of metapopulations in different habitats within the same hotspot lends support to the inclusion of poor-species richness habitats into the same hotspot. The conservation of jointed groundwater habitat types guarantees the protection of the ensemble of the evolutionary processes which are in need to be preserved for maintaining the hottest 'cores' into the hotspots.

Data availability statement

The data that support the findings of this study are available in the Supplementary material Data 1.

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Supplementary material (available online as Appendix ecog-05323 at <www.ecography.org/appendix/ecog-05323>). Appendix 1.