SANTA ROSALIA 50 YEARS ON

Stygobiotic crustacean species richness: a question of numbers, a matter of scale

Fabio Stoch · Diana M. P. Galassi

Abstract Species richness in ground water is still largely underestimated, and this situation stems from two different impediments: the Linnaean (i.e. the taxonomic) and the Wallacean (i.e. the biogeographical) shortfalls. Within this fragmented frame of knowledge of subterranean biodiversity, this review was aimed at (i) assessing species richness in ground water at different spatial scales, and its contribution to overall freshwater species richness at the continental scale; (ii) analysing the contribution of historical and ecological determinants in shaping spatial patterns of stygobiotic species richness across multiple spatial scales; (iii) analysing the role of β-diversity in shaping patterns of species richness at each scale analysed. From data of the present study, a nested hierarchy of environmental factors appeared to determine stygobiotic species richness. At the broad European scale, historical factors were the major determinants in explaining species richness patterns in ground water. In particular, Quaternary glaciations have strongly affected stygobiotic species richness, leading to a marked latitudinal gradient across Europe, whereas little effects were observed in surface fresh water. Most surface-dwelling fauna is of recent origin, and colonized this realm by means of post-glacial dispersal. Historical factors seemed to have also operated at the smaller stygoregional and regional scales, where different karstic and porous aquifers showed different values of species richness. Species richness at the small, local scale was more difficult to be explained, because the analyses revealed that point-diversity in ground water was rather low, and at increasing values of regional species richness, reached a plateau. This observation supports the coarse-grained role of truncated food webs and oligotrophy, potentially reflected in competition for food resources among co-occurring species, in shaping groundwater species diversity at the local scale. Alpha-diversity resulted decoupled from γ-diversity, suggesting that β-diversity accounted for the highest values of total species richness at the spatial scales analysed.

Keywords Ground water · Species richness · Stygobionts · Crustacea · Spatial scale

This paper is dedicated to the late Prof. Janine Gibert (University of Lyon, France), who along her life, with great passion, highly promoted research in groundwater ecosystems, representing a key-reference scientist worldwide.

F. Stoch (✉) · D. M. P. Galassi
Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila, Italy
e-mail: fstoch@faunaitalia.it

Introduction

Ground water has long been considered a special, unfavourable milieu, where few, highly specialized species (the stygobionts) took refuge; for this reason, several generations of ecologists considered the subterranean domain very poor in species richness (Danielopol, 1992). This paradigm gave rise to many debates, mainly dealing with models of colonization of subterranean waters and their role as refugia (Rouch & Danielopol, 1987; Boutin & Coineau, 1990; Botosaneanu & Holsinger, 1991; Notenboom, 1991; Stoch, 1995). Contrary to the traditional view of ground water as species-poor environment, an emphasis on their unexpectedly high species richness has been given in several contributions (e.g. Danielopol & Rouch, 1991; Stoch, 1995; Rouch & Danielopol, 1997; Galassi, 2001; Gibert & Deharveng, 2002). These higher estimates of stygobiotic species richness probably reflected the broad spatial scales at which species richness has been examined (Sket, 1999b; Culver & Sket, 2000). Indeed, the number of stygobionts in a single groundwater site is low, if compared at least to surface freshwater diversity. On this regard, Culver & Sket (2000) defined a subterranean diversity hotspot as a site containing 20 or more stygobiotic and troglobiotic species, a number that is exceeded in even the most species-poor surface aquatic sites (Malard et al., 2009).

Paradigm or matter of fact, few attempts have been made to give an answer to the basic fundamental question that an ecologist can ask (Tilman, 1982), paraphrasing Hutchinson's (1959) "Homage to Santa Rosalia": *why are there so few species in ground water?*

Unfortunately, species richness in ground water is still largely unknown and the way in which it is distributed only sketchily understood (Gibert & Deharveng, 2002; Culver, 2005; Galassi et al., 2009a; Gibert & Culver, 2009). This situation stems from the low level of knowledge of groundwater biodiversity as a whole, as well as from lack of contributions focused on the analysis of the hierarchical partitioning of groundwater diversity at different spatial scales (Culver, 2005; Ferreira et al., 2005; Pipan & Culver, 2005; Deharveng et al., 2009; Malard et al., 2009).

That biodiversity is sensitive to scale is an empirical observation, widely proved from the largest global scale, through the assessment of biodiversity hotspots (Myers et al., 2000), and the latitudinal Rapoport (1982)'s rule, to the local scale (Hutchinson, 1959). According to the different spatial scales under which biodiversity can be examined, a hierarchy of environmental factors appears to determine such a biodiversity, although the basic dichotomy lies in the distinction between ecological and historical factors (Whittaker et al., 2001; Colwell et al., 2004). The spatial-scale dependence in analysing patterns and processes leading to a given biodiversity in fresh water has been largely emphasized and extended to groundwater ecosystems by Gibert et al. (1994) and Wilkens et al. (2000).

Although it is widely recognized that both evolutionary processes and real-time ecological constraints (e.g. Gibert & Deharveng, 2002; Castellarini et al., 2005; Galassi et al., 2009b; Martin et al., 2009) give rise to groundwater biodiversity patterns, how they interact is far from being known. Until now, spatial patterns of groundwater biodiversity have been basically interpreted under an ecological perspective. Only occasionally have patterns been explained by also evaluating the role of historical events (e.g. Stoch, 1995; Wilkens et al., 2000; Galassi et al., 2009b; Martin et al., 2009).

Within this fragmented frame of knowledge of subterranean biodiversity, this contribution is aimed at (i) assessing species richness in ground water at different spatial scales, and its contribution to overall freshwater species richness at the continental scale; (ii) analysing the contribution of historical and ecological determinants in shaping spatial patterns of stygobiotic species richness across multiple spatial scales; (iii) analysing the role of β-diversity in shaping patterns of species richness at each scale analysed.

Methods

Species richness assessment

Groundwater species richness was analysed by examining the total number of freshwater invertebrates presently recorded worldwide (Freshwater Invertebrate Assessment: Balian et al., 2008) and in European water bodies, as listed in the Fauna Europaea Web Service (2004). Crustacea were selected as the target group, and stygobiotic species

were separated from surface ones. The Crustacea were selected not only on the basis of their high representation in ground water, but especially because there is relatively good information available on their ecological preferences, distribution data at the species level and a validated checklist (Deharveng et al., 2009) constructed under the PASCALIS (Protocols for the Assessment and Conservation of Aquatic Life In the Subsurface) project at the European scale (Gibert & Culver, 2009).

Data were assembled by integrating the different data sets with check lists available at the broad European scale (Limnofauna Europea: Illies, 1978; Stygofauna Mundi: Botosaneanu, 1986) and the PASCALIS data set (Deharveng et al., 2009), critically revised to assess the ecology of the species and update species lists using recent literature.

Spatial scales

Following a hierarchical spatial criterion (Whittaker et al., 2001), the groundwater Crustacea data set was analysed at four spatial scales: the continental scale (following the bioregion concept adopted in the EU Water Framework Directive), the stygoregional scale (i.e. a biogeographical unit, according to Hahn, 2009), the regional scale (i.e. a relatively large area that experienced a set of similar historical events, according to Malard et al., 2009), the local scale, ranging from aquifer units, to the smaller hydrogeological zones (unsaturated and saturated zones into the aquifer unit) and, finally, to the single sampling site (Malard et al., 2009).

Europe was selected as test-area at the continental scale; the data set derived from Fauna Europaea Web Service (2004) was used for geostatistical analyses. Data from European Russia were discarded (except for the Kaliningrad Region included among the European countries), due to poor information available on groundwater fauna for that area. The analysis suffered from the limits imposed by the use of political geographical units of the European continent (Whittaker et al., 2005); unfortunately, limnofaunistic regions (as defined in Limnofauna Europaea by Illies, 1978) and stygobiological regions (as proposed in Stygofauna Mundi by Botosaneanu, 1986) do not match with each other, making comparisons among epigean fresh water and ground water almost impossible.

Within the European scale, the stygoregional scale was represented by the eastern Alpine area in Italy (Stoch, 2008). Eight karstic areas were selected in the pre-Alpine longitudinal band from the eastern Italian–Slovenian border to Piedmont. The data set was obtained from the CKmap database (Ruffo & Stoch, 2006), enriched by additional species records assembled by one of us (F. Stoch, unpublished). Within the eastern Alpine stygoregion, the regional scale was represented by the Lessinian massif, where patterns of stygobiotic species richness were analysed into the PASCALIS project (Gibert & Culver, 2009). The data set was the same used by Galassi et al. (2009b). At this spatial scale, karstic and porous aquifers, as well as subsurface and deep saturated hydrogeological zones, were selected at the successively lower spatial scales. Finally, caves and single sites in alluvial sediments (hyporheic samples and wells) within the aquifers were selected as the smallest spatial scale.

Statistical analysis

Data sets were stored in MSAccess 2000 and Excel databases; maps at the three spatial scales were obtained using ArcMap version 9.1. Statistical analyses were performed using GeoDa^{TM} version 0.9.5i (Anselin, 2005) and SAM (Rangel et al., 2006) geostatistical software. The statistical significance of global and local (LISA, i.e. Local Indicators of Spatial Association) autocorrelation measurements (Moran's I) was assessed by 1,000 Monte Carlo permutations.

At the European scale, species richness for each country was corrected for the area effect, applying Heino's (2002) correction, where: $SR_c = SR_{obs}/A^z$ where SR_{obs} is the raw species count for each country, A is the area, and z is the constant in a typical species–area relationship. Since z value has been found to vary between 0.12 and 0.17 in continental environments, the intermediate value of 0.15 was selected for this study, according to Heino (2002). The palaeoecological data set used applied the mean annual temperature condition during the Last Glacial Maximum (LGM, $\sim$21000 years BP) following the CCSM model (Braconnot et al., 2007).

At the stygoregional, regional, and aquifer scales, sample-based rarefaction curves (Gotelli & Colwell, 2001) were calculated to compare species richness across the different areas, to minimize the effect of sampling effort. The software EstimateS version 8.02

(Colwell, 2005) was used for calculation of accumulation curves; Chao2 and Michaelis–Menten estimators of species richness were selected (Colwell, 2005), and mean dissimilarities between and within areas using the Sorensen's index were calculated as well.

Hierarchical additive partitioning of species richness (Crist et al., 2003) was used for dissecting species diversity into individual components. The total diversity (γ) has been partitioned into the average diversity within sampling units (α) and among samples (β) so that $\gamma = \alpha + \beta$. To extend across multiple hierarchical spatial scales ($i = 1, 2, 3\ldots n$ hierarchical levels), α-diversity was calculated for each level as the average diversity within the spatial units, being α_i and β_i the additive partitioning of total species richness within level $i < n$; at each sampling level $\gamma_i = \alpha_{i+1}$ and $\beta_i = \alpha_{i+1} - \alpha_i$; then, the additive partition of total diversity was: $\gamma = \alpha_1 + \Sigma_i \beta_i$. Partition software (Crist et al., 2003) was used to assess the statistical significance of species richness partition.

Results

Stygobiotic crustacean species richness

Crustacean species richness of European fresh water (Table 1) amounts to 2,285 species described so far; 1,111 species are epigean, while 1,174 (i.e. 51.4%) are stygobiont. Moreover, the number of stygobiotic species may be highly underestimated. Plotting the number of European surface and stygobiotic crustacean species discovered per year (Fig. 1a), it can be observed that most stygobiotic species were described after 1930, when more than one half of the epigean species was already known. Cumulative species counts (Fig. 1b) demonstrated that the description rate of epigean species remained unchanged after 1850, while the cumulative curve for stygobiotic species become steeper after 1930, and the discovery rate was rapidly increasing up to the present. There was no evidence of any asymptote, suggesting that we are far from having described the whole European crustacean fauna, and that the number of stygobiotic species could be dramatically higher than that of epigean ones.

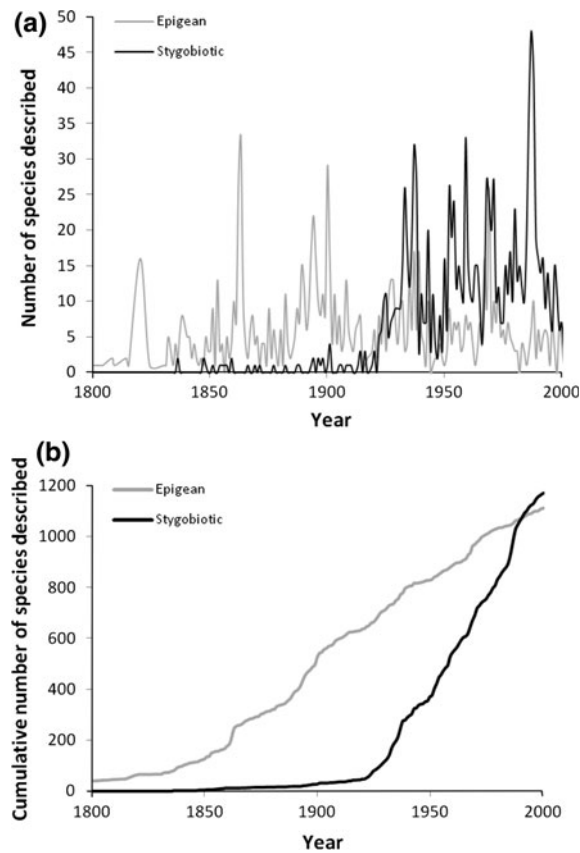### Species richness at the European scale

Patterns of species richness

By mapping the distribution of the 2,285 freshwater crustaceans across European countries (Fig. 2a), it is observable that species richness values were almost evenly distributed, with lower species densities in northern Europe and in the easternmost European countries. Being the number of data stored in the database clearly dependent on the area of each country (log-transformed values, Pearson's $r = 0.754$, $P < 0.001$), they were corrected applying Heino's (2002) transformation. The distribution of surface crustacean

Table 1 Distribution of major invertebrate groups in fresh water (including all habitat types) and in ground water, in both terms of total number of species and percentage of total species richness

Freshwater invertebrate groups	Freshwater species				Stygobiotic species			
	World		Europe		World		Europe	
	Species	%	Species	%	Species	%	Species	%
Rotifera	1,948	1.8	1,288	7.6	49	1.0	36	2.0
Nematoda	1,890	1.7	622	3.7	33	0.7	24	1.3
Annelida	1,761	1.6	612	3.6	78	1.6	70	3.9
Mollusca	4,998	4.5	809	4.8	350	7.3	279	15.5
Crustacea	11,990	10.8	2,285	13.5	3,400	71.2	1,174	65.0
Acari	6,149	5.6	1,028	6.1	650	13.6	100	5.5
Insecta	75,874	68.5	9,681	57.0	18	0.4	2	0.1
Other groups	6,109	5.5	654	3.9	197	4.1	120	6.6
Total	110,719		16,979		4,775		1,805	

For species count in ground water only stygobiotic species were considered. Data were derived from Fauna Europaea Web Service (2004), Balian et al. (2008), Botosaneanu (1986), integrated herein of recent data updated to 2009

Fig. 1 Number of species discovered per year against year (**a**) and cumulative species count against year of description (**b**) for European surface and stygobiotic crustaceans

species richness at the European scale did not follow a significant latitudinal gradient ($n = 43$ countries; $r = 0.195$; $P = 0.419$), nor displayed a significant global spatial autocorrelation (Moran's $I = 0.043$, $P = 0.28$). European countries were classified using LISA into two significantly different ($P < 0.05$) spatial clusters of countries with similar species richness (Fig. 2b). The Central-European, species-rich cluster displayed a species density of epigean crustaceans more than double than that of species-poor countries. In sharp contrast with the observed trends for epigean crustaceans, stygobiotic crustacean species richness, classified into three spatial clusters (Fig. 2c), showed a strong latitudinal gradient ($r^2 = 0.700$; $P < 0.001$).

The most species-rich countries (France, Spain, Italy and part of the Balkan Peninsula) may be considered as 'hot areas' of stygobiotic species richness; conversely, 'cold areas' were located in northern

and north-eastern European countries (including Scandinavia and Iceland). The clusters of countries included in 'hot' and 'cold' areas, respectively (Fig. 2c), were statistically significant (LISA analysis, $P < 0.05$). Such a distribution of stygobiotic species follows the palaeoclimatic conditions during the Last Glacial Maximum (Fig. 2d), along an increasing gradient of species richness from the northern area covered by the ice cap, passing through the permafrost zone and finally reaching the not-glaciated areas to the South. Differences in species richness between the three latitudinal bands were dramatically high; 'cold areas' in northern Europe harbour 11 stygobiotic species (approximately 0.04 species/km^2), against 210 species known for intermediate countries (0.8 species/km^2) and 956 species known for the 'hot' southern countries (3.9 species/km^2).

Species turnover and additive partitioning of species richness

Average species richness per country, β-diversity and total species diversity were calculated for each latitudinal band using additive partitioning of stygobiotic species richness. Beta-diversity at the continental level (i.e. changes of diversity among different latitudinal bands) decreased from southern to northern latitudes, indicating a high species turnover across Europe. Hierarchical additive partitioning of stygobiotic crustacean species richness at the European scale (Fig. 3) revealed that β-diversity accounted for the most part of the overall species richness in Europe (64.7%), reflecting the high dissimilarity observed among latitudinal bands. Stygobiotic β-diversity at the country level (31.5%) was approximately one half of β-diversity at the continental level. The contribution of each spatial level (latitudinal bands and countries) to total species richness increased with its relative size.

The stygoregional scale

Patterns of species richness

The eight unsaturated karstic aquifers analysed in the stygoregion of north-eastern Italy were distributed along the E–W pre-Alpine band (Fig. 4a). Most of the study area was not covered by the ice sheet during the Last Glacial Maximum, as showed in Fig. 4a. Seventy
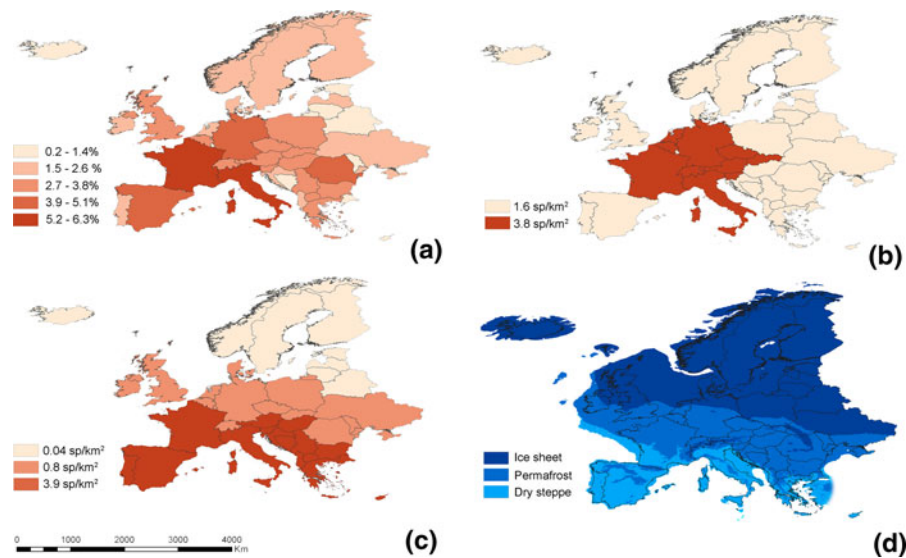
Fig. 2 Distribution of crustaceans at the European scale: **a** percentage of total crustacean species richness for each country after area correction (Heino's formula); **b** clusters of countries with similar surface crustacean species richness (LISA, $P < 0.05$); **c** clusters of countries with similar stygobiotic crustacean species richness (LISA, $P < 0.05$); **d** extent of areas covered by glacial ice shields, by permafrost soil with cold steppe-tundra, and areas free from ice covered by dry steppe during the Last Glacial Maximum (LGM; ~21000 years BP)
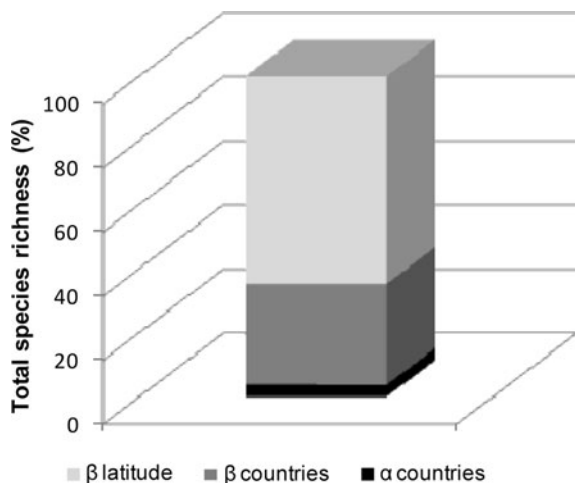

Fig. 3 Hierarchical additive partitioning of stygobiotic crustacean species richness at the European scale. *Bars* show the percentage of total species richness explained by α- and β-components at two spatial hierarchical levels: countries and latitudinal bands. Observed β-diversities significantly differ from a random distribution of species richness among countries and latitudinal bands (Partition software, $P < 0.01$)

stygobiotic crustacean species were collected from 138 caves of this stygoregion. Michaelis–Menten estimator was used to compare species richness among different aquifers, because regional-species accumulation

curves (i.e. cumulative species richness vs. number of sites sampled within an aquifer) did not reach an asymptote in any region. No relationships have been observed between average local species richness (LSR) and distance from the eastern stygoregional border ($r = 0.609$, $P = 0.14$), while aquifer species richness (RSR) decreased from East to West (Fig. 5a). The second-order polynomial model provided a better fit than the linear model, with the second-order term statistically significant ($P < 0.05$), suggesting a maximum of species richness in the eastern part of the stygoregion or immediately outside its border, in Slovenia.

A significant correlation ($r = 0.783$, $P = 0.02$) between RSR and the number of exclusive species (i.e. strict endemics within an aquifer) indicated that the eastern aquifers host a higher number of regional endemics, along with higher species richness values. The southern border of the Würmian glaciers, together with the southernmost extension of the karstic areas, marked by the interface between limestone and alluvial sediments of the Padanian Plane, resulted strictly related to the high level of endemicity among stygobiotic crustaceans, as exemplified in Fig. 4b. The distributional pattern exhibited by the sphaeromatid isopod genus *Monolistra* is mirrored by the

Fig. 4 The stygoregion of north-eastern Italy. **a** Distribution of the karstic areas analysed in the stygoregion and dendrogram based on average dissimilarity (scale on the right). *Grey areas* represent karstic areas; *dotted line* represents the southern border of Alpine ice sheet during the Last Glacial Maximum. **b** Influence of the LGM on the recent distribution of the isopod genus *Monolistra* in the same stygoregion
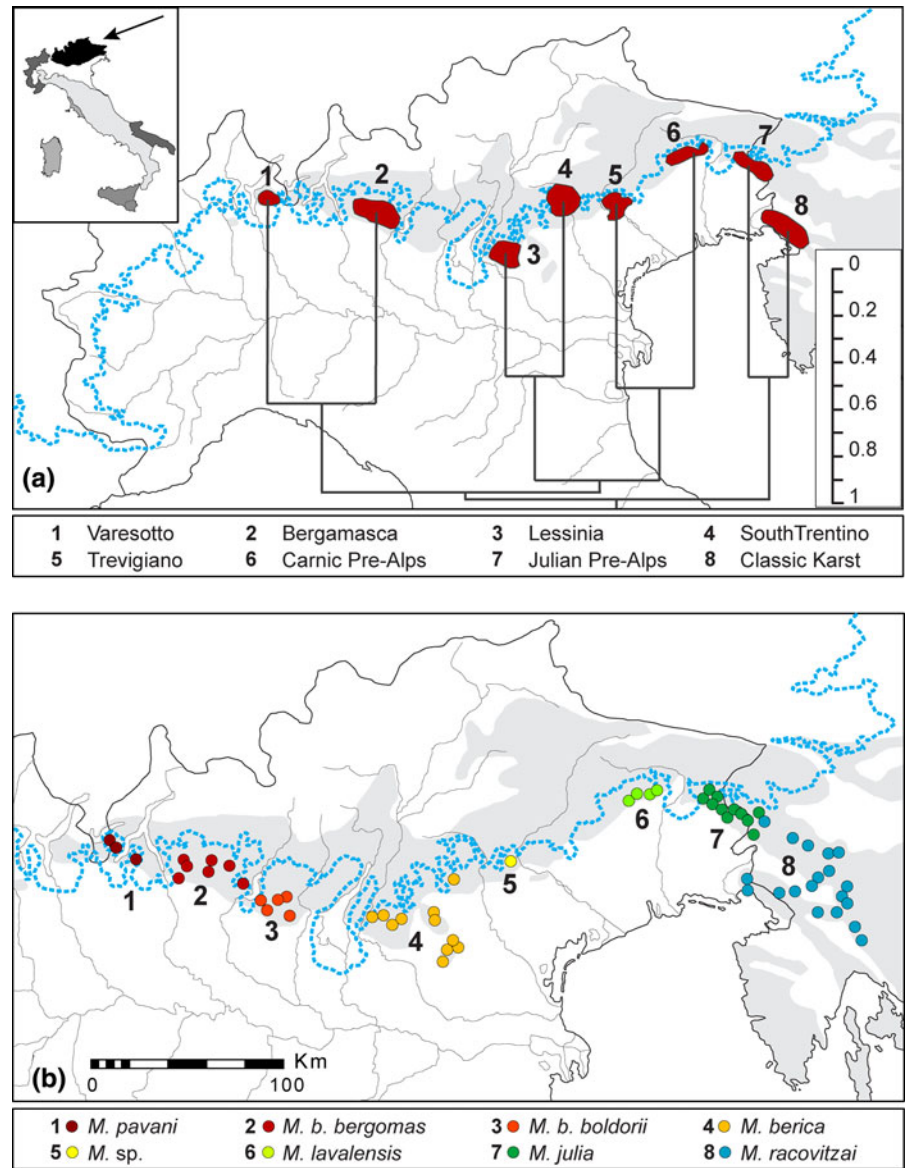

distribution of several other genera and species-groups of stygobiotic crustaceans (Ruffo & Stoch, 2006).

Species turnover

The high degree of endemicity suggests a high species turnover following the E–W pre-Alpine ridge. In fact, the dissimilarity in species composition among aquifers of the stygoregion markedly increased from the eastern aquifer close to the Slovenian border to the westernmost ones (Fig. 5b). The Michaelis–Menten model ($r^2 = 0.948$, $P < 0.001$) revealed a steep slope

in dissimilarity values in the first 100 km from the eastern aquifer; dissimilarity values close to 1 were maintained in the remaining aquifers located at the western part of the stygoregion. Values of species dissimilarity among aquifers were used to perform a cluster analysis that identified strong dissimilarities between regional crustacean assemblages (Fig. 4a). However, the lowest dissimilarity coefficient between two aquifers was everywhere very high in the stygoregion, never below 0.5. The relationship between species dissimilarity and geographic distance was statistically significant (a second-order polynomial

Fig. 5 a Relationship between distance from the eastern border of the stygoregion of north-eastern Italy and species richness (log-transformed) of the study regional units (karstic areas; *error bars* represent standard errors of estimated regional species richness; second-order polynomial regression fitted to data); **b** dissimilarity between each region and the easternmost area, the Classic Karst (asymptotic curve given by Michaelis–Menten equation fitted to data)
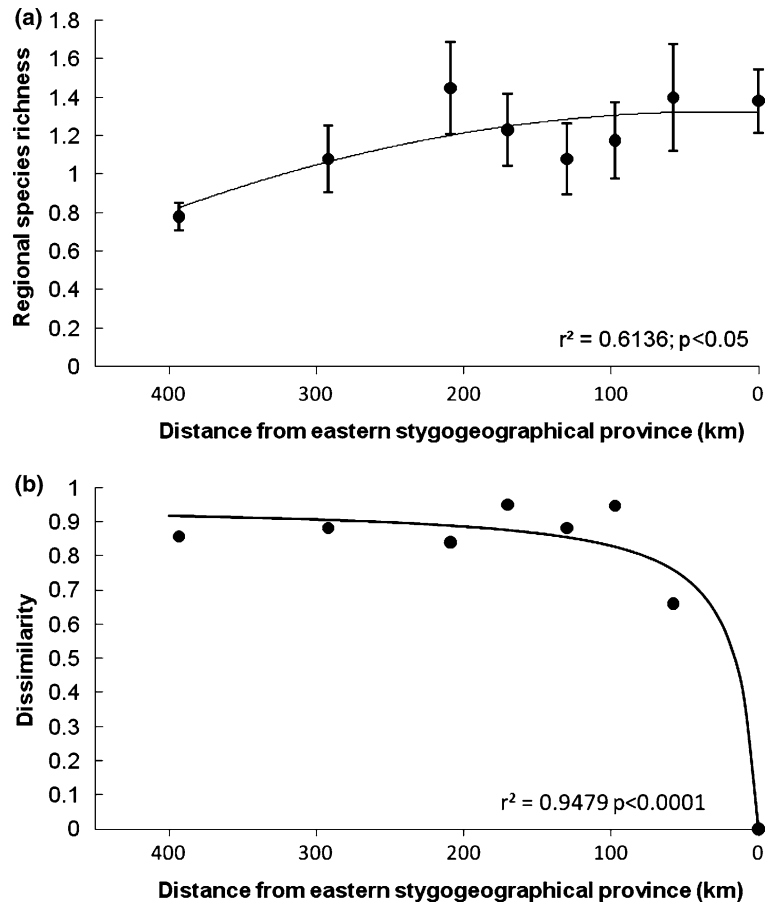
regression representing the best fit, with $r^2 = 0.385$ and $P < 0.001$); however, the relationship between RSR and average dissimilarity among caves within an aquifer (Fig. 6a) was statistically not significant ($r^2 = 0.070$, $P = 0.73$), confirming a high species dissimilarity among caves within the same aquifer independently of its RSR.

Local versus regional species richness relationship

LSR of the unsaturated karstic aquifers did not increase linearly with their RSR (Fig. 6b). The linear correlation was statistically not significant (log-transformed values, $r^2 = 0.291$, $P = 0.167$), while the second-order polynomial model provided a better fit ($r^2 = 0.707$, $P < 0.05$). Both the first- and second-order terms were statistically significant ($P < 0.05$).

The fitted curve suggested a maximum of LSR within the observed range of RSR.

Additive partitioning of species richness

Species diversity of stygobiotic crustaceans at the smallest spatial scales analysed in the whole stygo-region (mean RSR = 17.4 species) was considerably lower than among habitats-diversity measured at the same spatial scale (52.6), which made by far the highest contribution to total species richness (75.2%), reflecting the high dissimilarity observed among regional aquifers. Beta-diversity at the cave level (13.8) was considerably higher than α-diversity at the same level (mean LSR = 3.6). The contribution of each spatial level of the north-eastern stygoregion in Italy (aquifers and caves) to total species richness increased with its size (Fig. 7).

Fig. 6 **a** Relationship between regional species richness and mean dissimilarity within regional units (karstic areas; *error bars* represent standard error; *dotted line* fitted to data); **b** local (=cave) species richness (log-transformed; *error bars* represent standard errors; second-order polynomial regression fitted to data)
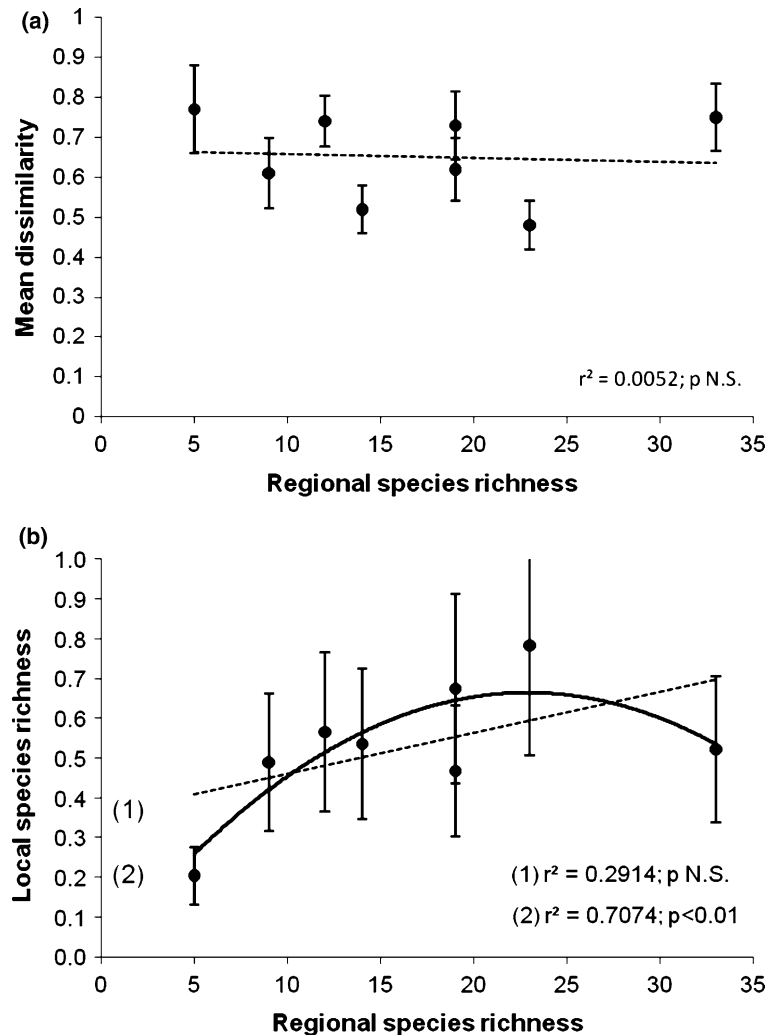

From the regional to the local scale

Patterns of species richness

A total of 64 stygobiotic species of crustaceans are at present known from ground water of the Lessinian massif region (Fig. 8). Differences in species richness among the four hydrogeological zones, namely: (1) unsaturated and (2) saturated karstic aquifers (with 29 and 28 species, respectively), (3) hyporheic zone of the porous aquifer (with 28 species) and (4) saturated porous zone (with 20 species), were slight.

Species richness was higher in the karstic aquifer of the region (57 species) than in the porous one (48 species). Species richness estimates using species rarefaction curves showed that Chao2 estimator stabilized in both aquifers after approximately 20 sampling sites (Fig. 9). After the same number of sampling sites, the uniques (i.e. the number of species found in a single site) decreased as well, indicating a similar sampling efficiency in both aquifers. For this reason, the observed difference in species richness cannot be attributed to sampling bias.

Patterns of point-species richness at the Lessinian regional scale are presented in Fig. 8. The spatial distribution of sampling sites was not uniform across the study area. Karstic sites rather followed the aggregate topology of the karst, whereas alluvial sites showed a clumped distribution, reflecting the location of upwelling stream bed sectors. Point-species richness showed a statistically significant (Moran's $I = 0.132$, $P < 0.001$) global autocorrelation.
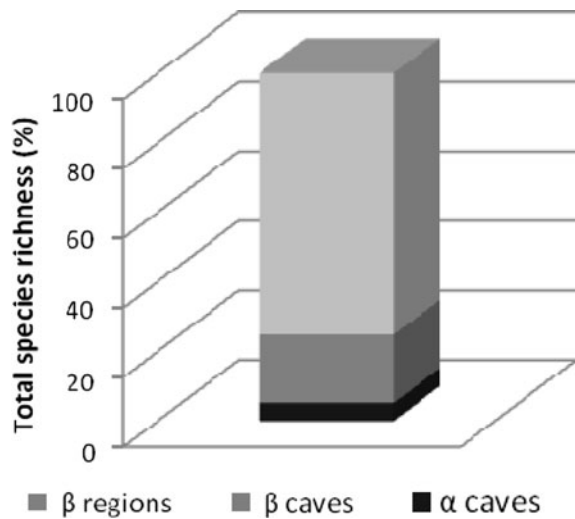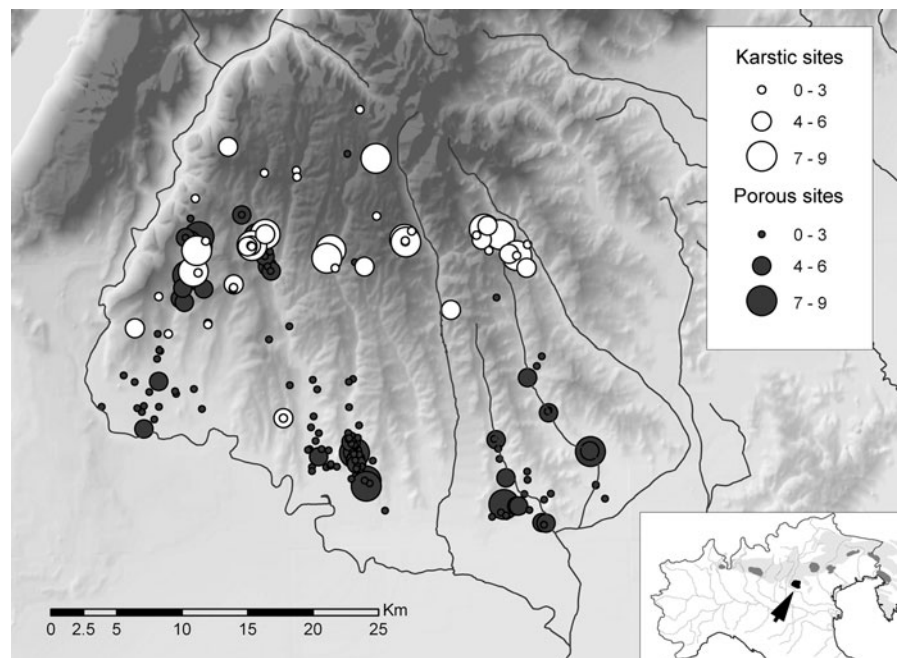
Fig. 7 Hierarchical additive partitioning of stygobiotic crustacean species richness at the stygoregional scale. *Bars* show the percentage of total species richness explained by α- and β-components at two spatial hierarchical levels (regional units and cave units). Observed richness at each level significantly differs from random distribution of samples ($P < 0.01$)

Additive partitioning of species richness

Point diversity of stygobiotic crustaceans was quite low (2.3 ± 1.8; 4.1% of total species richness), much lower than β-diversity at the same spatial scale

(23.2%). Beta-diversity at the hydrogeological-zone level was quite low (8%), while β-diversity at the aquifer level (27.5%) highly contributed to total species richness (Fig. 10).

Discussion

Species richness in ground water: a question of numbers

Currently, as far as fresh water is concerned, invertebrates are represented by approximately 111,000 species worldwide (Balian et al., 2008); among them, insects are the dominant group in surface fresh water, with about 76,000 species followed by crustaceans, with 12,000 species.

By comparing the taxonomic composition of species richness at the World and European scales, insects dominate everywhere in surface fresh water (from approximately 76,000 in World fresh water to about 9,700 in European fresh water, accounting for 68.5 and 57% of total species richness—TSR, respectively). They are followed by the crustaceans (from 12,000 species to 2,285, accounting for 10.8 and 13.5% of TSR, respectively), all the remaining groups representing only a small fraction of TSR

Fig. 8 Distribution of stygobiotic crustaceans along sampling sites within the Lessinian region (northeastern Italy). Circle sizes proportional to point-species richness
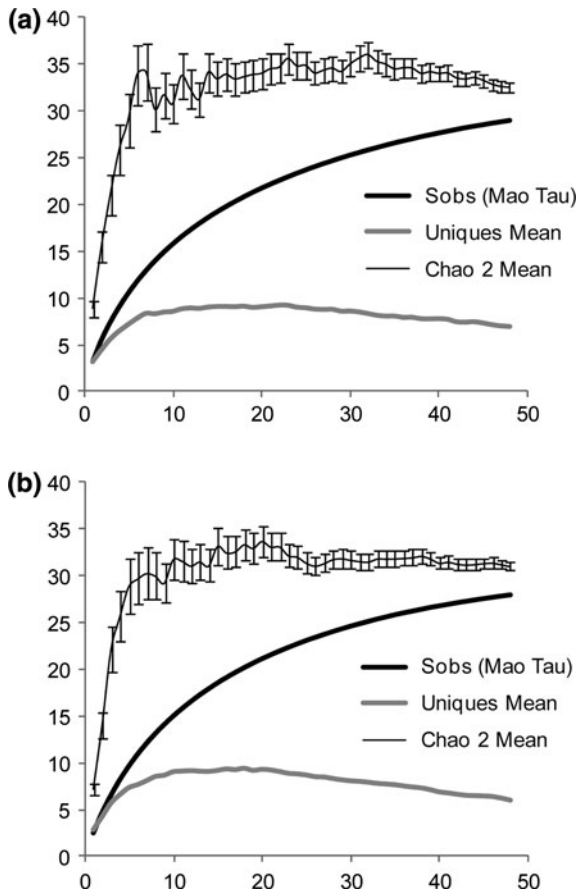
Fig. 9 Species accumulation curves for stygobiotic crustaceans in the Lessinian ground water at increasing sample size: **a** karstic aquifer; **b** porous aquifer. S_{obs} species rarefaction curves of observed species richness (Mau Tau, mean values of 100 randomizations); *Uniques* curve of the number of uniques, i.e. species present in a single sample (mean values); *Chao2* estimated species richness using Chao2 formula (mean values; *error bars* represent standard errors)

Fig. 10 Hierarchical additive partitioning of stygobiotic crustacean species richness at the regional scale (Lessinian massif). *Bars* show the percentage of total species richness explained by α- and β-components at three hierarchical spatial levels: sampling sites, hydrogeological zones (hzones: unsaturated zone of karstic aquifers; saturated zone of karstic aquifers; hyporheic zone of porous aquifers; saturated zone of porous aquifers) and aquifers (karstic and porous). Observed richness of α- and β-components significantly differ from random distribution of samples ($P < 0.01$)

(Table 1). The Crustacea are mostly represented in fresh water by the Copepoda, with approximately 2,800 species (Boxshall & Defaye, 2008), the Ostracoda, with about 2,000 species (Martens et al., 2008), and the Amphipoda, with some 1,800 species (Väinölä et al., 2008).

Species richness of crustaceans in subsurface environments may be higher or comparable at least to that observed in surface fresh water (Stoch, 1995; Rouch & Danielopol, 1997; Galassi et al., 2009a). The high radiation and subsequent diversification of crustaceans in ground water are indisputable. Their taxonomic diversification in surface fresh water parallels that in groundwater, where they are the most species-rich group. Sket (1999a, b) stated that stygobiotic diversity is mainly crustacean diversity. In ground water, the Crustacea contribute to about 70% of the overall species richness and are predominantly represented by the Copepoda, the Amphipoda and, to less extent, by the Ostracoda, collectively outnumbering all remaining invertebrate groups living in this environment (Galassi et al., 2009a; Stoch et al., 2009) (Table 1).

Among stygobiotic crustaceans, there are some taxonomic groups which are exclusively known from ground water, lacking any surface representative, or being exceptionally represented in surface fresh water. The Syncarida (with 240 stygobiotic species) are almost completely absent from surface fresh water, suggesting that the stygobisation process may have started at the origin of their evolutionary history, as hypothesized also for the harpacticoid copepod family Parastenocarididae (Schminke, 1981). The Spelaeogriphacea are exclusively known with four stygobiotic species. A similar situation is observable within the Thermosbaenacea with 18 stygobiotic species (Jaume, 2008) and in the copepod order Gelyelloida with only two stygobiotic species presently known; just to list few representative examples.

Basically, there is robust ground to infer that crustacean stygobiotic species richness is still largely underestimated, in part for sampling incompleteness, in part as consequence of the Linnaean and the Wallacean shortfalls (i.e. inadequacies in taxonomic and distributional data) (Lomolino et al., 2006), which operate together, firstly, in species richness underestimation, and secondly, in creating artefacts is species distribution. This bias is the inexorable product of the alarming decline of taxonomy (Crisci, 2006) that has profound implications for assessing the extent of groundwater biodiversity (how many stygobiotic species are there) and understanding the geographical distribution of species (where a stygobiotic species is present and how large is its geographical range). The Linnaean shortfall clearly emerged examining the discovery rate of epigean versus hypogean crustaceans; the increase in species discovery among stygobionts is far from having reached a plateau and the rate of species description shows several lag-phases in the recent history.

In spite of the poor overall LSR in ground water, why are there so many crustacean species in this environment? Several arguments have been presented (Stoch, 1995; Danielopol et al., 2000; Gibert & Deharveng, 2002) for explaining the success of the Crustacea in ground water, all advocating the lack of competition, due to the absence of insects (Sket, 1999b; Culver & Sket, 2000; Ferreira et al., 2007). Being dependent upon air for breathing or reproducing, aquatic insects are extremely rare in ground water (Danielopol et al., 2000). The absence of insects leaves many habitats and potential niches empty.

Arguments to explain the lower species richness in ground water revolve around the fact that the underground environment has been colonized by those epigean surface populations able to cope with the different selective pressures in ground water by means of morphological and physiological preadaptive and exaptive traits; moreover, this environment is characterized by a 'truncated food web' (Gibert & Deharveng, 2002), because primary productivity is missing, determining low-energy food webs and the assumed low turnover in community composition, with a few exceptions represented by chemoautotrophic groundwater ecosystems (Engel, 2007), where stygobiotic diversity may be higher than in the heterotrophic ground water.

Indeed, it is almost impossible to explain the crustacean dominance in ground water by interpreting the radiation and adaptation processes at the wide subphylum taxonomic rank. For instance, not all the crustacean taxa successfully colonized ground water. As a matter of fact, some groups have never entered ground water or, alternatively, are known from this environment with only a few specialized species. This is the case of the Cladocera, with 10 stygobiotic species worldwide (Brancelj & Dumont, 2007), mostly belonging to the genus *Alona*, out of a total of 620 species (Forrò et al., 2008), and the Copepoda Calanoida, with 9 stygobiotic species known (Brancelj & Dumont, 2007) out of a total of 552 freshwater species (Boxshall & Defaye, 2008). The poor taxonomic diversification of these crustacean groups in ground water is in sharp contrast with their relatively high diversity in surface fresh water and may be explained by their preference for the planktonic habitats. The planktonic life style allows them to colonize almost exclusively the saturated karst (e.g. subterranean lakes), and habitat availability for these species is strictly dependent on the degree of development of the saturated karst in different geographical areas, and, no less important, on the degree of connection between surface standing waters and the limnic ground water. Moreover, their high potential for dispersal, also by means of resting stages (Shurin et al., 2009), may prevent isolation and then speciation by vicariance.

Other crustacean groups (Copepoda Cyclopoida and Harpacticoida, Ostracoda, Isopoda and Amphipoda) have supremacy in fresh ground water situations. Not unlikely, the predominant benthic and inbenthic life styles, together with the widespread heterochrony observable in these groups (Coineau, 2000; Galassi et al., 2009a), may represent the basic explanation for their success in ground water. The diverse array of structural plans observed in stygobiotic copepods and amphipods, and, to less extent, in ostracods and isopods, is attributable to their intrinsic phylogenetic disparity, which has offered the opportunity to answer the different selective pressures exerted by the heterogeneous ground water.

Many stygobiotic copepods, amphipods, isopods and syncarids exhibit reductions in body plan and appendage morphology, which can be regarded as the result of a number of paedomorphic heterochronic events: post-displacement, progenesis and neoteny.

The recurrent heterochrony observable in the evolutionary history of the Crustacea may have favoured miniaturization and consequently a high potential to enter small fissures of karstic aquifers, survive in the capacitive subsystems or in small and large pools and trickles of the epikarst, or stably colonize the interstitial voids in subsurface alluvial sediments, which all represent the major routes for entering the true groundwater realm.

Species richness in ground water: a matter of scale

From the European scale to the stygoregional scale

An exhaustive explanation of the diversity patterns should cover a wide range of phenomena, at various scales of analysis, and cannot be expressed in a single argument. In particular, a top-down, global-to-local, macro-to-micro scale approach is necessary to modelling richness variations (Whittaker et al., 2001, 2005; Willis & Whittaker, 2002).

At the European scale, the distribution pattern of stygobiotic crustaceans differs from that of surface freshwater species, the latter being almost homogeneously distributed along the latitudinal gradient. This fact may be explained by considering that several surface crustacean species are limnic (Hof et al., 2008); and especially planktonic microcrustaceans show high aptitude to dispersal (Shurin et al., 2009). They may have re-colonized the water bodies at northern latitudes in the last 20000 years (Hewitt, 1999), following the strong gradient of limnicity (ratio of total lake area over total country area) (Lehner & Döll, 2004). In Europe, limnicity ranges from over 9% in Nordic countries, such as Sweden, to less than 0.5% in Greece (UNEP/IETC, 2000). A weak and less steep latitudinal gradient of species richness in fresh water has also been observed by Hillebrand (2004), and, more importantly, significantly weaker gradients were found in lakes than in streams in Europe. For example, in northern and central Europe, most of the crustacean diversity is built up by planktonic cladocerans and copepods, and, to less extent, by amphipods (Hof et al., 2008). It is not a case that in these areas there is also the highest value of limnicity. Hof et al. (2008) demonstrated that the correlation between widespread,

limnic species richness and latitude is not linear, showing a maximum at intermediate latitudes and dropping down towards both northern cold and southern warm and more arid countries, denoting a strong influence of current climatic conditions.

On the contrary, the strong latitudinal gradient displayed by groundwater species may be explained using climate simulations for the Last Glacial Maximum; i.e. past climatic conditions. Quaternary glaciations have led to massive extinctions of crustaceans in the Northern Hemisphere; stygobiotic species survived in the less drastic climatic conditions offered in southern Europe, in the more protective ground water. While the epigean survivors re-colonized the water bodies to north by means of post-glacial dispersal, this 'occasion' was denied to most stygobionts, due to the fragmented and isolated nature of the groundwater habitats (Rundle et al., 2002; Castellarini et al., 2007; Galassi et al., 2009b; Malard et al., 2009; Martin et al., 2009).

Araújo et al. (2008) found that the distribution of narrow-ranging species of amphibians and reptiles is markedly constrained by the mean annual freezing conditions in the Last Glacial Maximum, whereas widespread species are more constrained by current mean annual freezing conditions. This holds true for crustacean as well, considering the small species range of most stygobiotic crustaceans, mainly due to the high degree of endemicity in subterranean waters (Gibert et al., 1994; Wilkens et al., 2000; Lefébure et al., 2007; Galassi et al., 2009a, b; Trontelj et al., 2009).

Even at the stygoregional scale, the distribution of narrow-ranging stygobiotic species is markedly constrained by the mean annual freezing conditions in the LGM, and not by current mean annual freezing conditions. Species distribution clearly follows the ice sheet border of Last Glacial Maximum, whereas the current mean 0°C isotherm lies at higher altitudes on the Alps (Araújo et al., 2008). The prevailing role of historical factors in shaping stygobiotic crustacean diversity at the stygoregional scale examined is clearly demonstrated by several other evidences: (a) the decrease of species diversity among aquifers along a gradient from the species-rich Slovenian aquifers (hotspot of European stygodiversity after Deharveng et al., 2009) to the poorer western aquifers, more heavily influenced by Würmian glaciations and more peripheral to the main karstic areas of the eastern part; (b) the steep increase of species

dissimilarity (i.e. higher species turnover) among aquifers within the same stygoregion; (c) the fact that species-rich aquifers also host the highest number of endemics, supporting evidences for long-term isolation among aquifers which may have led to independent evolutionary histories of the aquifer assemblages.

From the regional to the local scale: the relationship between local and regional species richness

Our results revealed that at whatever value of species richness measured at regional scale, the local diversity (here interpreted as point-diversity) attained a ceiling (plateau), with low values of LSR measured at each cave. This fact suggests that LSR reached its maximum and did not follow the increase of regional species richness, contrasting the general tendency of a linear relationship (type I relationship described by Gaston, 2000). Similar results were obtained by Malard et al. (2009) at a larger scale in Europe, demonstrating that local (aquifer) species richness did not always increase with regional species richness; however, in this case, historical factors may have shaped this local versus regional species richness relationship (Malard et al., 2009). On the contrary, a linear relationship between local and regional species richness holds true for surface zooplankton (Shurin et al., 2000), characterized by high dispersal ability.

Historical factors seem to have operated at the stygoregional scale as well as at the regional scale, here exemplified by the Lessinian massif, where karstic and porous aquifers, differing in their relative geological history, showed different values of species richness. If aquifer species diversity seems to be mainly determined by historical events, point-species diversity saturation (or pseudosaturation) could require different explanations. Winkler & Kampichler (2000) argued the difficulty of distinguishing between true saturation and pseudosaturation. In particular, if saturation may be subject to local biotic and abiotic rules, pseudosaturation may be the reflection of stochastic equilibrium, high local extinction rates, endemicity, regional heterogeneity or inadequate sampling. In ground water, the high rate of endemicity and the high regional heterogeneity are undeniable (Gibert & Deharveng, 2002; Gibert & Culver, 2009), while high local extinction rates are unlikely in this conservative environment (if we exclude the effects of anthropogenic disturbance). Therefore, larger regions are expected to include more landscape features and, correspondingly, more specialists and endemics. Hence, regional species richness may include species not adapted to a given local habitat, originating pseudosaturation of point-diversity (Cornell, 1993). Moreover, low species turnover and supposed empty niches (Stoch, 1995; Sket, 1999a, b) may reinforce the observed pattern. However, the role of endemicity in pseudosaturation remains controversial; on the contrary, the high number of rare, locally endemic species (Gibert & Deharveng, 2002), as well as the high incidence of cryptic species (Lefébure et al., 2007; Trontelj et al., 2009) is certain, and may lead to an underestimation of regional species richness and not of point-species richness. Finally, spurious asymptotes can derive from methodological artefacts. Caley & Schluter (1997) emphasized the importance of adapting locality size, as well as sample size, to the range of the study areas. If area increases, constant sampling effort will likely detect a decreasing part of the total number of species at the point-site scale (Dole-Olivier et al., 2009a).

Even if pseudosaturation cannot be ruled out as an explanation of the observed patterns, instead of true saturation, the low point-species richness at the local scale, compared to the high regional species richness, remains unquestionable (Deharveng et al., 2009; Malard et al., 2009).

Dissecting spatial diversity at different spatial scales

The hierarchical additive partition of stygobiotic crustacean diversity performed at the European, stygoregional and regional scales, respectively, clearly confirmed that most of γ-diversity is explained by β-diversity, whereas α-diversity contributed to total diversity from 2.5% (at the regional and local scales) to 5.1% (at the stygoregional scale). The high values of β-diversity may seem a paradox in species-poor groundwater assemblages. However, at the macro-scale, the high β-diversity has been explained by considering the effects of historical determinants (Malard et al., 2009). The high level of endemicity (linked to habitat fragmentation and isolation) and the low dispersal ability of stygobionts are the major responsible of the high values of γ-diversity and the

high species turnover among aquifers and regions; i.e. β-diversity appeared to be more important than α-diversity in shaping γ-diversity. For this reason, the contribution of β-diversity increased with the size of the hierarchical levels analysed (aquifer, region, stygoregion and continent).

At local scale, the value of β-diversity was high at the sampling-site level as well. In addition to arguments about the potential pseudosaturation discussed above, which may have accounted for low α-diversity values, we must admit that theoretical positions on the expected relationships between α- and β-diversities are conflicting (Jost, 2007). Previous analyses at the local scale support the contention that, although not unlikely ecological factors may play some role in building species richness at local scale, differences in water chemistry and other environmental parameters analysed in the Lessinian area (Galassi et al., 2009b) seemed to have not affected point-species richness in ground water (Dole-Olivier et al., 2009b; Malard et al., 2009). In theory, food and spatial niche availability in the truncated food webs (Gibert & Deharveng, 2002) may be explanatory variables accounting for differences in species richness observed between different areas of highly heterogeneous aquifers; i.e. they may explain the low levels of measured point diversity. The high spatial heterogeneity was demonstrated for both karstic (Brancelj, 2002; Pipan et al., 2006) and porous (Rouch, 1988, 1991, 1992, 1995; Rouch & Lescher-Moutoué, 1992) aquifers. These statements were confirmed by Galassi et al. (2009b), who demonstrated a strong habitat segregation of stygobiotic fauna in the Lessinian massif, and by the present study, which evidentiated a high species dissimilarity among caves within the same aquifer in the eastern Alpine stygoregion, independent of regional species richness.

At the local site-scale, the relationship between food availability and species richness is still open to question, and unfortunately this argument alone cannot satisfactorily explain the values of point-species richness. For instance, an increase in organic matter availability in ground water leads to an increase in allochthonous species (i.e. the stygoxenes) along with the decrease in stygobiotic species richness (Malard et al., 1994; Paran et al., 2005). Conversely, in other situations, stygobiotic species (especially bacterial biofilm-feeders) resulted concentrated in local patches at the cm^2-scale, corresponding to habitats with the highest microbial activity (Galassi et al., 1999). For this reason, the role of the main ecological determinants of the low point-diversity in ground water remains poorly understood.

Conclusion

Being the Crustacea the dominant group in ground water, in this contribution the spatial distribution of their species richness across a range of spatial scales has been examined. From data of the present study, a nested hierarchy of historical and ecological factors appears to determine stygobiotic species richness. At the continental and regional scales, historical factors are the major determinants of species richness in ground water. Quaternary glaciations have strongly affected the distribution of stygobiotic species richness; however, additional historical factors probably affected stygobiotic species richness among not glaciated areas, differing in palaeogeographical and palaeogeological features. For instance, differences in the age of the underlying geological formations into the same stygoregion may be reflected in different magnitudes of long-term vicariant events and potential retention of multiple disjunct refugia, which may have led to comparatively higher species richness in some geographical compartments (Deharveng et al., 2009). Historical factors seem to have operated also at the smaller regional scale, where different karstic aquifers, located in the same stygoregion, but differing in paleogeographical and palaeoclimatic conditions, showed different values of species richness.

At the local scale, results are less clear. The almost widespread truncated food webs in ground water (Gibert & Deharveng, 2002), together with different habitat preferences of stygobiotic species, may represent the coarse-grained explanations of the crucial question governing this contribution: *why are there so few species in ground water*? This answer may apparently be in line with classic community assembly theories which are synthetically focused on interspecific competition and niche differentiation among species which built up the community (Gotelli & Graves, 1996; Losos, 2008). The analysis of patterns of species richness at the local scale may hypothetically suggest that some (if not all) of the groundwater habitats may offer a small range of

successful strategies (Cornwell et al., 2006; Ulrich et al., 2009), which, together with oligotrophy, may led to poor species assemblages, composed by phylogenetic related species. Only a further approach, dealing with analysis of the functional diversity (Stegen & Swenson, 2009) in groundwater communities, together with the evaluation of phylogenetic diversity within and among groundwater assemblages (Hardy & Senterre, 2007; Graham & Fine, 2008; Ricklefs, 2008), will clarify the more significant ecological processes responsible for the observed patterns of point-species richness.

References

Anselin, L., 2005. Exploring Spatial Data with GeoDa™: A Workbook. Center for Spatially Integrated Social Science, Urbana: 226 pp.

Araújo, M. B., D. Nogués-Bravo, J. A. F. Diniz-Filho, A. M. Haywood, P. J. Valdes & C. Rahbek, 2008. Quaternary climate changes explain diversity among reptiles and amphibians. Ecography 31: 8–15.

Balian, E. V., C. Lévêque, H. Segers & K. Martens, 2008. Freshwater Animal Diversity Assessment. Hydrobiologia, Vol. 595. Springer, Dordrecht, The Netherlands.

Botosaneanu, L., 1986. Stygofauna Mundi—A Faunistic, Distributional and Ecological Synthesis of the World Fauna Inhabiting Subterranean Waters (Including the Marine Interstitial). E. J. Brill, Leiden, The Netherlands.

Botosaneanu, L. & J. R. Holsinger, 1991. Some aspects concerning colonization of the subterranean realm—especially of subterranean waters: a response to Rouch & Danielopol, 1987. Stygologia 6: 11–39.

Boutin, C. & N. Coineau, 1990. 'Regression Model', 'Modèle Biphase' d'évolution et origine des micro-organismes stygobies interstitiels continentaux. Revue de Micropaléontologie 33: 303–322.

Boxshall, G. A. & D. Defaye, 2008. Global diversity of copepods (Crustacea: Copepoda) in freshwater. Hydrobiologia 595: 195–207.

Braconnot, P., B. Otto-Bliesner, S. Harrison, S. Joussaume, J.-Y. Peterschmitt, A. Abe-Ouchi, M. Crucifix, E. Driesschaert, Th. Fichefet, C. D. Hewitt, M. Kageyama, A. Kitoh, A. Laîné, M.-F. Loutre, O. Marti, U. Merkel, G. Ramstein, P. Valdes, S. L. Weber, Y. Yu & Y. Zhao, 2007. Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum—Part 1: experiments and large-scale features. Climate of the Past 3: 261–277.

Brancelj, A., 2002. Microdistribution and high diversity of Copepoda (Crustacea) in a small cave in central Slovenia. Hydrobiologia 477: 59–72.

Brancelj, A. & H. J. Dumont, 2007. A review of the diversity, adaptations and groundwater colonization pathways in Cladocera and Calanoida (Crustacea), two rare and contrasting groups of stygobionts. Archiv für Hydrobiologie 168: 3–17.

Caley, M. J. & D. Schluter, 1997. The relationship between local and regional diversity. Ecology 78: 70–80.

Castellarini, F., M.-J. Dole-Olivier, F. Malard & J. Gibert, 2005. Improving the assessment of groundwater biodiversity by exploring environmental heterogeneity at a regional scale. In Gibert, J. (ed.), World Subterranean Biodiversity. Université Claude Bernard, Lyon, France: 83–88.

Castellarini, F., F. Malard, M.-J. Dole-Olivier & J. Gibert, 2007. Modelling the distribution of stygobionts in the Jura Mountains (eastern France). Implications for the protection of ground waters. Diversity and Distributions 13: 213–224.

Coineau, N., 2000. Adaptations to interstitial groundwater life. In Wilkens, H., D. C. Culver & W. F. Humphreys (eds), Subterranean Ecosystems of the World, Vol. 30. Elsevier, Amsterdam, The Netherlands: 189–211.

Colwell, R. K., 2005. EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples, Version 8.02. Available at http://purl.oclc.org/estimates.

Colwell, R. K., C. Rahbek & N. J. Gotelli, 2004. The mid-domain effect and species richness patterns: what have we learned so far? The American Naturalist 163: E1–E23.

Cornell, H. V., 1993. Unsaturated patterns in species assemblages: the role of regional processes in setting local species richness. In Ricklefs, R. E. & D. Schluter (eds), Species Diversity in Ecological Communities: Historical and Geographical Perspectives. University of Chicago Press, Chicago: 243–252.

Cornwell, W. K., D. W. Schwilk & D. D. Ackerly, 2006. A trait-based test for habitat filtering: convex hull volume. Ecology 87: 1465–1471.

Crisci, J. V., 2006. Making taxonomy visible. Systematic Botany 31: 439–440.

Crist, T. O., J. A. Veech, J. C. Gering & K. S. Summerville, 2003. Partitioning species diversity across landscapes and regions: a hierarchical analysis of α, β, and γ diversity. The American Naturalist 162: 734–743.

Culver, D. C., 2005. The struggle to measure subterranean biodiversity. In Gibert, J. (ed.), World Subterranean Biodiversity. Université Claude Bernard, Lyon, France: 27–28.

Culver, D. C. & B. Sket, 2000. Hotspots of subterranean biodiversity in caves and wells. Journal of Cave and Karst Studies 62: 11–17.

Danielopol, D. L., 1992. New perspectives in ecological contribution of dissolved organic carbon to an upland research of groundwater organisms. In Stanford, J. A. & J. J. Simons (eds), Proceedings of the First International Conference on Groundwater Ecology. American Water Resources Association, Bethesda, MD: 15–20.

Danielopol, D. L. & R. Rouch, 1991. L'adaptation des organismes au milieu aquatique souterrain. Réflexions sur

l'apport des recherches écologiques récentes. Stygologia 6: 129–142.

Danielopol, D. L., P. Pospisil & R. Rouch, 2000. Biodiversity in groundwater: a large-scale view. Trends in Ecology & Evolution 15: 223–224.

Deharveng, L., F. Stoch, J. Gibert, A. Bedos, D. Galassi, M. Zagmajster, A. Brancelj, A. Camacho, F. Fiers, P. Martin, N. Giani, G. Magniez & P. Marmonier, 2009. Groundwater biodiversity in Europe. Freshwater Biology 54: 709–726.

Dole-Olivier, M.-J., F. Castellarini, N. Coineau, D. M. P. Galassi, P. Martin, N. Mori, A. Valdecasas & J. Gibert, 2009a. Towards an optimal sampling strategy to assess groundwater biodiversity: comparison across six European regions. Freshwater Biology 54: 777–796.

Dole-Olivier, M.-J., F. Malard, D. Martin, T. Lefébure & G. Gibert, 2009b. Relationships between environmental variables and groundwater biodiversity at the regional scale. Freshwater Biology 54: 797–813.

Engel, A. S., 2007. Observations on the biodiversity of sulfidic karstic habitats. Journal of Cave and Karst Studies 69: 187–206.

Fauna Europaea Web Service, 2004. Fauna Europaea Version 1.1. Available online at http://www.faunaeur.org.

Ferreira, D., F. Malard, M.-J. Dole-Olivier & J. Gibert, 2005. Hierarchical patterns of obligate groundwater biodiversity in France. In Gibert, J. (ed.), World Subterranean Biodiversity. Université Claude Bernard, Lyon, France: 75–78.

Ferreira, D., F. Malard, M.-J. Dole-Olivier & J. Gibert, 2007. Obligate groundwater fauna of France: diversity patterns and conservation implications. Biodiversity and Conservation 16: 567–596.

Forrò, L., N. M. Korovchinsky, A. A. Kotov & A. Petrusek, 2008. Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. Hydrobiologia 595: 187–194.

Galassi, D. M. P., 2001. Groundwater copepods: diversity patterns over ecological and evolutionary scales. Hydrobiologia 453(454): 227–253.

Galassi, D. M. P., P. De Laurentiis & M.-J. Dole-Olivier, 1999. *Nitocrellopsis rouchi* sp. n., a new ameirid harpacticoid from phreatic waters of France (Copepoda: Harpacticoida: Ameiridae). Hydrobiologia 412: 177–189.

Galassi, D. M. P., R. Huys & J. W. Reid, 2009a. Diversity, ecology and evolution of groundwater copepods. Freshwater Biology 54: 691–708.

Galassi, D. M. P., F. Stoch, B. Fiasca, T. Di Lorenzo & E. Gattone, 2009b. Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. Freshwater Biology 54: 830–847.

Gaston, K. J., 2000. Global patterns in biodiversity. Nature 405: 220–227.

Gibert, J. & D. C. Culver, 2009. Assessing and conserving groundwater biodiversity: an introduction. Freshwater Biology 54: 639–648.

Gibert, J. & L. Deharveng, 2002. Subterranean ecosystems: a truncated functional biodiversity. BioScience 52: 473–481.

Gibert, J., D. L. Danielopol & J. A. Stanford, 1994. Groundwater Ecology. Academic Press, San Diego.

Gotelli, N. J. & R. K. Colwell, 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4: 379–391.

Gotelli, N. J. & G. R. Graves, 1996. Null Models in Ecology. Smithsonian Institution Press, Washington, DC.

Graham, C. H. & P. V. A. Fine, 2008. Phylogenetic beta diversity: linking ecological and evolutionary processes across space and time. Ecology Letters 11: 1265–1277.

Hahn, H. J., 2009. A proposal for an extended typology of groundwater habitats. Hydrogeology Journal 17: 77–81.

Hardy, O. J. & B. Senterre, 2007. Characterizing the phylogenetic structure of communities by an additive partitioning of phylogenetic diversity. Journal of Ecology 95: 493–506.

Heino, J., 2002. Concordance of species richness patterns among multiple freshwater taxa: a regional perspective. Biodiversity and Conservation 11: 137–147.

Hewitt, G. M., 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68: 87–112.

Hillebrand, H., 2004. On the generality of the latitudinal diversity gradient. The American Naturalist 163: 192–211.

Hof, C., M. Brändle & R. Brandl, 2008. Latitudinal variation of diversity in European freshwater animals is not concordant across habitat types. Global Ecology and Biogeography 17: 539–546.

Hutchinson, G. E., 1959. Homage to Santa Rosalia, or, why are there so many kinds of animals? The American Naturalist 93: 145–159.

Illies, J., 1978. Limnofauna Europaea, 2nd ed. Gustav Fischer Verlag, Stuttgart.

Jaume, D., 2008. Global diversity of spelaeogriphaceans & thermosbaenaceans (Crustacea; Spelaeogriphacea & Thermosbaenacea) in freshwater. Hydrobiologia 595: 219–224.

Jost, L., 2007. Partitioning diversity into independent alpha and beta components. Ecology 88: 2427–2439.

Lefébure, T., C. J. Douady, F. Malard & J. Gibert, 2007. Testing dispersal and cryptic diversity in a widely distributed groundwater amphipod (*Niphargus rhenorhodanensis*). Molecular Phylogenetics and Evolution 42: 676–686.

Lehner, B. & P. Döll, 2004. Development and validation of a global database of lakes, reservoirs and wetlands. Journal of Hydrology 296: 1–22.

Lomolino, M. V., B. R. Riddle & J. H. Brown, 2006. Biogeography. Sinauer Associates. Inc, Sunderland, MA.

Losos, J. B., 2008. Phylogenetic niche conservation phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecology Letters 11: 995–1007.

Malard, F., J.-L. Reygrobellet, J. Mathieu & M. Lafont, 1994. The use of invertebrate communities to describe groundwater flow and contaminant transport in a fractured rock aquifer. Archiv für Hydrobiologie 131: 93–110.

Malard, F., C. Boutin, A. I. Camacho, D. Ferreira, G. Michel, B. Sket & F. Stoch, 2009. Diversity patterns of stygobiotic crustaceans across multiple spatial scales in Europe. Freshwater Biology 54: 756–776.

Martens, K., I. Schön, C. Meisch & D. J. Horne, 2008. Global diversity of ostracods (Ostracoda, Crustacea) in freshwater. Hydrobiologia 595: 185–193.

Martin, P., C. De Broyer, F. Fiers, G. Michel, R. Sablon & K. Wouters, 2009. Biodiversity of Belgian groundwater fauna in relation to environmental conditions. Freshwater Biology 54: 814–829.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca & J. Kent, 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853–858.

Notenboom, J., 1991. Marine regressions and the evolution of groundwater dwelling amphipods (Crustacea). Journal of Biogeography 18: 437–454.

Paran, F., F. Malard, J. Mathieu, M. Lafont, D. M. P. Galassi & P. Marmonier, 2005. Distribution of groundwater invertebrates along an environmental gradient in a shallow water-table aquifer. In Gibert, J. (ed.), World Subterranean Biodiversity. Université Claude Bernard, Lyon, France: 99–106.

Pipan, T. & D. C. Culver, 2005. Estimating biodiversity in the epikarstic zone of a West Virginia cave. Journal of Cave and Karst Studies 67: 103–109.

Pipan, T., M. C. Christman & D. C. Culver, 2006. Dynamics of epikarst communities: microgeographic pattern and environmental determinants of epikarst copepods in Organ Cave, West Virginia. American Midland Naturalist 156: 75–87.

Rangel, T. F. L. V. B., J. A. F. Diniz-Filho & L. M. Bini, 2006. Towards an integrated computational tool for spatial analysis in macroecology and biogeography. Global Ecology and Biogeography 15: 321–327.

Rapoport, E. H., 1982. Areography. Geographical Strategies of Species. Pergamon Press, New York.

Ricklefs, R. E., 2008. Disintegration of the ecological community. The American Naturalist 172: 742–750.

Rouch, R., 1988. Sur la répartition spatiale des Crustacés dans le sous-écoulement d'un ruisseau des Pyrénées. Annales de Limnologie 24: 213–234.

Rouch, R., 1991. Structure du peuplement des Harpacticides dans le milieu hyporhéique d'un ruisseau des Pyrénées. Annales de Limnologie 27: 227–241.

Rouch, R., 1992. Caractéristiques et conditions hydrodynamiques des écoulements dans les sédiments d'un ruisseau des Pyrénées. Implications écologiques. Stygologia 7: 13–25.

Rouch, R., 1995. Peuplement des Crustacés dans la zone hyporhéique d'un ruisseau des Pyrénées. Annales de Limnologie 31: 9–28.

Rouch, R. & D. L. Danielopol, 1987. L'origine de la faune aquatique souterraine entre le paradigme du réfuge et la modèle de la colonization active. Stygologia 3: 345–372.

Rouch, R. & D. L. Danielopol, 1997. Species richness of microcrustacea in subterranean freshwater habitats. Comparative analysis and approximate evaluation. Internationale Revue gesamten Hydrobiologie 82: 121–145.

Rouch, R. & F. Lescher-Moutoué, 1992. Structure du peuplement des Cyclopides (Crustacea: Copepoda) dans le milieu hyporhéique d'un ruisseau des Pyrénées. Stygologia 7: 197–211.

Ruffo, S. & F. Stoch, 2006. Checklist and distribution of the Italian fauna. Memorie del Museo Civico di Storia Naturale di Verona, 2. Serie. Sezione Scienze della Vita 17: 1–303.

Rundle, S., D. Bilton, D. M. P. Galassi & D. Shiozawa, 2002. The geographical ecology of freshwater meiofauna. In Rundle, S. D., A. L. Robertson & J. M. Schmid-Araya (eds), Freshwater Meiofauna: Biology, Ecology. Backhuys Publishers, Leiden, The Netherlands: 279–294.

Schminke, H. K., 1981. Perspectives in the study of the zoogeography of interstitial Crustacea: Bathynellacea (Syncarida) and Parastenocarididae (Copepoda). International Journal of Spéléologie 11: 83–89.

Shurin, J. B., J. E. Havel, M. A. Leibold & B. Pinel-Alloul, 2000. Local and regional zooplankton species richness: a scale-independent test for saturation. Ecology 81: 3062–3073.

Shurin, J. B., K. Cottenie & H. Hillebrand, 2009. Spatial autocorrelation and dispersal limitation in freshwater organisms. Oecologia 159: 151–159.

Sket, B., 1999a. The nature of biodiversity in hypogean waters and how it is endangered. Biodiversity and Conservation 8: 1319–1338.

Sket, B., 1999b. High biodiversity in hypogean waters and its endangerment—the situation in Slovenia, the Dinaric Karst and Europe. Crustaceana 72: 767–780.

Stegen, J. C. & N. G. Swenson, 2009. Functional trait assembly through ecological and evolutionary time. Theoretical Ecology 2: 239–250.

Stoch, F., 1995. The ecological and historical determinants of Crustacean diversity in groundwaters, or: why are there so many species? Mémoires de Biospéléologie 22: 139–160.

Stoch, F. (ed.), 2008. Fauna. In Subterranean Waters, Hidden Biodiversity. Italian Habitats, Italian Ministry of the Environment and Territorial Protection, Friuli Museum of Natural History 20: 41–77.

Stoch, F., M. Arteau, A. Brancelj, D. M. P. Galassi & F. Malard, 2009. Biodiversity indicators in European groundwaters: towards a predictive model of stygobiotic species richness. Freshwater Biology 54: 745–755.

Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press, Princeton.

Trontelj, P., C. J. Douady, C. Fišer, J. Gibert, Š. Gorički, T. Lefébure, B. Sket & V. Zakšek, 2009. A molecular test for cryptic diversity in ground water: how large are ranges of macro-stygobionts? Freshwater Biology 54: 727–744.

Ulrich, W., I. Hajdamowicz, M. Zaleswski, M. Stańska, W. Ciurzycki & P. Tykarski, 2009. Species assortment or habitat filtering: a case study of spider communities on lake islands. Ecological Research. doi:10.1007/s11284-009-0661-y.

UNEP/IETC, 2000. Lakes and Reservoirs: Similarities, Differences and Importance. http://www.unep.or.jp/ietc/Publications/Short_Series/LakeReservoirs-1/index.asp.

Väinölä, R., J. D. S. Witt, M. Grabowski, J. H. Bradbury, K. Jazdzewski & B. Sket, 2008. Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. Hydrobiologia 595: 241–255.

Whittaker, R. J., K. J. Willis & R. Field, 2001. Scale and species richness: towards a general, hierarchical theory of species diversity. Journal of Biogeography 28: 453–470.

Whittaker, R. J., M. B. Araújo, P. Jepson, R. J. Ladle, J. E. M. Watson & K. J. Willis, 2005. Conservation biogeography: assessment and prospect. Diversity and Distributions 11: 3–23.

Wilkens, H., D. C. Culver & W. F. Humphreys, 2000. Subterranean Ecosystems. Ecosystems of the World, Vol. 30. Elsevier, Amsterdam, The Netherlands.

Willis, K. J. & R. J. Whittaker, 2002. Species diversity: scale matters. Science 295: 1245–1248.

Winkler, H. & C. Kampichler, 2000. Local and regional species richness in communities of surface dwelling grassland Collembola: indication of species saturation. Ecography 23: 385–392.