

# Diversity patterns of stygobiotic crustaceans across multiple spatial scales in Europe

FLORIAN MALARD\*, CLAUDE BOUTIN<sup>†</sup>, ANA I. CAMACHO<sup>‡</sup>, DAVID FERREIRA\*, GEORGES MICHEL<sup>§</sup>, BORIS SKET<sup>¶</sup> AND FABIO STOCH\*\*

\*UMR CNRS 5023, *Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon 1, Villeurbanne, France*

<sup>†</sup>UMR CNRS 5245, *Laboratoire d'Ecologie Fonctionnelle, Université Paul Sabatier, Toulouse, France*

<sup>‡</sup>*Museo Nacional de Ciencias Naturales, CSIC, Departamento Biodiversidad y Biología Evolutiva, Madrid, España*

<sup>§</sup>*Commission Wallonne d'Etude et de Protection des Sites Souterrains, Bruxelles, Belgium*

<sup>¶</sup>*Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani, Ljubljana, Slovenia*

\*\**Dipartimento di Scienze Ambientali, University of L'Aquila, Italy*

## SUMMARY

1. Using species distribution data from 111 aquifers distributed in nine European regions, we examined the pairwise relationships between local species richness (LSR), dissimilarity in species composition among localities, and regional species richness (RSR). In addition, we quantified the relative contribution of three nested spatial units – aquifers, catchments and regions – to the overall richness of groundwater crustaceans.
2. The average number of species in karst and porous aquifers (LSR) varied significantly among regions and was dependent upon the richness of the regional species pool (RSR). LSR–RSR relationships differed between habitats: species richness in karstic local communities increased linearly with richness of the surrounding region, whereas that of porous local communities levelled off beyond a certain value of RSR.
3. Dissimilarity in species composition among aquifers of a region increased significantly with increasing regional richness because of stronger habitat specialisation and a decrease in the geographic range of species among karst aquifers. Species turnover among karst aquifers was positively related to RSR, whereas this relationship was not significant for porous aquifers.
4. The contribution of a given spatial unit to total richness increased as size of the spatial unit increased, although 72% of the overall richness was attributed to among-region diversity. Differences in community composition between similar habitats in different regions were typically more pronounced than between nearby communities from different habitats.
5. We conclude by calling for biodiversity assessment methods and conservation strategies that explicitly integrate the importance of turnover in community composition and habitat dissimilarity at multiple spatial scales.

*Keywords:* additive partitioning, local richness, regional richness, subterranean fauna, species turnover

## Introduction

Identification of biodiversity patterns across multiple spatial scales is of interest to infer processes shaping

biodiversity (e.g. Loreau, 2000) but is also essential for designing efficient conservation strategies of biological communities (Summerville *et al.*, 2003). Two of the major research avenues have concerned the link

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Correspondence: Florian Malard, UMR CNRS 5023, Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon 1, Bât. Forel, 43 Bd 11 Novembre 1918, Villeurbanne cedex F-69622, France. E-mail: malard@univ-lyon1.fr

between local species richness (LSR) and regional species richness (RSR) (Cornell & Lawton, 1992), and more recently the relative contributions of nested spatial scales to total richness (Veech *et al.*, 2002). Studies that examined the LSR–RSR relationship for different biogeographical regions generally demonstrated that LSR increased linearly with RSR (Srivastava, 1999; Kiflawi, Eitam & Blaustein, 2003). This linear relationship was initially considered as an indication that regional processes (e.g. evolutionary and biogeographic processes) predominated over local processes (e.g. competition, disturbance regime) in shaping local communities. This interpretation has recently been criticised (Loreau, 2000; Valone & Hoffman, 2002; Mouquet *et al.*, 2003; Hillebrand, 2005; Fox & Srivastava, 2006).

Since Lande (1996) revived the idea that total species diversity in a set of samples ( $\gamma$ ) could be expressed as the sum of alpha ( $\alpha$ ) and beta ( $\beta$ ) diversity, additive partitioning has been used to analyse hierarchical patterns of species diversity in agricultural landscapes (Wagner, Wildi & Ewald, 2000; Fournier & Loreau, 2001), rainforests (DeVries, Walla & Greeney, 1999), temperate deciduous forests (Gering, Crist & Veech, 2003; Summerville *et al.*, 2003), mountainous regions (Fleishman, Betrus & Blair, 2003) and lakes and streams (Stendera & Johnson, 2005). Additive partitions of diversity yielded contrasting results in some, but not all, studies reporting that the contribution of a sampling unit to overall species richness increased as its size increased. Variation among studies in the allocation of species diversity across spatial scales may be due in part to differences in biological traits (e.g. resource requirements, mobility) of the species considered (Fleishman *et al.*, 2003; Summerville *et al.*, 2006).

The description and understanding of diversity patterns among groundwater invertebrates is much less advanced than for other well-studied organisms such as mammals, birds, butterflies and vascular plants (Qian & Ricklefs, 2000; MacNally *et al.*, 2004; Rodriguez & Arita, 2004). Although verbal models of groundwater community organisation repeatedly emphasised the role of processes operating at multiple scales (Gibert, Danielopol & Stanford, 1994a; Gibert, Stanford & Dole-Olivier, 1994b; Gibert *et al.*, 2000; Ward *et al.*, 2000), the LSR–RSR relationship has not been formally examined for groundwater fauna,

nor have attempts been made to quantify the contribution of nested spatial units to overall diversity. An exception is the analysis by Gibert & Deharveng (2002), who reported a positive linear LRS–RSR relationship based on a restricted data set and suggested that local subterranean terrestrial and aquatic communities were shaped by broad-scale processes.

Local processes, especially competition for food, may also be of importance in shaping community structure. Although low food supply makes groundwater one of the harshest environments on earth (Sket, 1999a; Christman & Culver, 2001), surprisingly, the idea that local and/or regional biodiversity patterns may be in part controlled by competitive interactions has generally been discounted (but see Culver, 1994; Datry, Malard & Gibert, 2005). Food shortage in groundwater may be so severe that it potentially sets an upper limit to the number of coexisting species in a locality. Alternatively, Ricklefs (2004) suggested that over evolutionary time, competitive exclusion within an entire region should foster habitat specialisation and reduce the average distributional extent of species, thereby leading to an increase in spatial turnover in community composition between localities.

Most richness of the stygobiotic fauna probably originates at the level of broad spatial units (i.e. catchment, region) (Sket, 1999a; Culver & Sket, 2000). Indeed, the number of stygobionts in a single groundwater locality (i.e. cave, aquifer) is extremely low at least compared to surface habitats. Culver & Sket (2000) defined a subterranean diversity hotspot as a site containing 20 or more stygobiotic and troglobiotic species (i.e. aquatic and terrestrial species that are restricted to the subterranean environment, respectively), a number that is exceeded in even the most species-poor surface aquatic site. There may be substantial differences in community composition among catchments within regions because hydrological barriers strongly constrain dispersal of groundwater organisms (Gooch & Hetrick, 1979). Finally, broad-scale variations among regions having distinct colonisation histories are presumably even more important in shaping groundwater invertebrate communities than are local differences among catchments within regions (Culver *et al.*, 2003).

Until recently the lack of comprehensive regional and local lists of groundwater species has severely

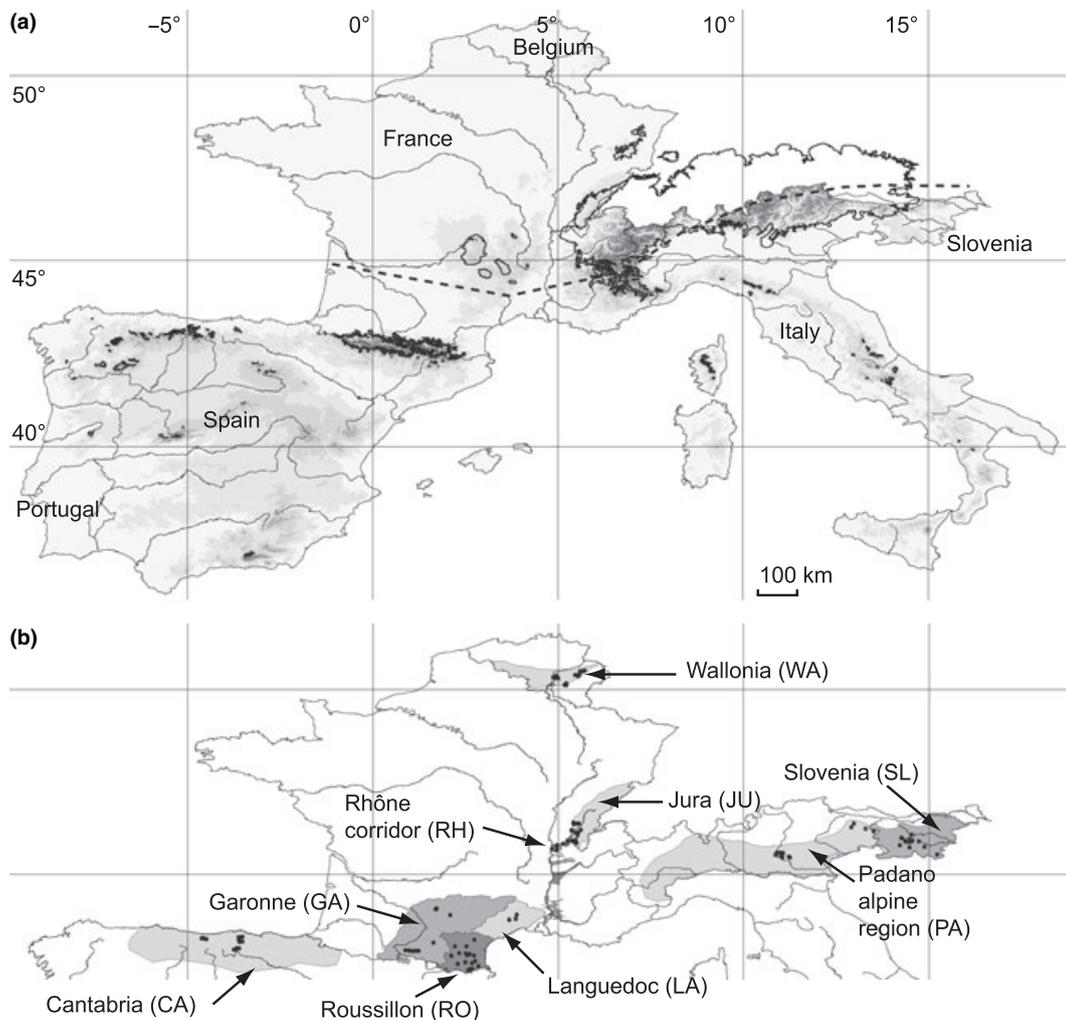
limited the examination of groundwater biodiversity patterns at multiple spatial scales (Culver & Fong, 1994; Pipan & Culver, 2007), although such lists are becoming increasingly available (Peck, 1998; Stoch, 2001; Proudlove *et al.*, 2003; Sket, Paragamian & Trontelj, 2004; Ferreira *et al.*, 2007; Hahn & Fuchs, 2009). In this study, we take advantage of an extensive survey carried out within the European research programme PASCALIS (Gibert, 2001) to determine the pairwise relationships between LSR, turnover in community composition among localities and RSR. In addition, we quantify the relative contribution of three nested spatial units – aquifers, catchments and regions – to the overall richness of groundwater crustaceans in western Europe and, based on this

information, we evaluate the implications of emerging biodiversity patterns for the assessment and conservation of groundwater fauna.

## Methods

### Study sites

Nine regions were chosen for this study (Fig. 1, Appendices 1 & 2). We refer to a region as a relatively large area that experienced a set of similar historical events (i.e. marine transgression/regression, glacial cover and tectonics). Groundwater fauna in Wallonia (WA), Slovenia (SL), Cantabria (CA) and the Jura (JU), Roussillon (RO) and Padano-alpine



**Fig. 1** (a) Physical geography of the study area showing elevation (grey patterns) and extent of glaciers (thick continuous black line) and permafrost (thick broken black line) during the late glacial maximum (c. 20 000 BP). Limits of glaciers and permafrost are from Hewitt (1999) and Buoncristiani & Campy (2004), respectively. (b) Location of regions and aquifers (black dots).

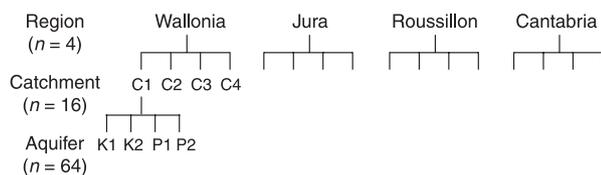
(PA), regions was sampled as part of the European research project PASCALIS (Gibert, 2001). Three additional regions – the Rhône River corridor (RH) and the Garonne (GA) and Languedoc (LA) regions – were selected because their groundwater fauna had been intensively studied at least since the 1970s (Ferreira *et al.*, 2007). A total of 111 well-studied aquifers were selected in the nine regions (Fig. 1, Appendices 1 & 2). An individual aquifer was considered as an appropriate spatial unit for estimating local richness. Indeed, aquifers are finite and continuous subsurface hydrological systems within which organisms can encounter each other and potentially interact within ecological time. We distinguished between two different types of aquifers that have long been considered as distinct groundwater habitats (Gibert *et al.*, 1994a,b). Karst aquifers form within soluble rock and conduct water principally via a connected network of fissures, whereas porous aquifers form within unconsolidated sediments (alluvium, colluvium, glacial till and outwash) and conduct water via a connected network of interstices. The number of aquifers selected in each region varied from three to nine for karst aquifers and four to eight for porous aquifers (Appendices 1 & 2). The Languedoc and Rhône River regions only comprised karst ( $n = 3$ ) and porous aquifers ( $n = 6$ ), respectively.

Four regions (WA, JU, RO and CA) were used for analysing the relative contribution of three nested spatial units (aquifer, catchment and region) to total richness (see Data analysis). In these regions, we selected four distinct river catchments and retained two karst aquifers and two porous aquifers per catchment (Fig. 2, Appendices 1 & 2). The boundaries of regions, catchments and aquifers were delineated in ARCGIS 8.3 (ESRI, Redlands, CA, USA) using multiple coverages including Landsat images, aerial

photographs, digital elevation models and topological, hydrological and geological maps. Polygon shape files of regions, catchments and aquifers are available upon request from the first author.

### Species lists

We assembled a species list of stygobionts as complete as possible for each aquifer using two distinct data sets. Stygobionts are obligate groundwater taxa that complete their entire life cycle exclusively in subsurface water (Gibert *et al.*, 1994a) and they represent approximately 8% of freshwater animal species in Europe (Sket, 1999b). The first data set consisted in a presence–absence species table arising from the sampling of 192 sites (i.e. springs, wells, caves and hyporheic sites) in four river catchments of a region. This data set was available for the six regions investigated as part of the PASCALIS project (WA, JU, RO, CA, PA and SL). Detailed information on the sampling design and methods are given in Malard *et al.* (2002). The second data set was a presence data base containing 10 183 occurrences of 1059 stygobionts collected in Belgium, France, Italy, Portugal, Slovenia and Spain (Deharveng *et al.*, 2009). This ‘European data base’ was built based on literature information, existing national data bases and personal collections. Both data sets were projected as point coverages in ARCGIS 8.3 and intersected with the polygon coverage of aquifers. Only those data points that intersected the polygons were analysed. Crustaceans were the only taxonomic group retained for analysis because other groups appeared to be unevenly studied in the different regions. Crustaceans are the dominant group in ground water and accounted for 71.9% and 80.3%, respectively, of the species and records included in the European data base. Epigeal species present in ground water were not considered in the analysis. Lists of stygobiotic crustacean species, records and distribution maps were checked by taxonomic group experts to avoid inconsistencies and synonymies among regions. The final presence–absence data matrix contained 111 aquifers and 373 crustacean species and subspecies belonging to 30 families and 78 genera (Table 1). The dominant families in terms of species richness were Cyclopidae (Cyclopoida), Niphargidae (Amphipoda), Canthocamptidae (Harpacticoida) and Candonidae (Ostracoda).



**Fig. 2** Spatial hierarchy considered in the additive partitioning of species richness. Levels 1 (aquifer), 2 (catchment) and 3 (regions) contained 64, 16 and 4 samples, respectively. Letters C, K and P designate catchment, karst aquifers and porous aquifers, respectively.

**Table 1** Number of genera and species within the crustacean families present in 111 aquifers distributed in 9 European regions

Order/class	Family	Genus	Species
Decapoda	Atyiidae	2	3
Amphipoda	Bogidiellidae	2	3
	Crangonyctidae	2	2
	Ingolfiellidae	1	2
	Niphargidae	4	58
	Pseudoniphargidae	1	4
	Salentinellidae	2	9
Isopoda	Asellidae	4	20
	Cirolanidae	3	4
	Microparasellidae	1	11
	Sphaeromatidae	2	14
	Stenasellidae	1	6
	Trichoniscidae	1	1
Thermosbaenacea	Thermosbaenidae	1	1
Bathynellacea	Bathynellidae	7	21
	Parabathynellidae	3	12
Calanoida	Diaptomidae	2	2
Cyclopoida	Cyclopidae	7	62
Harpacticoida	Ameiridae	3	13
	Canthocamptidae	11	51
	Ectinosomatidae	2	3
	Parastenocarididae	1	21
Cladocera	Chydoridae	1	2
Ostracoda	Candonidae	7	40
	Cyprididae	2	2
	Cypridopsidae	1	1
	Darwinulidae	1	1
	Limnocytheridae	1	1
	Metacyprinae	1	1
	Sphaeromicolidae	1	2

### Data analysis

*Effect of region and RSR on LSR.* ANOVA and Tukey's *post hoc* multiple comparisons were used to test for differences in LSR between regions and aquifer types (karst versus porous aquifers). LSR was the mean number of species in karst or porous aquifers of a region. A nested design was used to test for the effect of aquifer type (i.e. aquifer type nested within regions). Region and aquifer type were used as random and fixed factors, respectively.

Local species richness was plotted against RSR to determine whether species richness of an aquifer was limited by the supply of colonising species from the surrounding region (Srivastava, 1999). LSR–RSR relationships were assessed separately for karst (K) and porous (P) aquifers so that the regional pool of an aquifer type only comprised species which could colonise that particular type. We estimated RSR in

two different ways. First,  $RSR_1^*$  was estimated as the cumulative number of species in the selected aquifers of a region, excluding locally endemic species. The use of  $RSR^*$  instead of RSR (i.e. cumulative number of species in the aquifers of a region) was advocated by Cresswell, Vidal-Martinez & Crichton (1995) to ensure independence between estimates of local and regional richness. Secondly,  $RSR_2^*$  was calculated for each region as the total number of groundwater crustacean species listed in our European data base, again excluding locally endemic species. We fitted unconstrained linear and second-order polynomial regressions to the data and performed a *t*-test on the second-order term to test whether the second-order polynomial gave a better fit than the linear model. Ideally,  $RSR$ – $LSR$  regression lines should pass through the origin because a region with no species necessarily has a local richness of zero. However, constrained regressions extrapolate local richness beyond the range of the data and inflate  $R^2$  values (but see Srivastava (1999) for a discussion on the statistical merit of constraining regression lines). We did not attempt to account for differences in region size because RSR did not appear to increase with region size. The number of species in an aquifer did not correlate positively with aquifer area in any region except Cantabria.

*Relationships between species turnover and RSR.* We examined the relationship between species turnover among aquifers and RSR using linear regressions. Pairwise dissimilarity between aquifers *i* and *j* was measured using  $\beta_{sim}$  (Lennon *et al.*, 2001):

$$\beta_{sim} = \frac{\min(b,c)}{\min(b,c) + a}$$

where *a* is the number of species in common for a pair of aquifers *i* and *j* and *b* and *c* are the numbers of species exclusive to aquifer *i* and *j*.  $\beta_{sim}$  is considered as a narrow-sense measure of species turnover because it focuses more on compositional differences between aquifers than on differences in species richness (Lennon *et al.*, 2001; Koleff, Gaston & Lennon, 2003). It exhibits an upper limit of 1 when aquifers *i* and *j* have no species in common and a lower limit of 0 when all species belonging to aquifer *i* occurs in aquifer *j*. The average between-aquifer dissimilarity in species composition for each region

was calculated using all species (average  $\beta_{\text{sim}}$ ) and by excluding locally endemics (average  $\beta_{\text{sim}^*}$ ). Average  $\beta_{\text{sim}}$  and  $\beta_{\text{sim}^*}$  were linearly regressed against RSR and RSR\*, respectively. In order to test whether compositional differences between aquifer types increase with increasing RSR, the average dissimilarity between karst and porous aquifers of a region was regressed against RSR and RSR\*. Linear relationships between average dissimilarity and RSR were also assessed separately for karst and porous aquifers to examine differences between aquifer types. Significance for all statistical tests was accepted at  $\alpha = 0.05$ ; analyses were performed with the STATISTICA 6 software (Statsoft, Tulsa, OK, U.S.A.) and R software packages (R Development Core Team, 2006).

*Differences in community composition across scales.* Hierarchical cluster analysis with the  $\beta_{\text{sim}}$  measure of species turnover was used to examine patterns of dissimilarity among aquifers. Dissimilarity in species composition was calculated between all pairs of aquifers, and the UPGMA linkage method (unweighted pair-group method using arithmetic averages) was used to compute a hierarchical tree in R software (R Development Core Team, 2006).

Additive partitioning was applied to species richness data collected from a design involving three hierarchical levels (aquifer, catchment and region) to determine the contribution of each spatial scale to total richness. We restricted our analysis to four regions in order to obtain a balanced hierarchical design (Fig. 2). Total richness in a set of samples ( $S_T$ ) was additively partitioned into a within-sample component (alpha diversity,  $\alpha$ ) and a between-sample component (beta diversity,  $\beta$ ) as follows (Lande, 1996):

$$S_T = \alpha + \beta$$

where

$$\beta = \sum_i q_i(S_T - S_i), \quad \alpha = \sum_i q_i S_i,$$

where  $S_i$  is the number of species in sample  $i$  and  $q_i$  is the proportional weight associated with sample  $i$ .

Alpha and beta diversity were both expressed as number of species because  $\alpha$  was the average richness within the samples and  $\beta$  was the average amount of species not found in a single, randomly chosen sample. In the context of a hierarchy,  $\alpha$  at a given

scale was the sum of  $\alpha$  and  $\beta$  at the next lower scale (Veech *et al.*, 2002). Therefore, the following formulae apply to our hierarchical design:  $\alpha_{2(\text{catchments})} = \alpha_{1(\text{aquifers})} + \beta_{1(\text{aquifers})}$ ;  $\alpha_{3(\text{regions})} = \alpha_{2(\text{catchments})} + \beta_{2(\text{catchments})}$ ;  $\gamma = \alpha_{3(\text{regions})} + \beta_{3(\text{regions})}$ ; and after substitution,  $\gamma = \alpha_{1(\text{aquifers})} + \beta_{1(\text{aquifers})} + \beta_{2(\text{catchments})} + \beta_{3(\text{regions})}$ . Thus, it was possible to express the proportional contribution of each level in the hierarchical design to the total number of species ( $\gamma$ ).

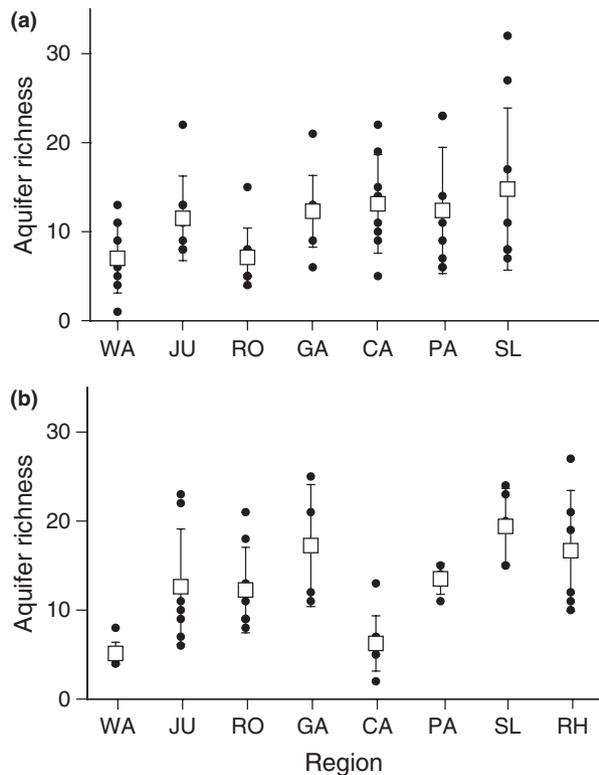
We used the reshuffling algorithm of the partition programme (Crist *et al.*, 2003) to test whether the observed diversity components ( $\alpha$  and  $\beta$ ) from our hierarchical design could have been obtained by a random distribution of sampling units at the next lower level. The randomisation procedure for each of the three levels of the hierarchy proceeded as follows: to test for the significance of  $\alpha_{1(\text{aquifers})}$  and  $\beta_{1(\text{aquifers})}$ , species were randomly allocated among aquifers that belonged to the same catchment. In a separate randomisation (test of  $\beta_{2(\text{catchments})}$ ), aquifers were randomly distributed among catchments that belonged to the same region. At last,  $\beta_{3(\text{regions})}$  was tested by randomly assigning catchments to any region. Randomisations were repeated 10 000 times to obtain a null distribution of  $\alpha$  and  $\beta$  components. Statistical significance was assessed by determining the proportion of null values that were greater or less than the observed components.

*Reserve system design.* We examined the effects of species distribution pattern on the design of groundwater biodiversity reserves using MARXAN 1.8.6 (University of Queensland, Brisbane, Australia), an optimisation package paying explicit attention to patterns of between-site (aquifer) complementarity (Ball & Possingham, 2001). The adaptive simulated annealing followed by the summed irreplaceability heuristic algorithm was used to determine the minimum number and location of aquifers for representing all species at least once (i.e. full representation goal). We examined the relationship between the proportion of aquifers selected in each region and RSR and average between-aquifer dissimilarity.

## Results

### *Differences in LSR between regions and habitats*

Local species richness of karst and porous aquifers varied significantly between regions (region effect:



**Fig. 3** Differences in average species richness ( $\pm$ SD) of karst (a) and porous (b) aquifers between regions: Wallonia (WA), Jura (JU), Roussillon (RO), Cantabria (CA), Padano-alpine region (PA), Slovenia (SL), Rhône River corridor (RH) and Garonne (GA) regions.

$F = 3.06$ ,  $P = 0.01$  for karst aquifers;  $F = 11$ ,  $P < 0.001$  for porous aquifers; Fig. 3). Species richness of the Walloon karst aquifers was significantly lower than that of Slovenian karst aquifers (Tukey's tests,  $P = 0.04$ ). Porous aquifers in Wallonia and Cantabria contained significantly fewer species than porous aquifers of all other regions (Tukey's tests,  $P < 0.05$ ). LSR of karst and porous aquifers were positively correlated across regions (Spearman's rank correlation coefficient = 0.94,  $P = 0.005$ ,  $n = 6$ ; Cantabria excluded). Porous aquifers contained in average more species than karst aquifers (hierarchical ANOVA, aquifer type nested in region,  $F_{7,88} = 3.04$ ,  $P = 0.006$ ).

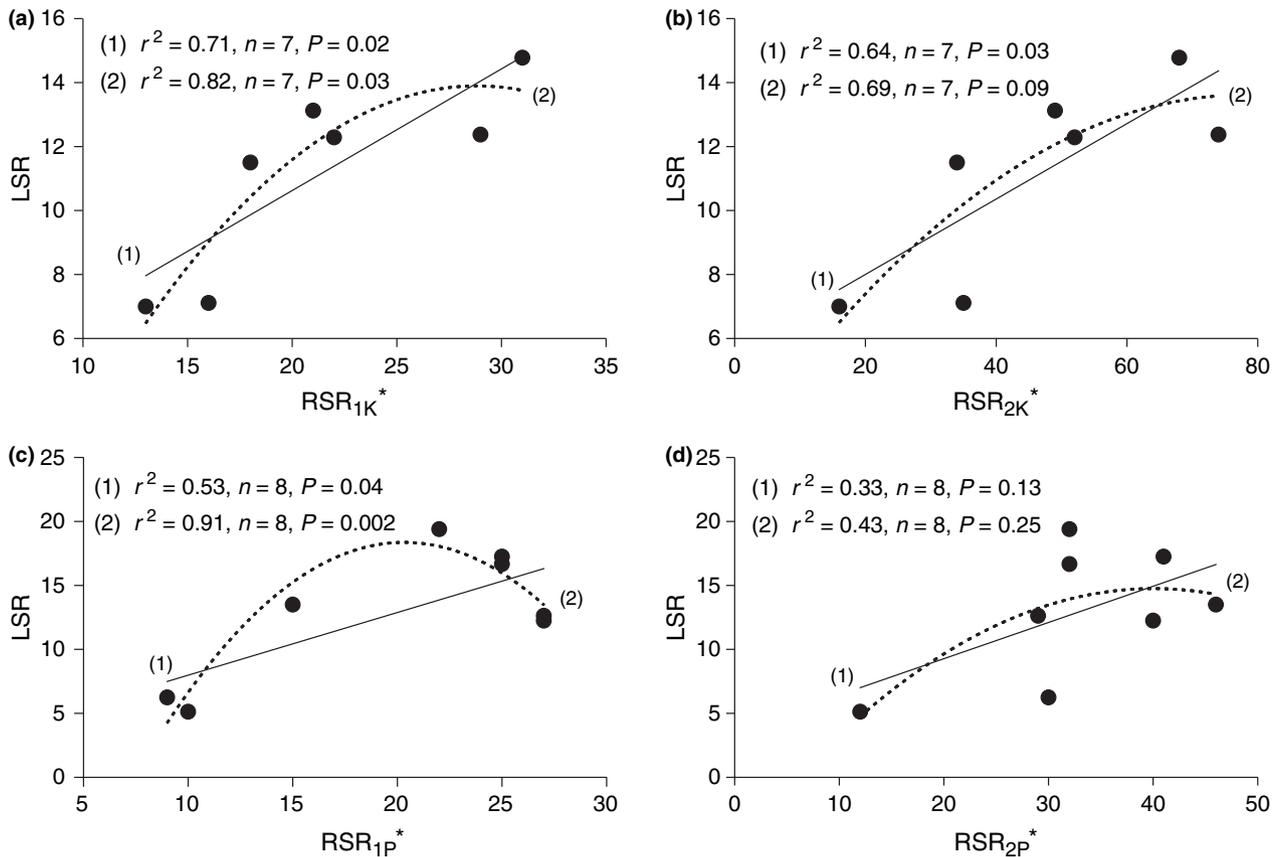
#### LSR–RSR relationships

None of the regional species–accumulation curves (cumulative species richness versus number of aquifers) performed with EstimateS (Colwell, 2005)

reached an asymptote, indicating that we did not exhaustively sample species richness in any of the regions. LSR of karst aquifers increased linearly with  $RSR_{1K}^*$  and  $RSR_{2K}^*$  (Fig. 4a,b, Table 2). The second-order term was not statistically significant for either relationship, indicating that the maximum of LSR was not reached within the observed range of RSR (13–31 species for  $RSR_{1K}^*$  and 16–74 species for  $RSR_{2K}^*$ ). LSR of porous aquifers showed a positive linear relationship with  $RSR_{1P}^*$ . However, the second-order polynomial model provided a better fit than the linear model, suggesting a maximum of LSR within the observed range of RSR (Fig. 4c, Table 2). The relationship between LSR of porous aquifers and RSR was no longer statistically significant with either linear or polynomial models when  $RSR_{2P}^*$  was used instead of  $RSR_{1P}^*$  (Fig. 4d, Table 2).

#### Relationships between species turnover and RSR

The average dissimilarity in species composition between aquifers of a region increased linearly with RSR (Fig. 5a, Table 3). This was in part due to an increase in the proportion of locally endemic species with RSR ( $y = 0.49x + 7.8$ ,  $r^2 = 0.82$ ,  $F = 23.4$ ,  $P = 0.004$ ,  $n = 7$ , for local endemics). However, the positive relationship between dissimilarity and RSR held true when excluding locally endemic species (i.e.  $\beta_{sim}^*$  versus  $RSR^*$ ). Average dissimilarity between aquifer types was positively correlated with RSR and  $RSR^*$ , indicating that habitat specialisation was more pronounced in species-rich regions (Fig. 5b, Table 3). Indeed, the proportion of species exclusive to a single habitat type increased with increasing  $RSR^*$  ( $y = -1.15x + 0.039x^2 + 40.9$ ,  $r^2 = 0.63$ ,  $F = 48.7$ ,  $P = 0.001$ ,  $n = 7$ ,  $48 \geq RSR^* \geq 18$ ). The average dissimilarity in species composition between karst aquifers was positively related to RSR (Fig. 5c, Table 3), whereas no significant linear relationships between dissimilarity and RSR were found for porous aquifers (Fig. 5d, Table 3). There was a significant positive linear relationship between the proportion of locally endemic species and RSR for karst aquifer ( $y = 0.54x + 21.9$ ,  $r^2 = 0.83$ ,  $F = 23.8$ ,  $P = 0.004$ ,  $n = 7$ ), whereas the same relationship was not significant for porous aquifers ( $r^2 = 0.0003$ ,  $F = 0.001$ ,  $P = 0.96$ ,  $n = 8$ ).



**Fig. 4** Relationships between local species richness (LSR) of karst (a & b) and porous (c & d) aquifers and regional species richness (RSR). RSR<sub>1K</sub>\* and RSR<sub>1P</sub>\* are the cumulative numbers of species (excluding local endemics) in selected karst aquifers and porous aquifers, respectively, of a given region. RSR<sub>2K</sub>\* and RSR<sub>2P</sub>\* refer to the total number of species (excluding local endemics) known from all sampling sites of a region in karst and porous aquifers, respectively.

#### Differences in community composition across scales

**Cluster analysis.** The hierarchical cluster analysis identified strong dissimilarities between regional crustacean assemblages, thereby grouping aquifers according to their regions (Fig. 6). RSR averaged  $54 \pm 25$  species ( $n = 9$ ), but the average number of species shared by any two regions was only  $6 \pm 5$  ( $n = 36$  comparisons) (Table 4). The proportion of regionally endemic species (i.e. species occurring in a single region) varied from 19% in the Rhône river corridor to 86% in Cantabria and increased with RSR ( $y = 35.3 \ln(x) - 79.4$ ;  $r^2 = 0.47$ ,  $F = 49.6$ ,  $P < 0.001$ ,  $n = 9$ ). Karst and porous aquifers formed distinct clusters in only three of the seven regions containing distinct aquifer types (CA, SL and JU) (Fig. 6). The average dissimilarity between karst and porous aquifers belonging to the same region ( $\beta_{sim} = 0.58$ ) was distinctly lower than the average dissimilarity be-

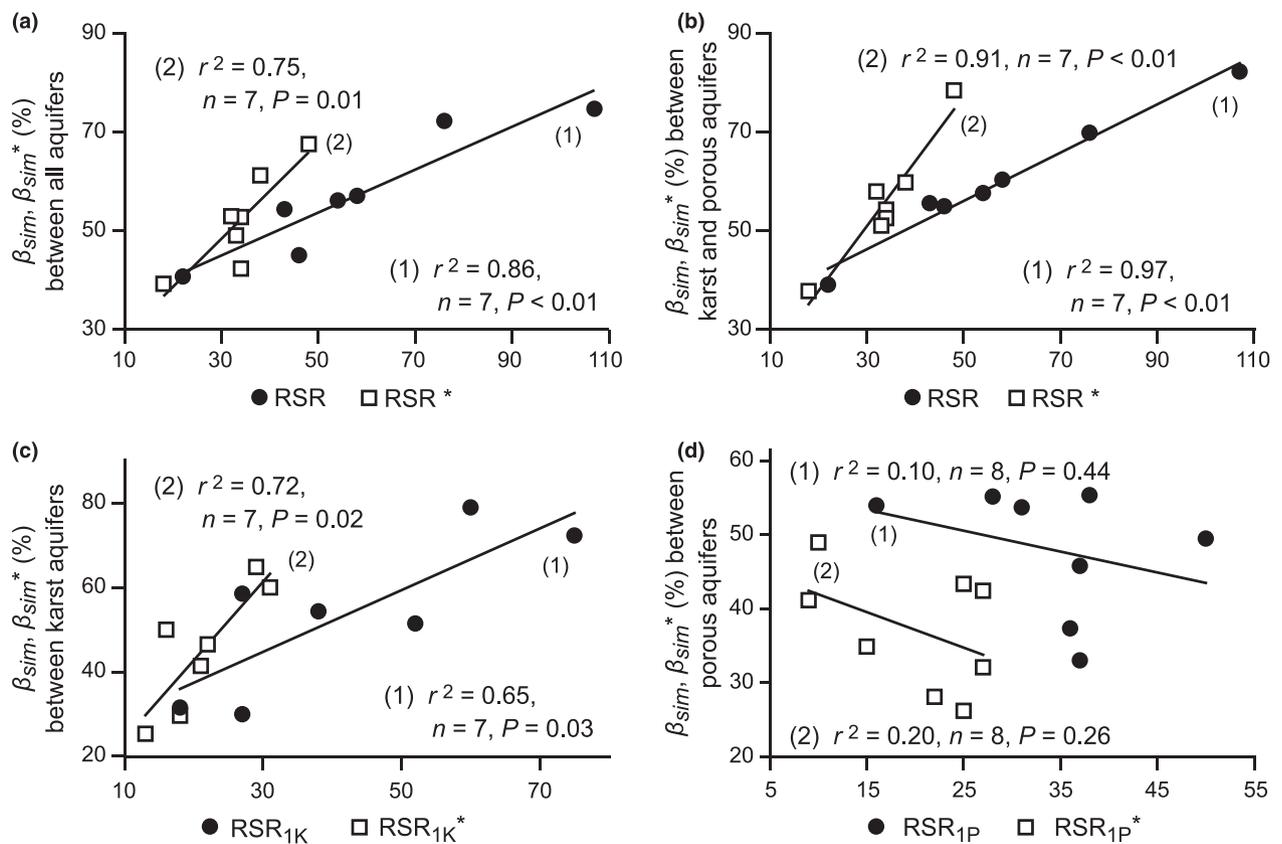
tween aquifers of the same type belonging to distinct regions ( $\beta_{sim} = 0.96$ ).

#### Additive partitioning of species richness

The four regions selected for the additive partitioning of species richness comprised a total of 150 species. Alpha diversity of stygobiotic crustaceans at the spatial level of the region ( $\alpha_{3(\text{regions})} = 42.2$  species) was considerably lower than beta diversity at that level ( $\beta_{3(\text{regions})} = 107.7$ ). In contrast, both alpha and beta diversities were similar at the spatial levels of catchment ( $\alpha_{2(\text{catchments})} = 21.2$ ;  $\beta_{2(\text{catchments})} = 21.1$ ) and aquifer ( $\alpha_{1(\text{aquifers})} = 9.4$ ;  $\beta_{1(\text{aquifers})} = 11.8$ ). The contribution of each spatial level to overall species richness increased as its size increased (Fig. 7):  $\beta_{3(\text{regions})} > \beta_{2(\text{catchments})} > \beta_{1(\text{aquifers})} > \alpha_{1(\text{aquifers})}$ . However, beta diversity between regions made by far the highest contribution to total richness

**Table 2** Coefficients with standard errors (SE), *t* statistic and significance level (*P*) of the linear and second-order polynomial regression models for the relationship between LSR and RSR (see Fig. 4)

Habitat	RSR	Model	<i>n</i>	Term	Coefficient	SE	<i>t</i>	<i>P</i> -value
Karst	RSR <sub>1K</sub> *	Linear	7	Constant	3.0	2.4	1.24	0.27
				First order	0.4	0.1	3.47	0.02
		Polynomial	7	Constant	-10.7	8.9	-1.21	0.29
	RSR <sub>2K</sub> *	Linear	7	Constant	5.6	2.0	2.84	0.04
				First order	0.1	0.0	2.99	0.03
		Polynomial	7	Constant	2.3	4.5	0.53	0.63
Porous	RSR <sub>1P</sub> *	Linear	8	Constant	3.1	4.0	0.77	0.47
				First order	0.5	0.2	2.59	0.04
		Polynomial	8	Constant	-27.0	6.6	-4.09	0.009
	RSR <sub>2P</sub> *	Linear	8	Constant	3.6	5.6	0.65	0.54
				First order	0.3	0.2	1.73	0.13
		Polynomial	8	Constant	-5.6	11.5	-0.49	0.64
	RSR <sub>2P</sub> *	Linear	8	Constant	3.6	5.6	0.65	0.54
				First order	0.3	0.2	1.73	0.13
		Polynomial	8	Constant	-5.6	11.5	-0.49	0.64
RSR <sub>2P</sub> *	Linear	8	Constant	3.6	5.6	0.65	0.54	
			First order	0.3	0.2	1.73	0.13	
	Polynomial	8	Constant	-5.6	11.5	-0.49	0.64	
RSR <sub>2P</sub> *	Linear	8	Constant	3.6	5.6	0.65	0.54	
			First order	0.3	0.2	1.73	0.13	
	Polynomial	8	Constant	-5.6	11.5	-0.49	0.64	
RSR <sub>2P</sub> *	Linear	8	Constant	3.6	5.6	0.65	0.54	
			First order	0.3	0.2	1.73	0.13	
	Polynomial	8	Constant	-5.6	11.5	-0.49	0.64	

**Fig. 5** Relationships between species turnover among aquifers (average  $\beta_{sim}$  expressed as a percentage) and regional species richness (RSR). Dissimilarity between (a) all aquifers of a region, (b) karst and porous aquifers, (c) karst aquifers and (d) porous aquifers.  $\beta_{sim}$  and  $\beta_{sim}^*$  were regressed against RSR and RSR\*, respectively.

**Table 3** Coefficients with standard errors (SE), *t* statistic and significance level (*P*) of the linear regression models for the relationship between turnover in species composition among aquifers and RSR (see Fig. 5)

Aquifers	Local endemics	<i>n</i>	Term	Coefficient	SE	<i>t</i>	<i>P</i> -value
All	Yes	7	Constant	32.0	4.8	6.60	0.001
			RSR	0.4	0.1	5.67	0.002
	No	7	Constant	19.2	8.6	2.22	0.08
			RSR*	1.0	0.2	3.92	0.01
Karst and porous	Yes	7	Constant	31.5	2.1	14.90	<0.001
			RSR	0.5	0.0	14.62	<0.001
	No	7	Constant	11.4	6.2	1.84	0.12
			RSR*	1.3	0.2	7.37	0.001
Karst	Yes	7	Constant	22.9	11.0	2.08	0.09
			RSR1K	0.7	0.2	3.08	0.03
	No	7	Constant	5.7	11.8	0.48	0.64
			RSR1K*	1.9	0.5	3.51	0.02
Porous	Yes	8	Constant	57.6	12.1	4.77	0.003
			RSR1P	-0.3	0.3	-0.82	0.44
	No	8	Constant	46.7	8.3	5.66	0.001
			RSR1P*	-0.5	0.4	-1.23	0.26

( $\beta_{3(\text{regions})} = 71.8\%$ ). The proportion of species unique to an aquifer, a catchment, and a region was 29%, 41% and 89%, respectively. The observed diversity within aquifers ( $\alpha_1$ ), between catchments ( $\beta_2$ ) and between regions ( $\beta_3$ ) was always greater than expected by chance ( $P < 0.001$ ), whereas the observed diversity between aquifers ( $\beta_1$ ) was not significantly different ( $P = 0.13$ ) from a random distribution of species among aquifers.

The proportions of richness components varied little among regions (Fig. 7). Between-catchment diversity ( $\beta_2$ ) was typically higher than both between-aquifer ( $\beta_1$ ) and within aquifer ( $\alpha_1$ ) diversity. The contribution of between-catchment diversity ( $\beta_2$ ) to regional richness increased in the order Wallonia, Jura, Roussillon and Cantabria, whereas that of within-aquifer diversity ( $\alpha_1$ ) showed the reverse order.

#### Reserve system design

Of a total of 111 aquifers, 62 were needed to capture all species at least once, although a high proportion of species rapidly accumulated in relatively few aquifers (Fig. 8). The full representation goal was achieved by selecting dissimilar proportions of aquifers in each region. The proportion of selected aquifers in a region increased linearly with RSR ( $y = 1.04x - 1.67$ ,  $r^2 = 0.82$ ,  $F = 22.7$ ,  $P = 0.005$ ,  $n = 7$ ) and  $\beta_{\text{sim}}$  ( $y = 2.37x - 77.12$ ,  $r^2 = 0.93$ ,  $F = 70.5$ ,  $P < 0.001$ ,

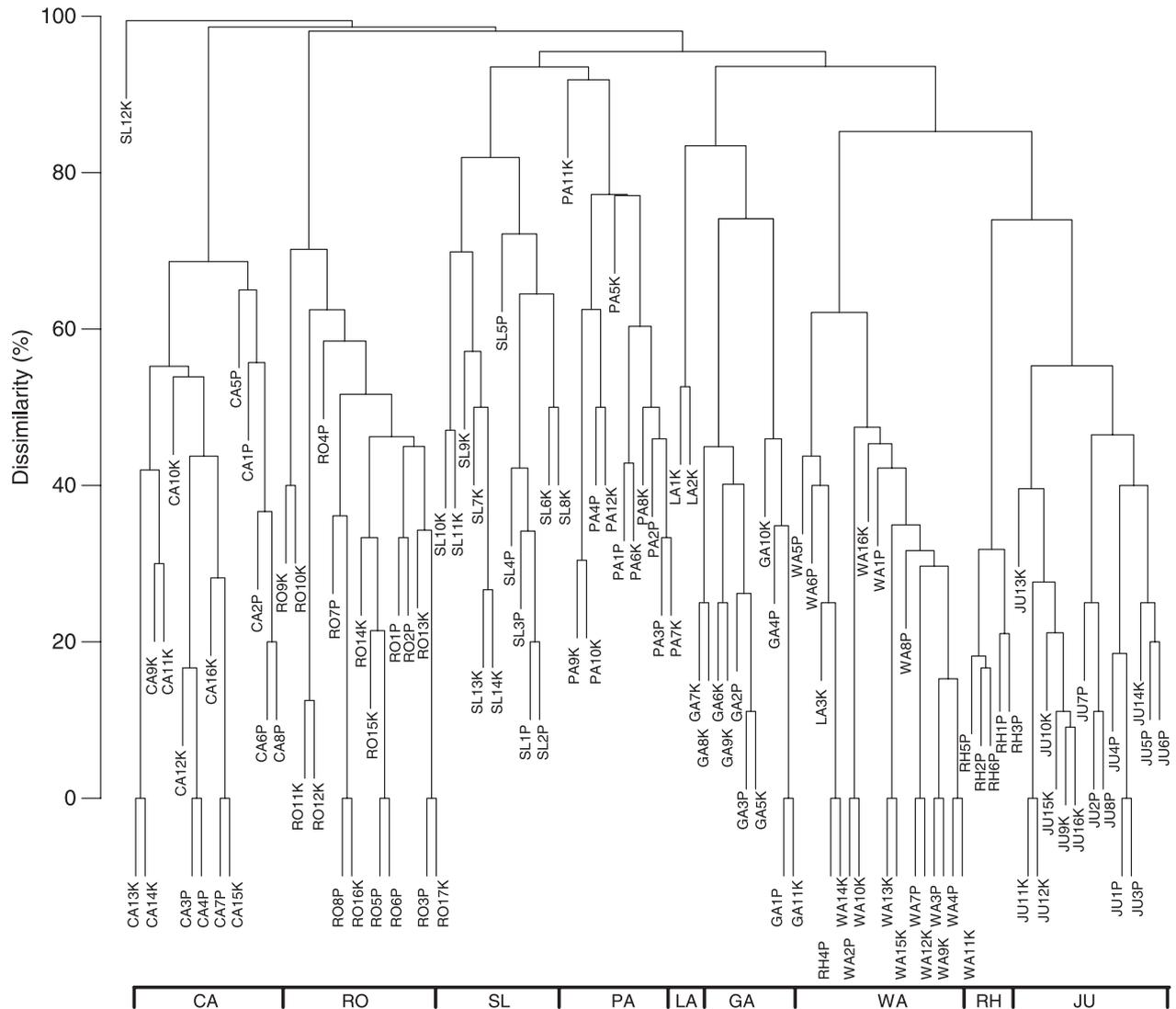
$n = 7$ ). Of a total of 373 species, 303 (81%) were represented in only 24 species-rich aquifers unevenly distributed in distinct regions: six aquifers in Slovenia; four in the Padano-alpine region; three each in Cantabria, the Garonne Basin and Roussillon; two in the Jura and one in Wallonia, the Languedoc and Rhône River corridor. These 24 species-rich aquifers comprised 16 karst aquifers (27% of karst aquifers) and only seven porous aquifers (i.e. 14% of porous aquifers).

## Discussion

### Emerging patterns of groundwater biodiversity

Several interesting patterns emerge from the present study on relationships between local richness and species turnover and regional richness of groundwater communities across nine European regions. Some regions (e.g. Slovenia) contained on average significantly more species of crustaceans per aquifer than others (e.g. Wallonia). Species richness of karst and porous aquifers covaried among regions but the average number of species in porous aquifers was higher than that of karst aquifers. This latter result is counterintuitive because karst aquifers offer older and more heterogeneous habitats than porous aquifers.

The average number of species per aquifer (LSR) was dependent upon the richness of the regional species pool (RSR), but the relationship between LSR

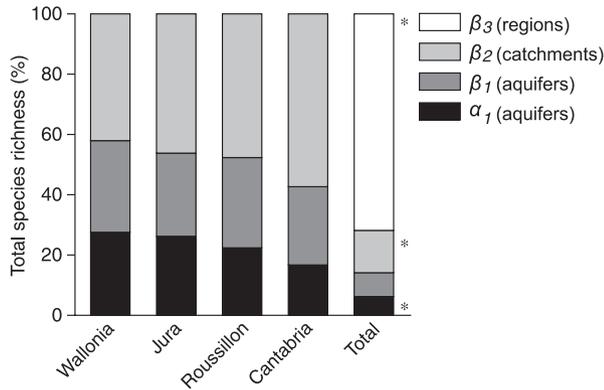


**Fig. 6** Dendrogram showing dissimilarity in species composition between 111 aquifers belonging to 9 distinct regions: Wallonia (WA), Slovenia (SL), Cantabria (CA), the French Jura (JU), Roussillon (RO), the Padano-alpine region (PA), the Rhône River corridor (RH), and the Garonne (GA) and Languedoc (LA) regions. Letters K and P designate karst and porous aquifers, respectively. See Appendices 1 & 2 for aquifer codes.

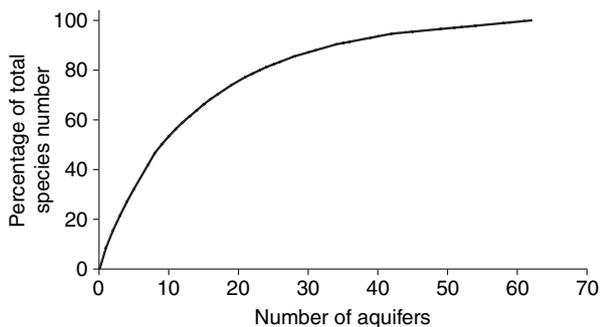
**Table 4** Number of species shared between any 2 regions

Region	Wallonia	Jura	Rhône corridor	Languedoc	Roussillon	Garonne	Cantabria	Padano-alpine region	Slovenia
Wallonia	22–8								
Jura	12	46–16							
Rhône corridor	9	22	37–7						
Languedoc	6	11	13	41–22					
Roussillon	2	3	4	6	43–30				
Garonne	5	6	8	9	5	54–39			
Cantabria	2	1	1	1	2	3	58–50		
Padano-alpine region	4	8	4	2	2	2	2	76–54	
Slovenia	3	6	6	4	1	2	5	16	107–84

Numbers in the diagonal indicate the number of species and regionally endemic species in each region.



**Fig. 7** Additive partitioning of stygobiotic crustacean richness at the scale of 4 individual regions and for all regions (total). Bars show the percentage of total species richness explained by alpha and beta components of richness at three spatial hierarchical levels: aquifer, catchment and region. Asterisks indicate that the observed richness at a level was significantly different from a random distribution of samples ( $P < 0.05$ ).



**Fig. 8** Species-accumulation curve for 62 aquifers representing all species at least once.

and RSR varied between habitats. Species richness in karstic local communities increased linearly with the richness of the surrounding region, whereas that of porous local communities levelled off beyond a certain value of RSR. The difference between habitats held true whatever the method used for estimating the regional species pool. The use of  $RSR_2^*$  instead of  $RSR_1^*$  probably overestimated the regional species pool but it did not result in a pseudo-saturation effect for karst aquifers (Cornell & Lawton, 1992). LSR–RSR relationships are particularly sensitive to variation in the definition, scale and estimation of local and regional richness (Hugueny *et al.*, 1997; Srivastava, 1999; Loreau, 2000; Rivadeneira, Fernández & Navarrete, 2002; Fox & Srivastava, 2006). For example, studies of LSR–RSR relationships among stream invertebrates in Finland and Sweden came to dissim-

ilar conclusions about the shape of the relationship (i.e. linear versus curvilinear), probably because of methodological differences in the relative size of local habitats and regions (Heino, Muotka & Paavola, 2003; Stendera & Johnson, 2005). Nevertheless, our results add to an increasing body of literature demonstrating that variation in species richness between local communities is partly attributable to differences in the size of the species pool associated with each particular community (Ricklefs, 1987; Eriksson, 1993; Lawton, 1999; Gaston & Chown, 2005; Freestone & Harrison, 2006). If differences in regional richness largely reflect historical contingencies rather than consistent differences in local environmental conditions between regions, it follows that comparable environments located in distinct regions may harbour different numbers of species. Ideally, any comparative studies based on species richness data from aquifers belonging to distinct regions should therefore compensate for the effect of the regional species pool on local richness.

Studies which examined the relationship between species turnover and RSR have provided highly variable results, indicating that high regional richness does not necessarily result from high species turnover between sites (Koleff & Gaston, 2002; Heino *et al.*, 2003; Qian, Ricklefs & White, 2005). In the present study, species turnover among aquifers of a region increased with increasing regional richness. The higher turnover among aquifers of species-rich regions reflected greater habitat specialisation and a decrease in the geographic range of species among karst aquifers. The proportion of endemics occurring in a single karst aquifer increased with an increase in the regional species pool, but this relationship was not observed for porous aquifers. Christman *et al.* (2005) found that areas in North America showing high diversity of non-endemic terrestrial cave animals also had a correspondingly high number of species known from a single cave only. The increase in the regional pool of groundwater species in the present study was the result more of higher beta diversity between aquifers than a consequence of increased richness of the local communities. In Slovenia, one of the areas in the world with the richest groundwater fauna (Sket, 1999a), the contribution of between-karst aquifer diversity to RSR reached 80%.

There was a positive relationship between size of a landscape level and its contribution to overall species

richness. As observed in other studies (Fleishman *et al.*, 2003; Gering *et al.*, 2003), this scale-dependence in species richness could reflect a species–area relationship, new crustacean species being found as surveys included larger areas. However, distinct processes probably operate at each spatial scale to generate differences in richness and species composition (Gibert *et al.*, 1994a). Regional variation in richness and composition of communities potentially reflect differences in the pool of colonisers during early phases of evolution (i.e. different colonisers with distinct diversification capacities), whereas hydrological barriers between catchments within the same region may promote species diversification via allopatric speciation events (Gooch & Hetrick, 1979; Gorički & Trontelj, 2006).

Our finding that crustacean community composition varied over broader spatial scales was supported by our cluster analysis, which identified pronounced differences in species composition among regions. Regional factors had a much stronger influence on species composition than habitat features, because differences in community composition between similar habitats in different regions were typically more pronounced than between nearby communities from different habitats. Part of the disproportionately high regional contribution to total richness was due to the fact that selected regions were distant from each other. However, even contiguous regions such as Garonne and Roussillon regions or the Padano-alpine region and Slovenia shared a very low percentage of species. Sket (1999a) showed that the Dinaric karst in Slovenia shared only 22% of its species with south-eastern neighbouring regions of the Dinaric karst.

Regions are typically smaller and exhibit higher dissimilarities in species composition in ground water than in surface water (Culver *et al.*, 2003; Ferreira *et al.*, 2007). For example, Ferreira *et al.* (2007) showed that nearly 70% of the 380 stygobionts collected in France were restricted to the French landscape whereas the French fauna of Ephemeroptera and Odonata (i.e. 252 species) had 80% and 100% species in common with the Italian and Swiss fauna, respectively. Our results also suggested that the relative contribution of alpha and beta diversity to total diversity changed with spatial scale but we lack sufficient data to test statistically the scale dependence of alpha and beta diversity (Gering & Crist, 2002).

#### *Processes generating biodiversity patterns*

Biodiversity patterns emerging from the present cross-region comparison may have multiple though non-exclusive explanations. Therefore, the remainder of this section is necessarily speculative because patterns alone are never sufficient to infer the underlying processes (Loreau, 2000). Differences in regional richness may arise from historical factors (e.g. Pleistocene climate) or/and variation in current environmental conditions among regions (e.g. groundwater flow, organic matter supply). Culver *et al.* (2006) showed that regional richness hotspots of terrestrial cave invertebrates were distributed along a latitudinal ridge expanding in Europe from 42 to 46 °N and suggested that these hotspots could correspond to regions where surface productivity remained high over recent geological times (i.e. no extremely dry episodes like in southern Europe and no extremely cold episodes like in northern Europe). This scenario may also account for differences in regional richness of groundwater crustaceans in karst. The three most-species rich regions (SL, PA and CA) are located south of the Alps and Pyrenees could thus have experienced both an uninterrupted history of speciation and reduced extinction rate among narrowly distributed species during the Pleistocene. Rapid changes in climate during the Pleistocene undoubtedly caused many species to go extinct in northern areas (Gibert & Culver, 2005; Michel *et al.*, 2009). The U.K. and Belgium have only 16 and 33 stygobiotic species, respectively, whereas southern countries such as France, Italy, Slovenia and Spain contain at least 200 species (Sket, 1999a; Stoch, 2001; Proudlove *et al.*, 2003; Ferreira *et al.*, 2007; Deharveng *et al.*, 2009).

High species turnover in species-rich regions characterised by a long history of stable environmental conditions may simply be due to the fact that new species originate locally (i.e. multiple independent colonisation events) and spread only a short distance from their points of origin (Culver, Kane & Fong, 1995; Stoch, 1995; Trontelj *et al.*, 2009). Alternatively, habitat specialisation and reduction in geographical extent of species could reflect an increase in competitive pressure within a region characterised by a long history of speciation events (Ricklefs, 2004; Qian *et al.*, 2005; Harrison *et al.*, 2006). Community assembly rules also predict that distinct communities at

environmentally similar sites, which leads to high species turnover, are more likely to occur in regions with high productivity, large species pools and low dispersal rates (Chase, 2003).

Differences in diversity patterns between karst and porous communities suggest that composition of these communities might be controlled by different factors. Part of the variation potentially resides in the expectedly higher hydrological connectivity between porous aquifers (Ward & Palmer, 1994). Greater connectivity may promote dispersal, which reduces species turnover among localities and enables colonisation of most localities by competitively superior species (Chase, 2003). Dispersal may simultaneously decrease differences in species composition among localities and decrease extinction rates, resulting in a negative relationship between regional richness and species turnover (Hunter, 2005). Dissimilarities in species composition among porous aquifers were indeed lower than between karstic aquifers, although the difference was not statistically significant. Clearly, additional distribution data are needed to elucidate diversity patterns of fauna in porous aquifers and formulate testable scenarios that can explain the origin of these patterns.

#### *Implications for biodiversity assessment and conservation*

Determining conservation strategies requires that at least diversity patterns, if not the processes that determine them, are identified at multiple spatial scales (Summerville *et al.*, 2003). Many of the patterns revealed in the present study have important implications for the assessment and conservation of groundwater biodiversity. The additive partitioning of groundwater biodiversity indicates that groundwater species–accumulation curves are unlikely to saturate in spatially extensive sampling designs because beta diversity becomes increasingly large with increasing spatial scale. Interestingly, although groundwater ecologists deal with species-poor communities, they are confronted with the same dilemma as biologists involved in the conservation of hyperdiverse communities (Gering *et al.*, 2003). Complete inventories yielding saturated species–accumulation curves can only be achieved by intensive sampling of a limited spatial area (Rouch & Danielopol, 1997; Pipan & Culver, 2007) but such surveys provide poor

indications of the spatial variation in community composition. Extensive regional sampling will yield unsaturated accumulation curves (Castellarini *et al.*, 2007; Dole-Olivier *et al.*, 2009) but a better estimate of the contribution of species turnover to overall groundwater diversity.

Effective protection of groundwater fauna necessitates a conservation strategy that specifically considers patterns of species replacement at multiple spatial scales. Indeed, beta diversity was at least as important as alpha diversity in determining total richness at the spatial scales of the aquifer in the present analysis ( $\beta_{1(\text{aquifers})} = 56\%$ ), catchment ( $\beta_{2(\text{catchments})} = 50\%$ ) and region ( $\beta_{3(\text{regions})} = 72\%$ ). Several studies which examined patterns of diversity among a variety of organisms showed that regional diversity was influenced to a greater extent by beta diversity (Harrison & Inouye, 2002; Culver *et al.*, 2003; Pineda & Halfpeter, 2004; Rodriguez & Arita, 2004). A number of reserve selection algorithms paying explicit attention to between-site complementarity have recently been developed and could be used for maximising the representation of groundwater species in a network of aquifers at the European scale (Fischer & Church, 2005; Michel *et al.*, 2009).

A more precise delineation of groundwater ecoregions using methods such as clustering techniques (see e.g. Houghton, 2004; Proches, 2005; Hahn & Fuchs, 2009) is essential for conservation planning in Europe because the diversity of groundwater fauna arises largely from species turnover among regions. The global biogeographical scheme by Botosaneanu (1986) is one of the first approaches to regionalising groundwater organism distributions but it is yet much too coarse to be used for conservation purpose. The disproportionately high contribution of between-regions to overall richness implies that a high proportion of groundwater species in Europe can be protected by focusing conservation efforts on a few aquifers distributed in distinct regions. Indeed, the few attempts made to identify areas to be reserved for representing species at large spatial scales showed that a very small proportion of the groundwater landscape (<2%) was needed to capture the majority of species (Culver *et al.*, 2000; Ferreira *et al.*, 2007; Michel *et al.*, 2009). A proportionally higher number of sites should be selected in species-rich regions because of the higher contribution of beta diversity to overall regional diversity. Focusing conservation

effort on species-rich regions containing a high number of endemics is a meaningful strategy because the restriction of species distribution ranges increases vulnerability to extinction.

Conservation efforts of groundwater fauna have essentially been devoted to karst habitats, probably because of a long history of biological data collection in caves (Camacho, 1992; Juberthie, 1995). However, faunal dissimilarities among karst and porous habitats contribute importantly to regional richness. In the present study, species observed exclusively in karst and porous aquifers represented on average  $37 \pm 18\%$  and  $27 \pm 11\%$ , respectively, of the regional species pool ( $n = 7$  regions). The design of reserves based on the diversity of only one of these aquifer types or the other will provide very different scenarios for conservation areas because there is no significant relationship between regional richness of karst and porous communities ( $P = 0.16$ ,  $n = 7$  regions). Clearly, an effective conservation strategy needs to consider multiple groundwater habitats because their relative contribution to gamma diversity varies among regions.

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**Appendix 1** Name, code, location, area and species richness of selected karst aquifers in 8 European regions. Longitude and latitude are in decimal degrees (WGS 84). The 4 species richness numbers indicated for each region successively correspond to  $RSR_{1K}$ ,  $RSR_{1K}^*$ ,  $RSR_{2K}$  and  $RSR_{2K}^*$  (see text for details)

Region	Catchment	Aquifer	ID	Code	Longitude	Latitude	Area (km <sup>2</sup> )	Species richness
Cantabria	Ason Gandara	Upper Gandara	30	CA10K	3.58 W	43.19 N	42508	52,21,80,49
	Ason Gandara	Ason	29	CA9K	3.61 W	43.26 N	1.40	9
	Lamason deva	Deva	36	CA16K	4.56 W	43.30 N	34.67	19
	Lamason deva	Latarma	35	CA15K	4.51 W	43.27 N	12.95	11
	Matienzo Comellante	Comellante	32	CA12K	3.61 W	43.31 N	24.64	14
	Matienzo Comellante	Matienzo	31	CA11K	3.57 W	43.31 N	4.04	15
	Trema Nela	Nela	34	CA14K	3.67 W	42.98 N	1.96	10
	Trema Nela	Palomera	33	CA13K	3.66 W	43.03 N	0.89	5
							23.49	22

## Appendix 1 (Continued)

Region	Catchment	Aquifer	ID	Code	Longitude	Latitude	Area (km <sup>2</sup> )	Species richness
Garonne							30977	38,22,68,52
	Aveyron	La Madeleine	20	GA11K	1.70 E	44.07 N	0.14	6
	Aveyron	Amiel	19	GA10K	1.74 E	44.09 N	4.16	13
	Salat	Goueil di Her	18	GA9K	0.88 E	42.97 N	4.17	12
	Salat	Jouan d'Arau	17	GA8K	1.10 E	42.94 N	0.06	12
	Salat	Moulis	16	GA7K	1.11 E	42.94 N	4.59	13
	Salat	Baget	15	GA6K	1.00 E	42.96 N	9.92	21
Jura							1.06	9
							8455	27,18,43,34
	Albarine	Dorvan	76	JU12K	5.42 E	45.90 N	4.77	22
	Albarine	Charvieux	75	JU11K	5.51 E	45.87 N	4.90	8
	Oignin	Martignat	78	JU14K	5.62 E	46.21 N	1.34	8
	Oignin	Corberan	77	JU13K	5.50 E	46.13 N	6.40	8
	Suran	Marais	74	JU10K	5.42 E	46.41 N	0.53	13
Languedoc	Suran	Drom-Ramasse	73	JU9K	5.36 E	46.21 N	19.61	13
	Valouse	Valfin	80	JU16K	5.51 E	46.38 N	2.77	11
	Valouse	Arinthod	79	JU15K	5.57 E	46.38 N	5.35	9
							9311	41,21,56,36
	Herault	Cent-Fons Fontanilles	8	LA2K	3.69 E	43.79 N	144.55	19
	Lez	Lez	7	LA1K	3.86 E	43.81 N	172.29	26
	Vidourle	Sauve	9	LA3K	3.89 E	43.93 N	53.47	24
Padano-alpine							47488	60,29,105,74
	Alpone Tramigna	Alpone Tramigna	44	PA8K	11.23 E	45.49 N	104.66	6
	Arzino	Arzino	47	PA11K	12.92 E	46.28 N	125.58	11
	Fumane	Fumane	41	PA5K	10.93 E	45.61 N	41.01	14
	Squaranto	Squaranto	43	PA7K	11.07 E	45.58 N	87.81	9
	Tagliamento	Tagliamento	48	PA12K	12.98 E	46.38 N	56.89	6
	Torre-Natisone	Natisone	46	PA10K	13.52 E	46.18 N	161.05	23
	Torre-Natisone	Torre	45	PA9K	13.31 E	46.25 N	80.64	23
Valpantena	Valpantena	42	PA6K	11.02 E	45.60 N	140.17	7	
Roussillon							10571	27,16,46,35
	Agly	Verdouble	107	RO13K	2.52 E	42.88 N	106.26	7
	Agly	Fenouillède	106	RO12K	2.55 E	42.83 N	543.08	15
	Aude	Lacamp	105	RO11K	2.54 E	43.07 N	208.93	8
	Aude	Bugarach	104	RO10K	2.34 E	42.89 N	104.82	8
	Aude	Bugary Canyon	103	RO9K	2.13 E	42.81 N	374.62	5
	Tech	La Fou	111	RO17K	2.58 E	42.43 N	16.27	5
	Tech	Amélie	110	RO16K	2.69 E	42.47 N	17.54	4
	Têt	Villefranche	109	RO15K	2.30 E	42.60 N	64.32	7
	Têt	Corbère	108	RO14K	2.68 E	42.62 N	12.79	5
Slovenia							16303	75,31,112,68
	Borovnicica	Borovnicica karst	88	SL8K	14.38 E	45.89 N	43.10	11
	Dolenjska	Bela Krajina	92	SL12K	15.23 E	45.55 N	262.47	8
	Dolenjska	Suha Krajina	91	SL11K	14.90 E	45.76 N	523.21	27
	Dolenjska	Sica-Krka	90	SL10K	14.74 E	45.90 N	23.48	17
	Iska	Iska karst	87	SL7K	14.49 E	45.89 N	88.71	8
	Notranjska	Krizna jama - Bloke	94	SL14K	14.48 E	45.74 N	36.09	15
	Notranjska	PPJS	93	SL13K	14.22 E	45.80 N	7.49	32
	Podlipscica	Podlipscica karst	89	SL9K	14.26 E	46.00 N	17.52	7
Zelimejlsica	Zelimejlsica karst	86	SL6K	14.55 E	45.91 N	64.35	8	
Wallonia							7402	18,13,21,16
	Amblève	Comblain	64	WA16K	5.60 E	50.48 N	15.55	6
	Amblève	Vallon de chantoirs	63	WA15K	5.73 E	50.51 N	7.26	4
	Lesse	Boisne (Han)	60	WA12K	5.20 E	50.12 N	4.60	11
	Lesse	L'homme souterraine	59	WA11K	5.23 E	50.17 N	17.49	13
	Meuse	Spontin	58	WA10K	5.01 E	50.31 N	10.19	5

## Appendix 1 (Continued)

Region	Catchment	Aquifer	ID	Code	Longitude	Latitude	Area (km <sup>2</sup> )	Species richness
	Meuse	Crupet	57	WA9K	4.96 E	50.37 N	4.02	9
	Ourthe	Ferrières	62	WA14K	5.58 E	50.40 N	2.93	1
	Ourthe	Houmart	61	WA13K	5.47 E	50.40 N	3.86	7

**Appendix 2** Name, code, location, area and species richness of selected porous aquifers in 8 European regions. Longitude and latitude are in decimal degrees (WGS 84). The 4 species richness numbers indicated for each region successively correspond to  $RSR_{1p}$ ,  $RSR_{1p}^*$ ,  $RSR_{2p}$ , and  $RSR_{2p}^*$  (see text for details)

Region	Catchment	Aquifer	ID	Code	Long.	Lat.	Area (km <sup>2</sup> )	Species richness
Cantabria							42508	28,9,49,30
	Ason Gandara	Gandara alluvium	22	CA2P	3.51 W	43.20 N	3.15	6
	Ason Gandara	Ason alluvium	21	CA1P	3.60 W	43.26 N	7.16	7
	Lamason deva	Deva/Cares alluvium	28	CA8P	4.62 W	43.29 N	7.02	13
	Lamason deva	Lamason alluvium	27	CA7P	4.48 W	43.27 N	5.01	5
	Matienzo Comellante	Comellante alluvium	24	CA4P	3.60 W	43.31 N	1.36	6
	Matienzo Comellante	Clarion alluvium	23	CA3P	3.51 W	43.33 N	1.26	2
	Trema Nela	Nela alluvium	26	CA6P	3.73 W	42.98 N	4.11	5
	Trema Nela	Trema alluvium	25	CA5P	3.60 W	43.01 N	2.92	6
Garonne							30977	38,25,54,41
	Ariège	Ariege alluvium	10	GA1P	1.65 E	43.17 N	169.22	11
	Salat	Nert alluvium	12	GA3P	1.25 E	42.95 N	5.70	25
	Salat	Lachein alluvium	11	GA2P	0.99 E	42.96 N	0.06	21
	Tarn	Tarn alluvium	13	GA4P	2.09 E	43.91 N	69.62	12
Jura							8455	36,27,38,29
	Albarine	Upper Albarine alluvium	68	JU4P	5.48 E	45.94 N	2.53	23
	Albarine	Lower Albarine alluvium	67	JU3P	5.38 E	45.93 N	6.38	22
	Oignin	Upper Oignin till	70	JU6P	5.53 E	46.07 N	26.69	13
	Oignin	Montréal alluvium	69	JU5P	5.55 E	46.15 N	12.86	10
	Suran	Villereversure alluvium	66	JU2P	5.39 E	46.18 N	6.03	9
	Suran	Upper Suran alluvium	65	JU1P	5.43 E	46.37 N	4.62	7
	Valouse	Valouse alluvium	72	JU8P	5.54 E	46.38 N	8.13	11
	Valouse	Arintnod till	71	JU7P	5.56 E	46.38 N	17.28	6
Padano-alpine							47488	31,15,62,46
	Alpone Tramigna	Alpone Tramigna alluvium	40	PA4P	11.27 E	45.47 N	33.62	14
	Fumane	Fumane alluvium	37	PA1P	10.90 E	45.56 N	2.45	14
	Squaranto	Squaranto alluvium	39	PA3P	11.06 E	45.48 N	6.58	15
	Valpantena	Valpantena alluvium	38	PA2P	11.03 E	45.51 N	13.54	11
Rhône valley							4071	37,25,44,32
	Ain	Ain alluvium	1	RH1P	5.26 E	45.92 N	164.51	19
	Rhône	Saint-Priest alluvium	6	RH6P	4.96 E	45.67 N	96.10	12
	Rhône	Décines alluvium	5	RH5P	4.97 E	45.72 N	54.05	11
	Rhône	Lower Rhône alluvium	4	RH4P	4.85 E	45.70 N	47.46	10
	Rhône	Upper right Rhône alluvium	3	RH3P	5.14 E	45.85 N	128.87	27
	Rhône	Upper left Rhône alluvium	2	RH2P	4.97 E	45.80 N	44.35	21
Roussillon							10571	37,27,50,40
	Agly	High Verdoube	98	RO4P	2.63 E	42.86 N	13.37	9
	Agly	Tautavel	97	RO3P	2.74 E	42.82 N	70.91	11
	Aude	Limoux	96	RO2P	2.23 E	43.07 N	136.13	9
	Aude	Lower Aude River	95	RO1P	2.75 E	43.25 N	346.98	13
	Tech	Piémont Albères	102	RO8P	2.86 E	42.54 N	121.89	18
	Tech	Vallespir	101	RO7P	2.63 E	42.45 N	18.83	9
	Têt	Prades	100	RO6P	2.50 E	42.64 N	37.61	8
	Têt	Millas	99	RO5P	2.77 E	42.68 N	245.18	21

## Appendix 2 (Continued)

Region	Catchment	Aquifer	ID	Code	Long.	Lat.	Area (km <sup>2</sup> )	Species richness
Slovenia							16303	50,22,60,32
	Borovnicica	Borovnicica alluvium	83	SL3P	14.37 E	45.91 N	8.97	20
	Iska	Iska alluvium	82	SL2P	14.50 E	45.95 N	7.73	24
	Podlipscica	Podlipscica alluvium	84	SL4P	14.29 E	45.99 N	6.68	15
	Sava	Ljubljanskopolje	85	SL5P	14.53 E	46.80 N	60.55	23
	Zelimejscica	Zelimejscica alluvium	81	SL1P	14.57 E	45.93 N	3.23	15
Wallonia							7402	16,10,18,12
	Amblève	Amblève alluvium	56	WA8P	5.63 E	50.49 N	3.58	5
	Amblève	Sprimont colluvium	55	WA7P	5.66 E	50.50 N	14.82	5
	Lesse	Wavreille colluvium	52	WA4P	5.24 E	50.12 N	2.20	4
	Lesse	Lesse alluvium	51	WA3P	5.19 E	50.13 N	2.14	5
	Meuse	Champalle alluvium	50	WA2P	4.87 E	50.33 N	4.36	8
	Meuse	Evrehailles colluvium	49	WA1P	4.93 E	50.32 N	1.12	5
	Ourthe	Aisne alluvium	54	WA6P	5.55 E	50.36 N	1.20	5
	Ourthe	Ourthe Lembrée alluvium	53	WA5P	5.53 E	50.39 N	2.43	4