Groundwater biodiversity in Europe

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SUMMARY

1. The spatial patterns of groundwater biodiversity in Europe remain poorly known, yet their knowledge is essential to understand local variation in groundwater assemblages and to develop sound conservation policies. We explore here the broad-scale distribution of groundwater biodiversity across Europe, focussing on obligate subterranean species.
2. We compiled published distributional data of obligate subterranean aquatic taxa for six European countries (Belgium, France, Italy, Portugal, Slovenia and Spain), and conducted a detailed biological survey of six regions (one in Belgium, two in France, one in Italy, one in Slovenia and one in Spain). Based on this data set, we mapped spatial patterns of biodiversity in Europe on a cell grid with 0.2 × 0.2 resolution.
3. As of mid-2006, the total number of described stygobiotic species in the six countries was 930 and the total number of genera with at least one described stygobiotic species was 191. The total number of sampling sites where at least one stygobiont had been collected was 4709, distributed in 1228 of the 4668 grid cells covering the study area.
4. Groundwater stygobiotic biodiversity was dominated by Crustacea with 757 species in 122 genera. Insects were represented by only two species of a single genus of dytiscid beetles restricted to south-eastern France.
5. The geographic distribution of stygobionts was extremely heterogeneous. Stygobionts were recorded in 26% of the 4668 grid cells and only 33 cells had more than 20 stygobiotic species. These 33 ‘hot-cells’ of groundwater species richness clustered in seven hotspots.
6. Endemicity was very high, with 43% of the total number of stygobiotic species restricted to a single cell, i.e. <500 km².
7. Hotspots defined by rarity, number of genera, number of genera with only one species known in Europe, or number of monospecific genera differed markedly in ranking from those based on species richness. However, a core of four hotspots emerged in all cases: one stretching across Slovenia and northeastern Italy, one in the French Pyrenees, one in the Cévennes in southern France and one in the Rhine River valley in northeastern France.

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8. Unevenness in stygobiont distribution cannot be explained solely by unevenness in sampling effort. This is indicated in particular by the fact that our comprehensive sampling survey roughly matched the level of taxonomic richness of the studied regions based on previously published information.

9. With sampling effort continuing, a twofold or higher increase in species richness can be expected in several Mediterranean areas, with a potential to discover up to 50% more new species than are currently known in the region.

Keywords: endemism, hotspots, rarity, species richness, stygobionts

Introduction

Conserving biodiversity in the face of human exploitation of natural resources is among the main challenges of the coming decades (Ehrlich, 1994; Wilson, 1994), increasingly recognized at the highest political levels. Any strategy designed from this perspective has to rely primarily on knowledge about the geography of biodiversity (Balmford & Gaston, 1999). The challenge today is far beyond the conservation of individual species, and the impediment is clear: at an operational level, the priority areas for conservation are relatively well known regarding threats, but very poorly known regarding their biological value, even in Europe where conservation efforts can build on a strong background in natural history and sizeable data sets. The compilation and georeferencing of the huge amount of distributional data scattered in the literature is a first step towards mapping such spatial patterns. Accomplishing this task is mandatory to establish a reliable, non-narrative background for selecting priority areas for biodiversity conservation policies. The present paper is a contribution to this global effort, targeted at groundwater habitats of western Europe, and based on the results of the European project PASCALIS between 2002 and 2005 (Gibert, 2001; Gibert et al., 2005).

Though low compared to surface freshwater, groundwater biodiversity has attracted much attention in recent years. This is primarily due to two important features of ground waters (Gibert & Deharveng, 2002). The first is the uniqueness of its fauna whose composition is vastly different from that of surface freshwater fauna (Ske\textsuperscript{t}, 1999a). Crustaceans are the most diversified in the former, insects are the most diversified in the latter. In this respect, ground waters are more similar to marine waters than to fresh waters. The second feature is that narrow endemism is the rule. Very few species are recorded across large areas; the vast majority is limited to a few aquifers. This is again in sharp contrast with surface water, but also with marine biodiversity.

Groundwater biodiversity has been documented for many taxa (Notenboom, 1988; Jubertie & Decu, 1994, 1998, 2001; Stoch, 1995; Danielopol, Rouch & Baltanas, 2002; Camacho, 2003), as well as for a number of sites, countries or regions worldwide (Bell\textsuperscript{e}s i Ros, 1987; Dole-Olivier et al., 1994; Jubertie & Decu, 1994, 1998, 2001; Malard, Gibert & Laurent, 1997; Ske\textsuperscript{t}, 1999b; Culver & Ske\textsuperscript{t}, 2000). Groundwater biodiversity patterns were examined at various scales by Marmonier et al. (1993) and Danielopol, Pospisil & Rouch (2000). The most exhaustive and global approach regarding the distribution of groundwater biodiversity at large scale is found in Botosaneanu (1986). None of these studies, however, addressed groundwater biodiversity through extensive and detailed mapping. The first effort in this direction, based on the PASCALIS data set, is the work of Ferreira et al. (2003) on the groundwater biodiversity of France.

The aim of the PASCALIS project was more ambitious: to integrate high-resolution distribution data for all stygobiotic taxa (i.e. true groundwater animals) of a large part of western Europe, in order to derive robust spatial patterns of biodiversity at this broad scale. Spatial patterns of biodiversity at such a scale are not available for groundwater biodiversity of any region of the world, although they have been recently documented on the basis of limited region comparisons for terrestrial cave fauna (Culver et al., 2006). They are poorly documented even for surface freshwater habitats (Groombridge & Jenkins, 2002). Knowledge on these patterns is needed to understand biogeographic events that have shaped present-day biodiversity at a regional scale. They may constitute powerful integrative tools to evaluate broad-scale
ecological determinants of biodiversity and to monitor water quality. They are essential for implementing sustainable conservation strategies for living organisms.

Based upon the large data set gathered in the PASCALIS project, the main objectives of the present analysis are: (i) to map the large-scale spatial patterns of groundwater biodiversity in Europe; (ii) to detect and characterise groundwater biodiversity hotspots in Europe; (iii) to explore the taxonomic composition of local and regional assemblages and (iv) to evaluate the main gaps in our knowledge.

Methods

Geographical and ecological coverage

A data base was compiled from three sources. First, we gathered published distributional data of groundwater fauna from six European countries (Belgium, France, Italy, Portugal, Slovenia and Spain), henceforth referred to as the PASCALIS area. Secondly, the data collected in six regions surveyed in detail during the PASCALIS project were used. These regions are Wallonia (part of the Walloon karst in Belgium), Roussillon (part of the Roussillon and eastern Pyrenees in France), Jura (part of the meridional Jura in France), Krim (the Krim Massif in Slovenia), Cantabria (part of the Cordillera Cantabrica in Spain) and Lessinia (the Lessinian Mountains in Italy). Thirdly, unpublished but reliable distributional records were provided by several taxonomic specialists. Records that we were unable to georeference accurately were discarded from the analyses. We estimate that 70\% (Portugal) to 95\% (Belgium, Italy, Slovenia) of distributional data published in the literature are included in the data base.

Our analyses focussed on stygobionts, which are groundwater taxa that complete their entire life cycle in the subterranean environment, never in surface waters. Since the classification of taxa as stygobionts or non-stygobionts is not always clear, assignments of problematic taxa to one category or the other were decided after discussion among experts within the PASCALIS consortium. A few species whose assignment remained problematic were excluded from analyses. Groundwater samples that did not contain stygobionts (absence data) were ignored. Today, the PASCALIS data set is by far the most comprehensive compilation of groundwater biodiversity in Europe, and the first to allow mapping biodiversity and endemism patterns at the European scale.

Base grid for mapping

A grid of 0.2 × 0.2 ° cells (312–403 km² from north to south) was constructed in the GIS software MapInfo 7.5 (Mapinfo Company, New York, NY, U.S.A). A total of 4668 grid cells covered the six PASCALIS countries. Each non-ambiguous record in the data base was georeferenced and assigned to a cell of this grid.

Data analyses

Biodiversity was measured in five ways.

1 Species richness. Species richness is the number of identified species, including those encountered during the PASCALIS project. Species of uncertain taxonomic status were discarded. Undescribed species collected during the PASCALIS survey and validated as new by the consortium taxonomists were included in the gap analysis described below. Total expected species richness at country-level was obtained by the Jack-knife 1 estimator (Heltsh & Forrester, 1983), based on the observed species richness by sampling site:

\[
S = s + \left(\frac{n - 1}{n}\right) \times k
\]

where \( S \) = Jack-knife estimate of species richness, \( s \) = observed total number of species present in \( n \) sampling sites, \( n \) = total number of sites sampled and \( k \) = number of narrow endemics. Narrow endemics are defined here as single-cell species, i.e. species that occur in one and only one grid cell. Jack-knife 1 has been recognised as robust, accurate and easy to compute (Hellmann & Fowler, 1999; Brose, Martinez & Williams, 2003).

2 The number of genera. Species flocks, which are frequent in the highly fragmented subterranean habitats, may hugely inflate species richness and distort our view of local biodiversity. Consequently, species richness was complemented by an evaluation of the number of taxonomic lineages. In the absence of phylogenies for most taxa, this evaluation was approximated by the number of recognised genera.

3 Taxonomic uniqueness. This measure of biodiversity was evaluated as the number of genera with only a
single species in Europe and also as the number of monospecific genera, taking into account both epigean and stygobiotic species. Taxonomic uniqueness may be seen, in the absence of phylogenies, as a surrogate of local fauna relictuality.

4 The number of single-cell species. The number of species present in only one cell of the sampling grid is a measure of local endemism.

5 The range-size rarity score. Range-size rarity is defined as the inverse of the total number of cells in which a species occurs across its range (Williams, 2000). The species range considered was approximated by the number of cells that contain the species in the PASCALIS area. The range-size rarity score of a cell is defined here as the sum of range-size rarity values of all the species present in this cell. This rarity score is a measure of endemism narrowness when occupancy area is small and non-fragmented at the scale of the sampling grid, as in most rare stygobionts.

Rarity scores and the number of single-cell species are powerful measures to evaluate the biodiversity value of an area. However, the rarity score of a cell strongly relies on sampling effort in surrounding areas. Border regions of the PASCALIS area (like the Jura and Slovenia) share species with adjacent regions that are not included in the data base, leading to an underestimation of the distribution range of some species and hence an overestimation of their rarity.

Mapping biodiversity and hotspots

Biodiversity measures were calculated for each grid cell from the pool of records for the cell. A biodiversity matrix (cells × biodiversity measures) was imported in the GIS and the different biodiversity measures were mapped on the grid.

Hotspots of biodiversity – originally defined as large areas particularly rich in species and under threat (Myers, 1988; Reid, 1998; Myers et al., 2000) – are increasingly delineated with finer resolution to help set priorities for conservation at local and regional scales. In this work, 'hot-cells' were defined as grid cells having more than 25% of the number of described stygobiotic species in the richest cell of the PASCALIS area. Biodiversity hotspots were defined as groups of adjacent hot-cells without reference to environmental threats; they would therefore correspond to potential hotspots according to the original definition. Cold-cells (and cold-spots) are more difficult to characterise, as they include both cells with low richness and cells in hotspots that are poorly sampled.

Gap analyses

Because non-stygobiotic fauna is not included, the data set does not give information on whether an empty cell was sampled but had no stygobionts, or was not sampled. More generally, to what extent the richness within a given geographic unit (here a grid cell) reflects sampling effort or real diversity is difficult to establish from the data set alone. In order to evaluate the gaps, four complementary approaches were used. First, cumulative species richness was plotted against the cumulative number of sampled sites in the different PASCALIS countries. Secondly, correlations between sampling effort, approximated by the number of sampled sites with at least one stygobiont, and biodiversity per grid cell were calculated. A strong correlation over the studied region would suggest that the observed gaps were real gaps. Thirdly, the minimum gap was measured as the difference between observed species richness, and potential (expected) species richness given by the Jack-knife 1 estimate. Finally, the impact of the sampling survey of the PASCALIS project was evaluated in each surveyed region by the proportion of species not previously recorded from the region and of species new to science encountered in this region. Because sampling was intensive and similar in the six PASCALIS regions, this approach is the most relevant to assess the minimum magnitude of a past local gap, as it does not rely on theoretical estimates of potential species richness.

Data management and statistical analyses

Data were stored and managed in the relational database PASCALIS developed under 4D 2000 running on a Macintosh G5. Unless specified otherwise, it is the number of described species that was used in measures and analyses. Data for building accumulation curves, biodiversity measures and statistics were obtained from estimates 7.5 (Colwell, 2005) and from built-in procedures of the database. DataDesk 6.2.1 (Velleman, 2005) was used to calculate correlations.
Results

By mid-2006, the total number of stygobiotic species recorded in the PASCALIS area was 930, which increases to 1047 when species not yet formally described are included. The corresponding number of genera was 191 and 205 respectively. A total of 4709 sampling sites distributed in 1228 grid cells contained at least one record of described stygobionts. When undescribed new species and non-georeferenced sampling sites are included, these numbers reached 5559 and 1231 respectively.

More than 70% of the species and 50% of the genera were Crustacea, the second dominant group being Mollusca (16% and 17%, respectively) (Table 1). Insects were represented by two stygobionts in the same genus of beetles limited to south-eastern France (Siettitia) and thus represented only 0.2% of the stygobiotic fauna.

Table 1 Contribution to total stygobiotic biodiversity of groundwater stygobionts taxa with more than 10 species in the PASCALIS area

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Proportion (%)</th>
<th>Genera</th>
<th>Species</th>
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</thead>
<tbody>
<tr>
<td>Malacostraca: Peracarida</td>
<td>20.7</td>
<td>33.4</td>
<td></td>
</tr>
<tr>
<td>Maxillopoda: Copepoda</td>
<td>16.4</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>Gastropoda: Prosobranchia</td>
<td>17.4</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Malacostraca: Syncarida</td>
<td>12.7</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>Clitellata: Oligochaeta</td>
<td>10.3</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Ostracoda</td>
<td>5.6</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Tricladida</td>
<td>2.8</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Arachnida: Acari</td>
<td>7.0</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Temocephalida</td>
<td>1.9</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Biodiversity distribution

Heterogeneity of stygobiont distribution is obvious (Fig. 1). Large areas have no recorded stygobionts while some have many sites with at least one species. The pattern of grid cell richness shows strong contrasts, with a large number of single-species cells and a small number of cells with many species (Fig. 2). Of the 4668 grid cells that cover the PASCALIS area (including those intersecting the borders), only 26% (1228) have at least one recorded stygobiont (1231 with undescribed species included). Forty per cent have only one stygobiont, more than

Fig. 1 Sampling sites in the PASCALIS area where at least one stygobiont was collected.

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70% have less than three. Only 2.7% (33 cells) have more than 20 stygobionts.

Hotspots of taxonomic richness. The spatial pattern of biodiversity for described stygobiotic species is illustrated on Fig. 3. The richest cells are unevenly distributed across the PASCALIS area. The 33 hot-cells with more than 20 described stygobionts are clustered in seven hotspots (Fig. 3a): Slovenia + northeastern Italy (three groups of 4, 2 and 7 adjacent cells with 79, 50, 49, 39, 34, 32, 28, 28, 24, 23, 21 and 21 described stygobionts), the Ariège area of the Pyrenees in south-western France (two adjacent cells with 52 and 21 stygobionts), the southern Jura and Rhône valley near Lyon in eastern France (eight adjacent cells with 33, 29, 28, 28, 26, 25, 22 and 21 stygobionts), the Cévennes in southern France (two groups of 1 and 3 adjacent cells with 30, 29, 24 and 22 stygobionts), the northern Italian Alps (4 adjacent cells with 27, 24, 22 and 21 stygobionts), the Rhine valley near Strasbourg in eastern France (1 cell with 23 stygobionts) and the Cordillera Cantabrica in northwestern Spain (1 cell with 22 stygobionts). Among these hotspots, Slovenia and the Ariège area include the four richest cells with more than 40 described stygobionts (Table 2).

The number of genera with described species produces a hotspot pattern similar to that based on the number of described species (Fig. 3b). However, some discrepancies are apparent (Table 2). In particular, the richest Pyrenean cell ranks second for the number of described species, but only fourth for the number of genera.

Hotspots of endemism, rarity and taxonomic uniqueness. Endemicity and rarity values were extremely high. The species with the narrowest distribution at our study scale (present in a single cell, i.e. within <400 km²) amounted to 43% of the total number of stygobiotic species, i.e. 396 of 930 (excluding undescribed species). There were 151 (16%) two-cell endemics and 75 (8%) three-cell endemics. In total, 95% of the species (884) were present in less than 20 grid cells (i.e. in <8000 km²). Two-cell endemics had their cells contiguous (by their sides or by their angles) in 54% of the cases. Three-cell endemics had their cells contiguous in 29% of the cases.

The regions with highest rarity scores match only roughly those with highest richness (Table 2, Fig. 4 versus Fig. 3). Cells in the Jura (in spite of having their rarity scores overestimated because of their proximity to the border of the PASCALIS area) and northern Italian Alps are eliminated in the rarity score map at the 50% threshold (Fig. 4). A cell appears in northern Portugal, which was absent in the species richness map. The rarity pattern is strongly modified when undescribed species are included, and the cells in the northern Italian Alps (but not the Jura) regain their hot-cell status (Table 2).

The analysis in terms of taxonomic uniqueness showed that only 168 cells had genera with a single European species and 135 had monospecific genera, i.e. 14% and 11% of the cells containing stygobionts. Cell ranking by genera with a single European species returned a pattern very different from cell ranking by species richness. In particular, several cells with relatively low species richness from southern Italy and the Cévennes ranked much higher (Fig. 5a). Conversely, a number of species-rich cells had low scores for genera with a single European species. Twenty-four cells contained two genera with a single European species, half of which had <17 described species. Among the 33 hot-cells, ten had only one or no genus with a single European species.

The pattern of monospecific genera was different as well (Fig. 5b). The highest cell score (five genera) was found in Slovenia. Four cells, one from Slovenia and three from the Cévennes, had four genera. Five cells had three genera, one in the Pyrenees and four in Slovenia. Southern Italy had no top-ranking cells in

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**Fig. 2** Correlation between number of cells, ranked by increasing species richness, and number of stygobiotic species per cell.
Fig. 3 (a) Map of stygobiotic species numbers in $0.2 \times 0.2$ ° grid cells distributed across six European countries. (b) Map of the numbers of genera having at least one stygobiotic species in $0.2 \times 0.2$ ° grid cells distributed across six European countries.
Table 2  Hot-cells (i.e. cells with more than 20 described species) ranked by decreasing order of described-species richness

<table>
<thead>
<tr>
<th>Cell centroid coordinates</th>
<th>Hotspot</th>
<th>No. described species</th>
<th>No. genera with a single European species</th>
<th>Monospecific genera</th>
<th>Rarity of described species</th>
<th>No. sampled sites</th>
<th>No. described + undescribed species</th>
<th>Rarity of described + undescribed species</th>
<th>Genera with a single European species (monospecific genera excluded)</th>
</tr>
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<tbody>
<tr>
<td>14.3 × 45.9 itsl</td>
<td>itsl</td>
<td>79</td>
<td>40</td>
<td>7</td>
<td>4</td>
<td>23.5</td>
<td>112</td>
<td>88</td>
<td>Mononisia, Proteus, Troglodiaptomus, Velkovrhia</td>
</tr>
<tr>
<td>1.1 × 42.9 pyr</td>
<td>pyr</td>
<td>52</td>
<td>27</td>
<td>3</td>
<td>3</td>
<td>21.5</td>
<td>59</td>
<td>52</td>
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<tr>
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<td>50</td>
<td>34</td>
<td>7</td>
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<td>90</td>
<td>68</td>
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<tr>
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<td>49</td>
<td>30</td>
<td>3</td>
<td>3</td>
<td>16.2</td>
<td>24</td>
<td>52</td>
<td>Frontipodopsis, Parvidrilus, Stygomononia</td>
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<tr>
<td>13.5 × 45.9 itsl</td>
<td>itsl</td>
<td>39</td>
<td>18</td>
<td>5</td>
<td>5</td>
<td>9.5</td>
<td>65</td>
<td>39</td>
<td>Proteus, Carinurella, Limnosbaena, Phreatica, Proteus, Troglodiaptomus</td>
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<td>20</td>
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<td>34</td>
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<th>Cell centroid coordinates</th>
<th>Hotspot</th>
<th>No. described species</th>
<th>No. genera with described species</th>
<th>No. genera with a single European species</th>
<th>Monospecific genera</th>
<th>Rarity of described species</th>
<th>No. sampled sites</th>
<th>No. described + undescribed species</th>
<th>Rarity of described + undescribed species</th>
<th>Monospecific genera</th>
<th>Genera with a single European species (monospecific genera excluded)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5 × 46.1 jura</td>
<td>26</td>
<td>17</td>
<td>1</td>
<td>0</td>
<td>3.0</td>
<td>54</td>
<td>33</td>
<td>5.1</td>
<td>Cavernocypris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5 × 46.3 jura</td>
<td>25</td>
<td>18</td>
<td>2</td>
<td>1</td>
<td>2.0</td>
<td>68</td>
<td>32</td>
<td>4.3</td>
<td>Cavernocypris, Faucheria, Henrignardia, Herauliella, Kieferiella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.7 × 43.7 cev</td>
<td>24</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>6.7</td>
<td>11</td>
<td>24</td>
<td>6.7</td>
<td>Cavernocypris, Cavernocypris</td>
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</tr>
<tr>
<td>10.9 × 45.7 itn</td>
<td>24</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>3.7</td>
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<td>34</td>
<td>8.5</td>
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<tr>
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<td>24</td>
<td>16</td>
<td>2</td>
<td>2</td>
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<td>8</td>
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<td>18</td>
<td>3</td>
<td>3</td>
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<td>8</td>
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<td>5.3</td>
<td>Niphargopsis, Troglochaetus</td>
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</tr>
<tr>
<td>7.7 × 48.5 rhin</td>
<td>23</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>8.2</td>
<td>17</td>
<td>23</td>
<td>8.2</td>
<td>Niphargopsis, Troglochaetus</td>
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<tr>
<td>−3.5 × 43.3 cant</td>
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<td>16</td>
<td>2</td>
<td>0</td>
<td>7.0</td>
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<td>34</td>
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<td>2</td>
<td>2</td>
<td>4.8</td>
<td>27</td>
<td>22</td>
<td>4.8</td>
<td>Herauliella, Niphargopsis</td>
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<tr>
<td>11.3 × 45.5 itn</td>
<td>22</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>2.0</td>
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<td>33</td>
<td>8.5</td>
<td>Troglochaetus</td>
<td></td>
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</tr>
<tr>
<td>4.9 × 45.7 jura</td>
<td>22</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>3.7</td>
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<td>22</td>
<td>3.7</td>
<td>Niphargopsis, Carinula, Proteus, Troglochaetus</td>
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<tr>
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<td>3</td>
<td>3.6</td>
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<td>21</td>
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<td>Parvidrilus</td>
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<tr>
<td>1.3 × 42.9 pyr</td>
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<td>12</td>
<td>1</td>
<td>1</td>
<td>5.3</td>
<td>17</td>
<td>21</td>
<td>5.3</td>
<td>Niphargopsis</td>
<td></td>
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<tr>
<td>11.1 × 45.5 itn</td>
<td>21</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>3.2</td>
<td>47</td>
<td>36</td>
<td>11.8</td>
<td>Vandelibathyrella</td>
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<tr>
<td>5.3 × 46.1 jura</td>
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<td>16</td>
<td>1</td>
<td>1</td>
<td>1.6</td>
<td>23</td>
<td>23</td>
<td>1.9</td>
<td>Niphargopsis</td>
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<tr>
<td>13.5 × 46.1 itsl</td>
<td>21</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>3.4</td>
<td>42</td>
<td>21</td>
<td>3.4</td>
<td>Niphargopsis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

cant, Cordillera Cantabrica; cev, Gévénes; itn, northern Italy; itsl, northeastern Italy and Slovenia; jura, Jura and Rhône valley near Lyon; pyr, Ariège region in the Pyrenees; rhin, Rhine valley near Strasbourg.
spite of its high number of genera with a single European species, because several of these genera have also marine species widely distributed outside Europe, and hence are not monospecific.

In spite of important differences in the ranking of hotspots based on different biodiversity measures, Spearman rank correlations calculated on the 33 hot-cells are high between number of stygobiotic species and number of genera ($r_s = 0.82$, $P < 0.01$), and between number of genera with a single European species and number of monospecific genera ($r_s = 0.80$, $P < 0.01$). They are still significant between number of stygobiotic species or genera and number of monospecific genera or genera with a single European species ($r_s = 0.51–0.56$, $P < 0.01$).

**Evaluating gaps**

**Gaps at the country level.** Accumulation curves were built for the whole PASCALIS area (Fig. 6) and separately by country (Fig. 7). Species richness increased rapidly, and in a very similar way, with increasing number of sampled sites for all countries pooled or considered separately, except for Belgium. The accumulation curve for Slovenia had initially a slightly steeper slope, but was less steep than curves for the other regions at about 150 sampling sites (Fig. 7a,b). The Jack-knife 1 estimates exhibited a similar pattern. Magnitude of the expected gain in species diversity with further sampling (Fig. 7c,d, Table 3) calculated for the different countries with the Jack-knife 1 estimator ranged from 30% to 83%, and was 39% for the data from all countries pooled together.

The curves of single-cell species (Fig. 7e,f) followed three clear-cut patterns: a plateau was reached rapidly, with slow or no increase in the number of single-cell species after 15 sampling sites (Belgium), a plateau was reached late, with a fast increase until 100–150 sampling sites, then a sudden slow-down (Slovenia), or a plateau was not reached (France, Italy, Portugal and Spain). The asymptotic patterns indicate that no or few additional single-cell species are expected when additional samples are taken. The monotonously increasing pattern indicates that further sampling effort should lead to many discoveries of single-cell species.

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**Fig. 4** Map of stygobiotic rarity scores in $0.2 \times 0.2$° grid cells distributed across six European countries.
Fig. 5 (a) Map of the number of genera with a single stygobiotic species known in Europe in 0.2 × 0.2° grid cells distributed across six European countries. (b) Map of the number of stygobiotic monospecific genera in 0.2 × 0.2° grid cells distributed across six European countries.
Taxonomic richness versus sampling effort in hot-cells. Sampling effort, approximated by the number of sampling sites per cell with at least one stygobiont, was compared to the number of species. Based on the 33 hot-cells of Table 2, it proved to be a poor predictor of taxonomic richness measures based on described species. Spearman’s rank correlation coefficients ($r_s$) were 0.26 with the number of described species, 0.14 with the number of genera and $-0.02$ with rarity. When undescribed species were included, the correlation with species number increased to 0.68 and became significant at $r_s = 0.01$.

Gaps as reflected in the impact of intensive sampling on biodiversity. The samples collected during the PASCALIS survey concerned 57 grid cells distributed in six regions (Table 4). The number of species added by this survey and the proportion of new species among them reflects primarily the magnitude of local gaps in knowledge about groundwater fauna before the survey, since sampling effort was roughly the same in all regions. Both measures point to very important gaps in all PASCALIS regions except Wallonia (Table 4). Species number increased from 55% to 168%. It was highest in Lessinia, lowest in the Krim, Jura and Roussillon. The proportion of new species ranged from 0% to 51%. It was highest in Cantabria and Lessinia, lowest in the Krim and Jura and zero in Wallonia. Spearman correlations between the biodiversity measures in Table 4, calculated on the 57 cells of the PASCALIS survey, gave a large range of values: 0.77 between the number of species before and after the PASCALIS survey, 0.71 between the number of species after the PASCALIS survey and the number of new species discovered during the PASCALIS survey, 0.49 between the number of species before the PASCALIS survey and the number of new species discovered during the PASCALIS survey. However, they were always significant at $P < 0.01$, suggesting that the overall biodiversity patterns changed little as a result of the PASCALIS survey at the scale of grid cells.

Discussion

In spite of some limitations, the PASCALIS data base assembled from a huge amount of scattered data and a large-scale survey across Europe offers a wealth of information. The present analysis of these data raises five issues relevant to major biological or conservation questions. They will be commented on below.

Crustacea versus Insecta in ground water

It has been stressed repeatedly that ground water strongly differs from surface water in the relative importance of Crustacea and Insecta (Stoch, 1995; Gibert & Deharveng, 2002; Ferreira et al., 2003). The pattern in Europe shown in the PASCALIS data set is clear: 757 species and 122 genera of stygobiotic Crustacea compare with only two species of dytiscid beetle of the genus *Siettitia*. Elsewhere in the world, only western Australia (Watts & Humphreys, 2003) has a large number of stygobiotic insects, all from the single beetle family Dytiscidae. This paucity of stygobiotic insects in spite of the dominance of insects in epigean aquatic assemblages is puzzling for two reasons. First, it does not match the pattern of terrestrial habitats, where insect diversity contributes in the same proportion to total diversity in subterranean and epigean assemblages. Secondly, facultative subterranean insect larvae of various groups are common in ground water, but none has evolved towards an obligate subterranean life-style. Evolutionary pathways to groundwater colonisation therefore appear to involve adaptive capabilities that are...
Fig. 7 Stygobiotic species accumulation curves by country. Left panels: all sampling sites included; right panels: enlargement of the left panels for the first 200 sampling sites; bold red line: Belgium; blue thin line: France; orange thin line: Italy; purple thin line: Slovenia; bold black line: Portugal; green thin line: Spain.
The geographic distribution of stygobiont biodiversity in Europe is extremely heterogeneous, with a few hotspots, as illustrated in the maps provided here (Fig. 5) and in other contributions of this special issue (Dole-Olivier et al., 2009; Malard et al., 2009; Michel et al., 2009; Stoch et al., 2009). Only one-quarter of the 0.2° x 0.2° grid cells covering the PASCALIS area have yielded stygobionts so far. Among them, only 33 have more than 25% of the number of species found in the richest cell and only four have more than 50% (Fig. 3a). Furthermore, there is a striking contrast between the outstanding richness of some cells and the low richness of adjacent cells in biogeographically homogeneous regions (e.g. the Pyreneo-Cantabric range). Finally, the relatively frequent disjunction in endemic species distribution area is most probably caused by local sampling gaps. This suggests that highly uneven sampling efforts contribute to the observed heterogeneity. Nevertheless, there is evidence for causes of heterogeneity other than sampling effort: (i) the correlation between species richness and sampling effort per cell for the richest cells of Europe was not particularly strong; (ii) sampled regions outside of the Mediterranean region never yielded species richness and rarity scores as high as the Slovenian, northern Italian or Pyrenean hotspots; (iii) the intensive sampling surveys of the PASCALIS project broadly confirmed our expectations based on previous sampling in the studied area in that all sampled regions except Wallonia had a rich subterranean fauna; (iv) the most important groundwater biodiversity hotspots are located in the European biodiversity ridge documented by Culver et al. (2006) for terrestrial subterranean fauna and (v) at a broad scale, the cells containing a species, even if not contiguous, nearly always cluster.

### Table 3 Groundwater biodiversity measures in six European countries

<table>
<thead>
<tr>
<th>Country</th>
<th>No. sampled cells</th>
<th>No. sampled sites</th>
<th>No. described species</th>
<th>No. species predicted by Jack-knife 1</th>
<th>Additional species expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>Belgium</td>
<td>17</td>
<td>155</td>
<td>33</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>France</td>
<td>566</td>
<td>1712</td>
<td>320</td>
<td>434</td>
<td>114</td>
</tr>
<tr>
<td>Italy</td>
<td>337</td>
<td>1580</td>
<td>288</td>
<td>394</td>
<td>106</td>
</tr>
<tr>
<td>Portugal</td>
<td>24</td>
<td>34</td>
<td>48</td>
<td>88</td>
<td>40</td>
</tr>
<tr>
<td>Slovenia</td>
<td>54</td>
<td>491</td>
<td>183</td>
<td>246</td>
<td>63</td>
</tr>
<tr>
<td>Spain</td>
<td>241</td>
<td>737</td>
<td>216</td>
<td>308</td>
<td>92</td>
</tr>
<tr>
<td>All</td>
<td>1228</td>
<td>4709</td>
<td>930</td>
<td>1291</td>
<td>361</td>
</tr>
</tbody>
</table>

The number and proportion of expected additional species refers to estimates of species discovered if further samples are taken.

### Table 4 Indicators of the magnitude of knowledge gaps in stygobiotic biodiversity in the sites sampled during the PASCALIS field surveys

<table>
<thead>
<tr>
<th>PASCALIS region</th>
<th>Observed number of stygobiotic species</th>
<th>No. species found in the PASCALIS survey</th>
<th>Proportion of species added by the PASCALIS survey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before PASCALIS</td>
<td>After PASCALIS</td>
<td>All</td>
</tr>
<tr>
<td>Cantabria</td>
<td>28</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>Jura</td>
<td>43</td>
<td>67</td>
<td>48</td>
</tr>
<tr>
<td>Krim</td>
<td>86</td>
<td>134</td>
<td>78</td>
</tr>
<tr>
<td>Lessinia</td>
<td>31</td>
<td>83</td>
<td>70</td>
</tr>
<tr>
<td>Roussillon</td>
<td>44</td>
<td>68</td>
<td>40</td>
</tr>
<tr>
<td>Wallonia</td>
<td>14</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>All</td>
<td>217</td>
<td>379</td>
<td>262</td>
</tr>
</tbody>
</table>

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Ranking and prioritising hotspots

Ranking hotspots with respect to their biodiversity is a powerful approach to prioritise areas for conservation. Ranking, however, depends on the way biodiversity is assessed. In the present study, we ranked hot-cells rather than hotspots. Pooling species of the different hot-cells constituting a hotspot would lead to a more accurate assessment of the richness of hotspots. However, the ability to compare biodiversity of areas of similar surface would be lost. Ranking would also differ significantly from that based on grid-cell biodiversity. Thus, considering both hot-cell and hotspot approaches yields the maximum of information.

Biodiversity measures were significantly correlated for the 33 hot-cells defined from our data set, but the richest cells often ranked differently when defined by stygobiota species number (the traditional approach), by narrowest-range species number (endemicity), by rarity scores or by number of monospecific genera (taxonomic uniqueness). Taxonomic uniqueness, rarity and endemicity should therefore be evaluated separately in spatial biological assessments, as none is accurately reflected by species richness. Narrowly distributed species that belong to monospecific genera combine the most valuable biological features, i.e. high vulnerability due to small ranges and high number of unique evolutionary features, often associated with extreme adaptive characters. Such taxa are high priority in conservation efforts, so that retaining their small occupancy areas in the areas selected for biodiversity conservation should be mandatory.

Improving biodiversity maps

Mapping the distribution of biodiversity on a grid as we did here provides invaluable spatial information at coarse resolution and allows easy comparisons between taxonomic groups. However, grid cells have no direct biogeographical or environmental meaning and hence are of limited relevance for ecological or conservation issues. Ferreira (2005), in a study of stygobiota diversity in France, used catchments and aquifers instead. This is probably the most adequate way to map stygobiota biodiversity. However, at the European scale this approach is limited by both a lack of data and considerable uncertainty about locations given in the literature. Grid-based and basin-based mapping of stygobiota biodiversity will therefore have to co-exist for some time, but the catchment-based approach should be favoured whenever possible.

Evaluating gaps

Evaluating the pattern of existing gaps is essential for estimating the accuracy and relevance of many studies in community ecology. Analysing gaps can also help assess the room for progress in our knowledge of biodiversity (Christman & Culver, 2001; Krow & Culver, 2001). Therefore, a first step towards understanding, monitoring and preserving groundwater assemblages is to evaluate the existing taxonomic and geographical gaps.

The magnitude of these gaps were estimated in different ways from our data set. Accumulation curves of expected species richness based on sampled sites as spatial units, and differences between estimated and observed species richness at country scale, both underestimate the gaps, as they rely on the Jack-knife 1 which, as all classical estimators, underestimates species richness, sometimes very significantly (Ugland, Gray & Ellingsen, 2003; Ugland & Gray, 2004). Sampling effort, on which the number of collected species is locally dependent, was found to be poorly related to the number of species at the European scale. Hence, comparing local sampling efforts alone would generate only limited information about gaps in biodiversity. Differences between observed richness before and after the PASCALIS surveys provide minimal but robust estimates of previous gaps in the knowledge of distributional patterns. Similarly, the numbers of new taxa discovered during the PASCALIS surveys are minimal estimates of previous taxonomic gaps. Both kinds of gaps were found to be more important in regions where biodiversity was higher. Therefore, at the current state of knowledge, it would be more productive to sample hotspots than cold spots to fill taxonomic and distributional gaps in the knowledge of European groundwater biodiversity.

The unevenness of biodiversity distribution and knowledge gaps across Europe are well illustrated at both the country and PASCALIS region levels. The expected increase in species richness from further samples ranges from 30% for Belgium to 83% for Portugal. The proportion of new stygobiota species contributed by the PASCALIS surveys was of the same order of magnitude, ranging from 29% in the Krim (0%
in Wallonia) to 51% in Cantabria. The overall increase in species richness resulting from the PASCALIS surveys ranged from 55% for the Roussillon to 168% for Lessinia. These values are much higher than those expected for any other aquatic ecosystem in Europe. They highlight the need to prioritise the study of groundwater biodiversity for a more balanced overview of European aquatic biodiversity.

To this end, a first step would be to strengthen the current data base in six major respects:

1. Improve the spatial coverage by including parts of biogeographical entities adjacent to countries covered by the PASCALIS project, especially the Swiss part of the Jura, the Swiss and Austrian parts of the Alps and the whole Rhône basin.
2. Lower the spatial coverage heterogeneity by sampling obvious geographical gaps, such as most parts of France, of the Iberian Peninsula, and of Italy.
3. Determine whether apparent cold spots are real low-diversity areas, or have been insufficiently sampled, as can be postulated from comparison with other biological data sets for central and northern France, respectively, and for parts of Spain and the south-western Alps (Williams et al., 2000).
4. Refine hotspot limits by sampling cells at their margins.
5. Document the distribution beyond the limits of the PASCALIS area of each stygobiotic species present in this area, in order to improve the current rarity and endemism estimates.
6. Favour biodiversity assessment at the catchment level as the most adequate geographic unit for subterranean stygobiologic fauna.

Acknowledgments

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References


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