



Protocols for the **AS**essment and **C**onservation of **A**quatic **L**ife **I**n the **S**ubsurface

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**PASCALIS D8 DELIVERABLE**  
**for Workpackage 7 :**  
**Statistical analyses and**  
**identification of indicators**

**Report on a set of biodiversity assessment tools including :**  
**- a standard protocol for regional biodiversity assessment**  
**- a set of indicators and predictors of groundwater biodiversity**

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## **INTRODUCTION :**

### **➤ MAIN TARGET AND INPUTS TO THE WORKPACKAGE :**

The main objectives of the PASCALIS WP7 are the statistical analysis of the input datasets (environmental parameters and species lists), the optimization of the field sampling protocol for biodiversity assessment, and the selection of indicators and predictors of biodiversity.

The input to the workpackage derive from WP3 (taxonomic list of PASCALIS species), WP5 (data set of environmental attributes of PASCALIS selected regions), and WP6A (data set of species lists for each sampling site).

Data were collected following a standardized sampling procedure in 6 regions distributed in southern Europe (WP4: Sampling design): the Walloon karst (Belgium), the meridional Jura (Eastern France), the Roussillon region (France), the Cantabria region (Spain), the Lessinian mountains (Italy), and the Krim massif (Slovenia). In each region, the sampling strategy involved the collection of stygobiont species and the measurement of environmental variables at 192 sites, which were evenly distributed among 4 habitats (1 - unsaturated zone of karst aquifers; 2 - saturated zone of karst aquifer; 3 - hyporheic zone, 4 – phreatic groundwaters in unconsolidated sediments) of 4 hydrogeographic basins of comparable area.

The environmental data set was provided by WP5 leader; the tables included for each sampling site the values of the following environmental variables: longitude, latitude, elevation, hydrogeological variables (i.e. geology and hydrological connectivity), physico-chemical variables (i.e. temperature, pH, specific conductance, dissolved oxygen, calcium, magnesium, nitrates, phosphates), land cover (following Corine Land Cover, IV level), and distance from the Wurmian glacier borders. Details concerning the measurement of variables can be found in the WP5 deliverable.

The species data set was provided by WP6A leader; the tables reported for each site the presence (1) or absence (0) of stygobiotic species. The following taxonomic groups were identified at the species level and included in the analysis: Annelida (Polychaeta and Oligochaeta), Gastropoda, Acari, Coleoptera and Crustacea, including Cladocera, Copepoda (Calanoida, Cyclopoida, Harpacticoida), Isopoda, Amphipoda, Bathynellacea and Thermosbaenacea.

Finally, the complete data set of all the stygobiotic species up to now known from the PASCALIS countries and Portugal was provided by the WP3 leader; it was compiled by the taxonomists involved in the PASCALIS project and included 830 species.

➤ **ORGANIZATION OF THE WORKPACKAGE :**

The workpackage deliverable was organized into 5 sections.

- 1) ***Section 1 - Optimization of field sampling strategy*** : Statistical analyses (SACs – Species Accumulation Curves; DCOA – DeCentred Correspondence Analysis) were applied to the species data sets in order a) to establish the number of sampling sites needed to obtain an accurate estimate of species richness in a given region; b) to test the efficacy of the stratified sampling strategy proposed in the PASCALIS manual for the assessment of stygobiotic diversity; c) to improve the sampling strategy in the different regions.
- 2) ***Section 2 - Biodiversity and environment relationships*** : Multivariate statistical analyses (OMI - Outlying Mean Index; PCA – Principal Component Analysis; RDA – Redundancy analysis) were applied to the environmental and species data sets in order to explore the relationship between the structure of obligate-groundwater assemblages and environmental gradients at a regional and European (PASCALIS countries) scale.
- 3) ***Section 3 – Partition of groundwater biodiversity*** : Additive partitioning of groundwater species diversity across nested spatial scales – aquifers, basins, and regions – using species-richness data collected in the six European regions was performed; a two-level nested analysis of variance was used to test the results.
- 4) ***Section 4 – Selection of biodiversity indicators*** : Sets of environmental parameters, species and higher level taxa were selected as indicators of biodiversity. The spatial scale ranges from the regional to the European level. Multiple regression models and statistically sound information criteria were used to select the indicators and assess their predictive power of species richness of groundwater assemblages.
- 5) ***Section 5 - Proposal of a method for assessing the conservation value of species*** : A standard method to build conservation indices based on the information stored in the WP3 database and on the grid cells used to map the distribution of species over Europe was developed. Indices to assess degree of endemism, range-size rarity, habitat selection and taxonomic isolation (included relictuality) were proposed. Mean values of endemism, rarity, and taxonomic isolation were used to assign a cumulative conservation value to each of the 830 species included in the database.

# **1 SECTION 1 : OPTIMISATION OF FIELD SAMPLING STRATEGY :**

## **SUMMARY :**

Estimation of species richness is an urgent and important step in conservation biology. The question is how to estimate the total number of species in front of the spatial and temporal variations. The aim of this study is to refine the sampling strategy proposed through PASCALIS protocol to obtain an accurate estimation of the stygobiont species richness at the regional scale.

Species Accumulation Curves (SACs) were built to evaluate the effect of the sample size (number of sites) on species richness estimation. These curves did not reach the saturation (asymptote level) in 4 of the 5 regions, in spite of the high sampling effort performed. This may be due to the rarity of most of the stygobiotic species: approximatively 50% of the species occurred in less than 3% of the sites.

The distribution of species through the different units of the sampling hierarchy was studied using the Decentred Correspondence Analysis (DCOA). The results showed that a stratified sampling in the karst and porous strata is an efficient strategy for the Walloon, Lessinian and Krim regions. For the Jura and Cantabrica regions, the stratified sampling scheme can be improved regarding other variables as source of heterogeneity such as, for the Jura, distance to the glacier and altitude. In all the regions and especially in the Cantabrica region an increased sampling effort is recommended for future monitoring studies of groundwater species richness.

## **1.1 INTRODUCTION :**

Groundwater fauna may be sampled using a large panel of techniques available (see PASCALIS Sampling Manual, Malard *et al.*, 2002). Unfortunately, critical problems arise when researchers have to assess species richness values in different kinds of groundwater habitats to cope with conservation issues. Planning an efficient sampling strategy is strongly constrained by difficulties in accessing the subterranean realm, especially in deep phreatic habitats. As a consequence, sometimes it may not be possible to distribute samples sites where it would be necessary, but only where access is possible through a limited number of outputs (springs, resurgences) or few “windows” (caves, wells).

Nevertheless the assessment of groundwater biodiversity is of paramount importance to help policy makers and propose a guide for groundwater protection. Apart a few attempts in particular habitats such as works by Boulton *et al* (2003, 2004) in the porous aquifer, up to now no sampling strategy have been proposed or tested to solve such a basic question. The sampling strategy applied in PASCALIS for the first time is based on a hierarchical scheme developed for assessing species richness in order to evaluate differences in species composition between different habitat units, basins and regions (Malard *et al.* 2002).

In order to evaluate and improve the efficiency of the sampling strategy proposed in the PASCALIS project, this section aims to answer the following questions:

- 1) How many sampling sites are needed to obtain an accurate estimate of species richness in a region?
- 2) Is the stratified sampling strategy proposed in PASCALIS project an appropriate protocol for a correct assessment of stygobiotic diversity ?
- 3) How can the sampling strategy be improved in the different regions ?

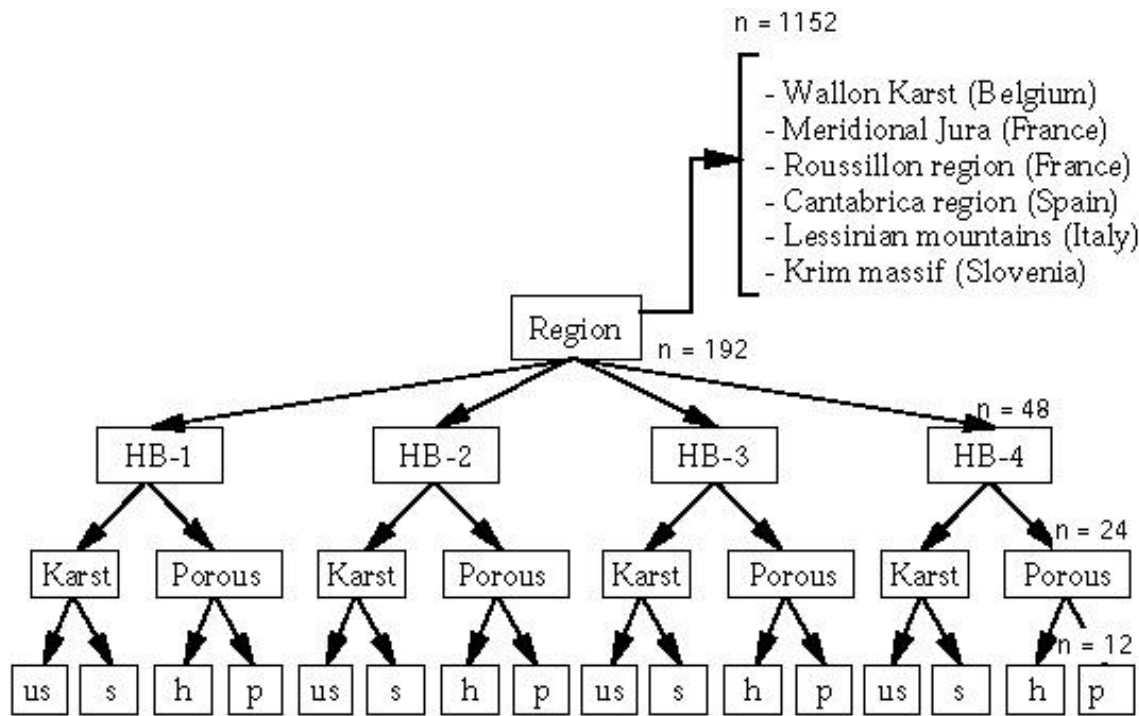
## **1.2 MATERIALS AND METHODS :**

### ➤ ***Hierarchical sampling scheme :***

The sampling scheme used within the framework of PASCALIS project is widely discussed in the sampling manual (Malard *et al.*, 2002). A region (level 1) contains several hydrogeographic basins (level 2) of which only 4 are retained for sampling. Each basin encompasses two distinct types of ground waters (level 3) that either flow in porous sediments (**P**) or in karstified rocks (**K**). At the last level of the hierarchy (level 4), subsurface water flowing through porous sediments has further been divided into 2 units: the hyporheic zone



(h) and phreatic zone (p). Similarly, we distinguished between subsurface water flowing in the vadose zone of karst aquifers (i.e., the unsaturated zone, us) and that flowing in the phreatic zone of karst (i.e., the saturated zone, s). Twelve sites were selected in each unit of the level 4 (h, p, us, s), corresponding to a total of **192 sampling sites** for the four hydrogeographic basins of a region (i.e., 12 sites x 4 habitats x 4 basins).



HB: Hydrogeographic basin; Porous: unconsolidated sediments; s: saturated zone of the karst; us: unsaturated zone of the karst; h: hyporheic zone; p: Groundwater flowing in unconsolidated sediments; n = number of sampling sites for each unit.

➤ Database :

Species richness is estimated using presence-absence data from the data sets assembled following the sampling protocol. The data collected in the Roussillon region were not suitable to be processed in this section, because of the lack of identification at the species level for two very speciose groups of copepods (Harpacticoida and Cyclopoida). This data set was ruled out for this section. Species codes used in this section are listed in *appendix 1*. The exact number of sites is provided in the table below:

Region	Number of sites	Number of species
Walloon karst (Belgium)	202	34
Meridional Jura (France)	192	67
Cantabrica (Spain)	189	61
Lessinian mountains (Italy)	197	89
Krim massif (Slovenia)	187	105

➤ **Statistical analyses :**

Species Accumulation Curves (SACs) were calculated making 100 times randomizations without replacement and the mean and the standard deviation computed for each step of the process using the EstimateS package (Colwell, 1997).

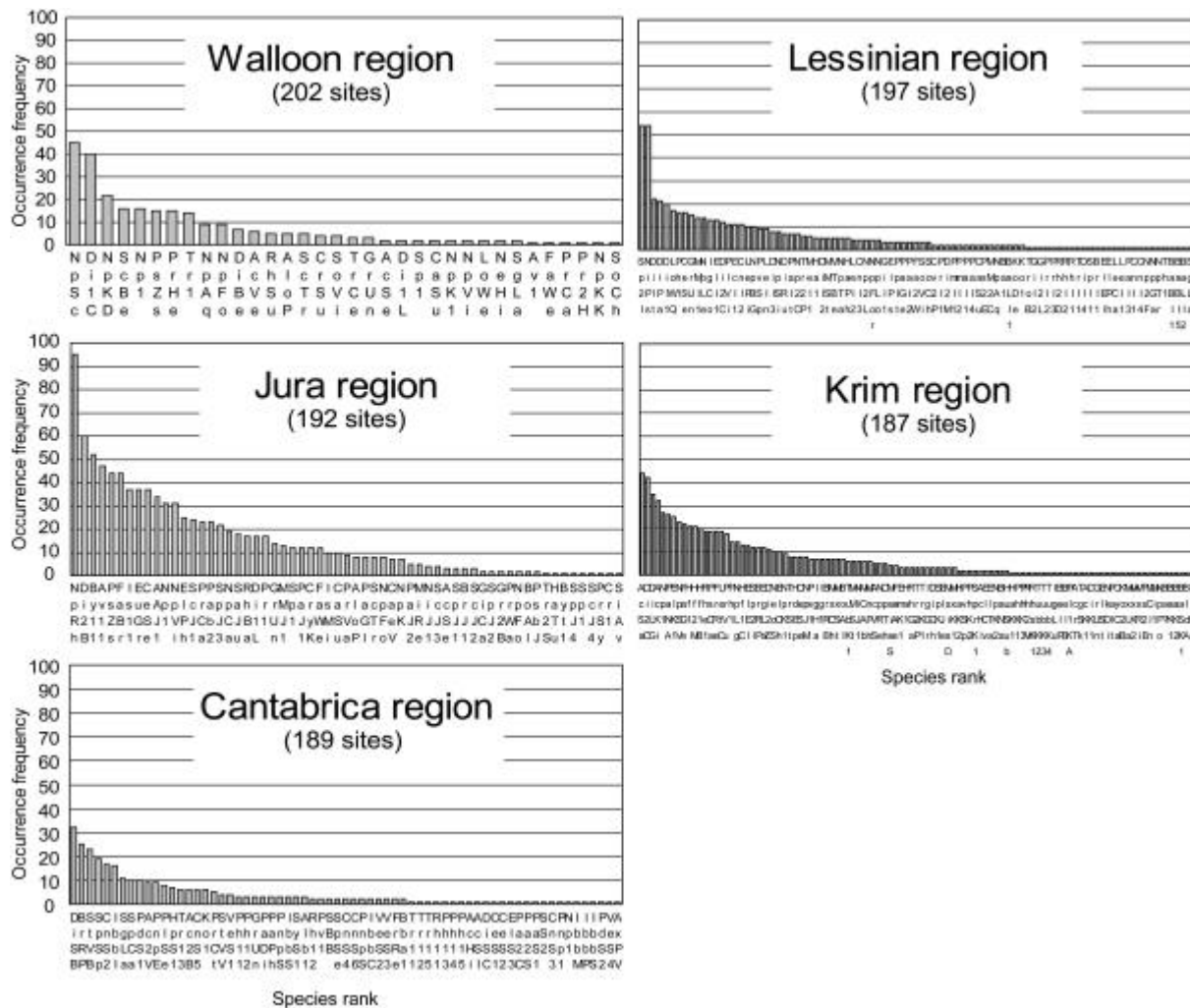
In order to describe the best sampling strategy Decentred Correspondence Analysis (DCOA) was performed using the ADE-4 statistical software (Thioulouse et al. 1997). This analysis, which belongs to the correspondence analysis on model group, allows to take into account as well the spatial-temporal heterogeneity as the heterogeneity of sampling effort (Dodélec et al. 1995). In this analysis the reference point does not correspond to the most abundant species (as it is the case in classical COA) but measures the distance of the species from a uniform distribution (i.e., species occurring in all sites). To explore the effect of the different hierarchical units on species richness patterns and hence on the sampling strategy, analysis between and within groups were done. The hierarchical units taken into account were: basins (between and within basins analysis), karst (K) and porous (P) strata (between and within KP analysis), basins and KP strata coupling (between and within Bas\_KP analysis) and unsaturated (us), saturated (s), hyporheic (h) and phreatic (p) zones (between and within usshp analysis). Only the most significant results are shown in the following section.

### **1.3 RESULTS :**

#### **1.3.1 General results :**

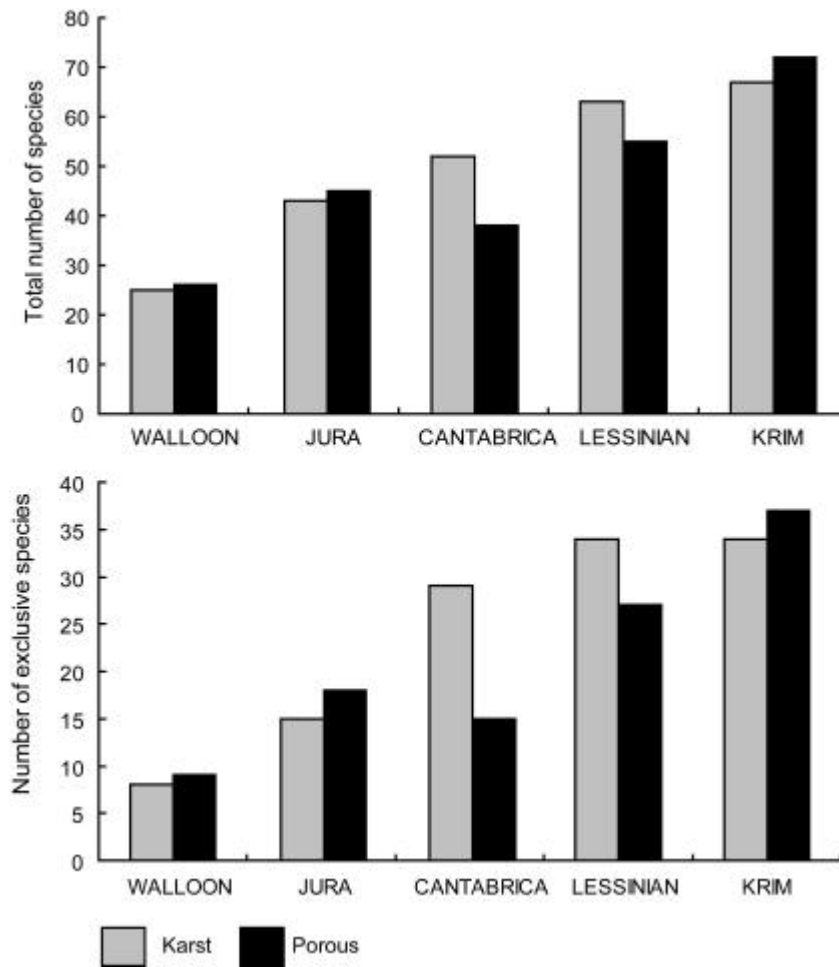
The number of collected species varies from 34 (within 202 sites) in the Walloon region to 105 species (within 187 sites) in the Krim region. The occurrence frequency of stygobionts evidenced the high percentage of rare species in groundwaters (Figure 1). A low frequency of occurrence is observed in all the regions studied: most of the species occurred in less than 10% of the sites. Only 8, 7, and 17 species respectively were collected in more than 10% of the sites in the Walloon, Cantabria, and Lessinia respectively, indicating the large dominance

of rare species within these regions while 25 and 28 species were collected in more than 10% of the sites in the Krim and Jura regions respectively. The meridional Jura gathers the most frequent species with 12 species collected in more than 30% of the sites. The number of empty samples, representing about 40% of the sites within the Walloon and Cantabria, is another proof of the rarity of stygofauna in those regions.

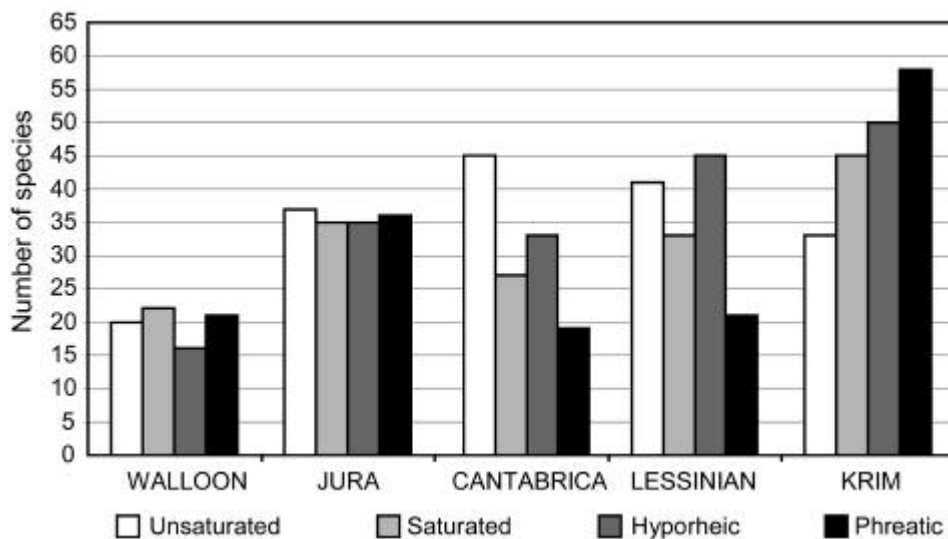


**Figure 1 :** Occurrence frequency of stygobiotic species from five regions of PASCALIS

The results of hierarchical sampling (K versus P) showed that both the total number and the exclusive number of species in the porous stratum was higher than in the karst stratum from the Walloon, Jura and Krim regions and conversely from the Cantabrica and Lessinian regions (Table 1, Figure 2A & B). The contribution of the four different zones (**us**, **s**, **h**, **p**) to the species richness was different for each region (Figure 3). Differences between these zones were less marked within the Walloon and Jura regions.

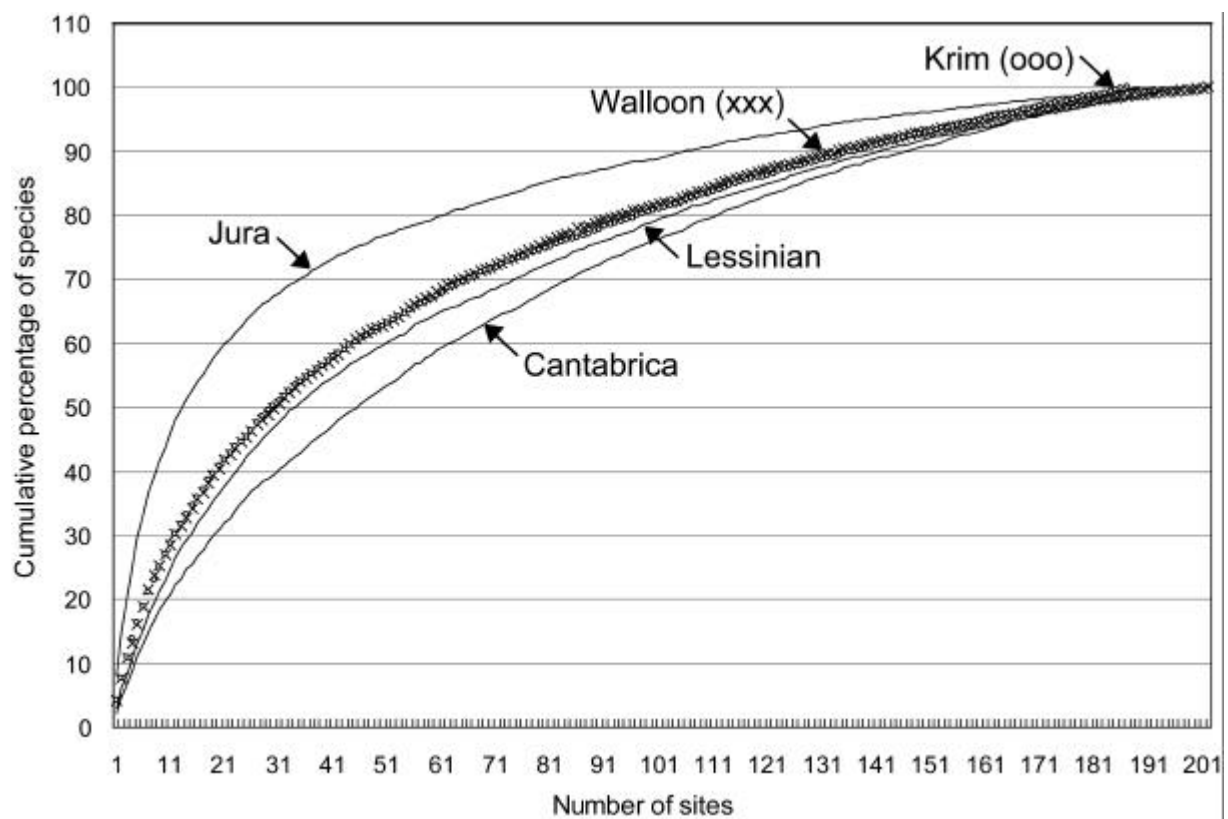


**Figure 2:** Occurrence of stygobionts from five regions of Europe. Upper panel: total species in the karst and porous strata. Lower panel: exclusive species of the karst and porous strata.



**Figure 3:** Occurrence of species in the unsaturated, saturated, hyporheic and phreatic zones from five regions of Europe.

In spite of the high sampling effort, the Species Accumulation Curves (SACs) do not reach the asymptotic level in 4 of the 5 regions (only a quasi-plateau is observed for the Jura) (Figure 4). The curves obtained for Walloon and Krim are very similar. Lack of saturation may be explained in three ways: 1) the number of samples is too low, 2) there is an important number of sites without stygobiotic species and 3) the sampling scheme did not comprises the overall sources of heterogeneity. A different stratification sampling taking into account other sources of heterogeneity could be studied. In the Jura region, 61 sites are needed to get 80% of the observed species richness, while more than 100 and 110 sites respectively are necessary for the Lessinian and Cantabrica regions.



**Figure 4:** Species accumulation curves for five regions in Europe.

Region	SPECIES							DCOA				Sampling strategy suggested
	Total	Karst		Porous		Exclusive		Be Bas	Be KP	Be BasKP	Be usshp	
		us/t	s/t	h/t	p/t	K	P					
WALLOON	34	2/20	2/22	4/16	4/21	8	9	NS	NS	NS	NS	Random sampling <sup>1</sup>
JURA	61	5/37	4/35	5/35	5/36	15	18	NS	S	S	S	Stratified sampling using K-P strata for all groups or alternatively stratifying in the K strata 3 groups: 1) B1, 2) B4 and 3) B3-B2 and in the P strata 2 groups: 1) B2 and 2) B1-B3-B4. Stratified sampling is although possible using us-s-h-p strata.
CANTABRICA	67	17/45	4/27	10/33	1/19	29	15	S	S	S	NS	Stratified sampling using K-P strata for two different groups: 1) B1, B2 and B3 together and 2) B4. An alternative may be to stratify using K-P strata grouping all basins and stratify on us-strate of Basin1 and h-strate of Basin 4.
LESSINIAN	89	25/41	5/33	20/45	6/21	34	27	NS	NS	NS	S	Stratified sampling using K-P strata or alternatively to stratify in particular zones. Stratified sampling is although possible using s and p together, h, us strata.
KRIM	105	13/33	18/45	8/50	15/58	34	37	NS	S	NS	S	Stratified sampling using K-P strata or alternatively stratifying at us-strate, s-strate and h-p strata together

us/t, s/t, h/t and p/t: Number of species found exclusively in the zone / total species in the zone

<sup>1</sup>If species related with isolated groups in the DCOA are important, stratified sampling on those sites is recommended or alternatively to follow current protocol increasing the sampling size. See comments in the Walloon Section.

**Table 1:** Suggested sampling strategies based on the results of Decentred Correspondence Analysis (DCOA).

### 1.3.2 Optimisation of the field sampling strategy for the Walloon Region (Belgium) :

As a rule, the number of occurrences for stygobiotic species is always low (Figure 1): the absolute frequency of occurrence is less than, or equal to, 3% of sites for 50 % of the species. The two most frequent species (*Niphargus schellenbergi* and *Diacyclops clandestinus*) were collected within less than 45% and 40% of the sites respectively; 26 species were collected within less than 10% of the sites and 6 species within 1% only. In addition, stygobionts are completely absent in 75 sites on a total of 202. These low values of occurrence could explain why different effects of different strata on the structure of stygobiotic assemblages are not statistically detectable.

On a total of 34 species observed in the Walloon Region, 25 and 26 species were present in the karst and porous strata, respectively, and 8 to 9 species were exclusive to each of the two

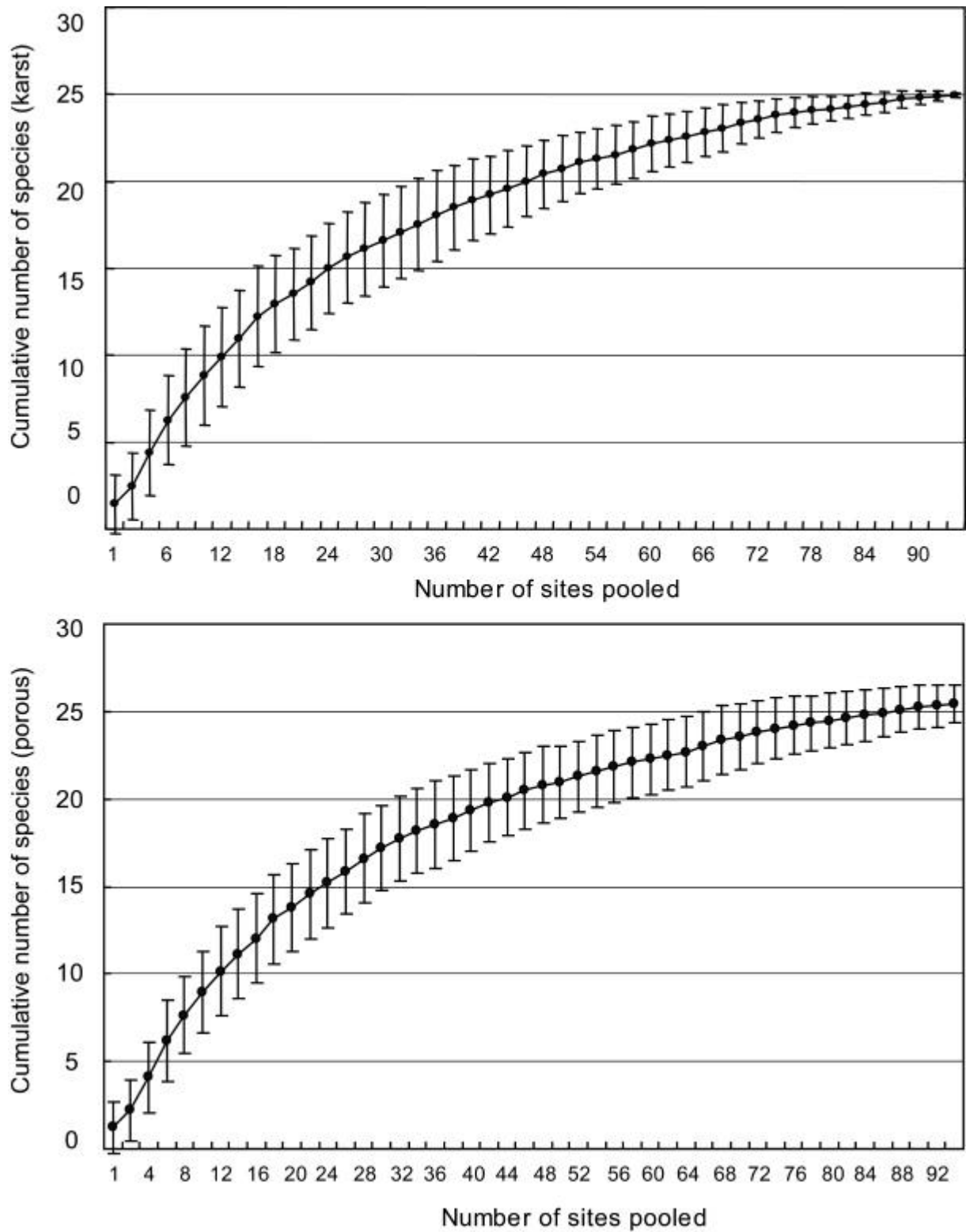
strata. Whatever the stratification considered (karst, porous, karst and porous), the SACs for observed species increase with the number of samples and does not seem to reach an asymptote (Figures 4 & 5). The 80% of observed species richness in both the karst and porous strata may be obtained by sampling 48 sites in each strata. The total number of stations sampled during this study (201) is not satisfactory as regards the best estimate of species richness.

The results of the DCOA for the Walloon Region showed no statistically significant differences in species composition whatever the stratification scheme considered (Table 1). Such lack of statistical significance is not entirely unexpected given that most stygobiotic species found in Belgium are probably ubiquitous, as also suggested in section 2 by the OMI (Outlying Mean Index) analyses. A few species are, however, exclusive to one of the considered zone, in accordance with their known biology. The cladoceran *Alona phreatica* (AloP) and hydrachnidians (*Stygomomonium latipes* - code: SgLa and *Soldanellonyx chappuisi* - code: SoCh) are mostly found in the hyporheos while the isopod *Proasellus cavaticus* (PrCa) and the cyclopoids *Speocyclops* indet (Sp1) and *Graeteriella unisetigera* (GrUn) are in this area exclusive of the karst (but this is not a general rule). Not taking this aspect into account in the sampling strategy could lead to a biased estimate of regional species richness.

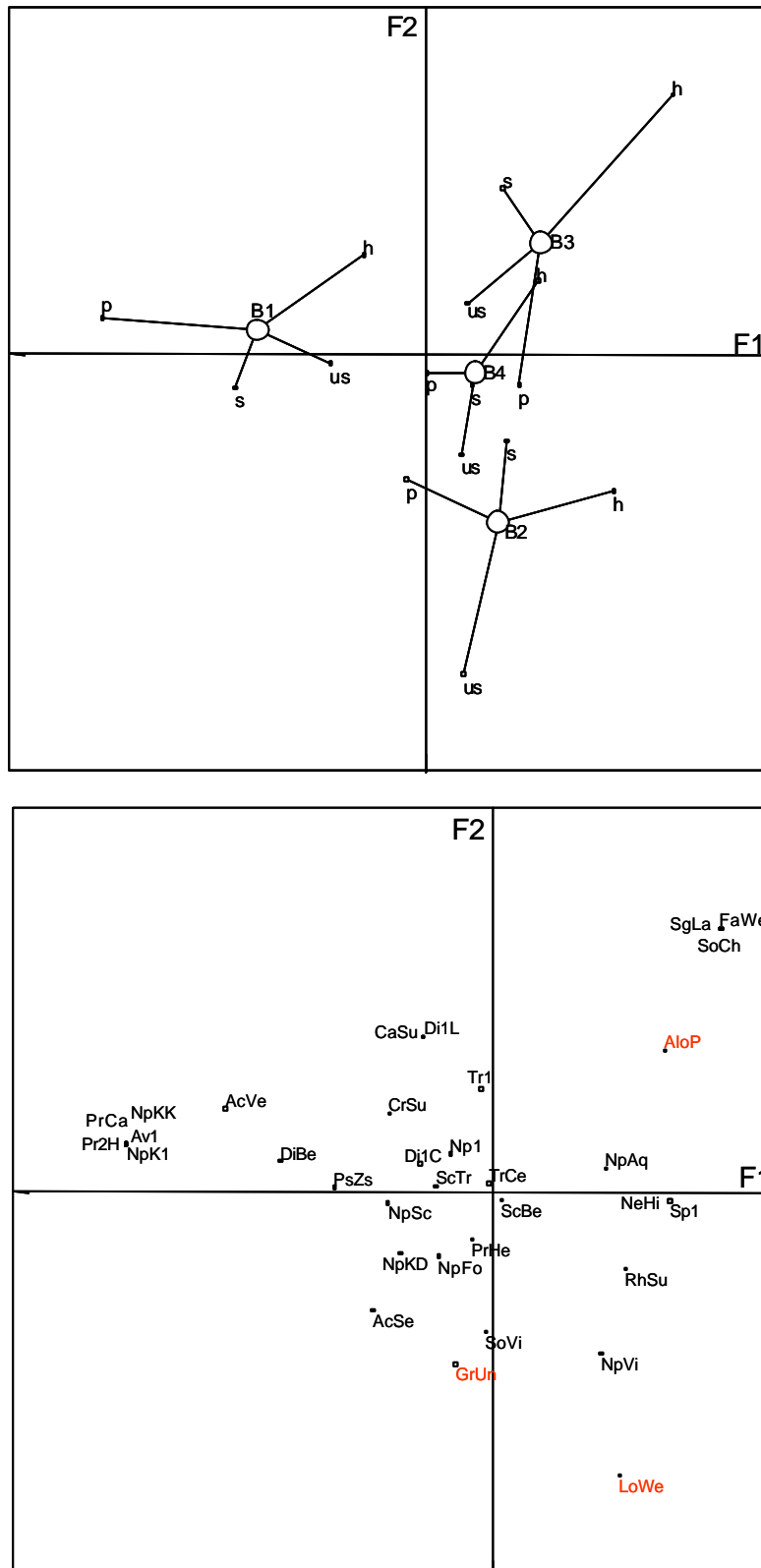
Three zones constantly stand out from analyses, namely B1-p, B2-us and B3-h which correspond to the phreatic zone of the Bocq river basin, the unsaturated zone of the Lesse River and the hyporheic zone of the Ourthe River, respectively. Moreover, the Bocq basin (B1) is clearly separated from the other three basins along the first axis (F1) of DCOA for the Basin effect (Figure 6A). It is worth noting that at least two of them are meaningful and could be a source of heterogeneity in analyses. The Bocq basin (B1), especially the phreatic zone, was mostly sampled via installations of the CIBE Water Company (piezometers, wells, water catchments), which provided an ideal access to ground water and an optimal sampling of its fauna. As a result, samples taken in this zone could be biased in comparison with other regions where an “open window” to a stygobiotic fauna was less evident. In addition, the Bocq basin has some environmental features quite apart from the other three basins (a. o. higher grain-size, two types of limestone with different dissolution rate). As to the Lesse basin, it is the richest region in terms of cave and karst phenomena. In this basin, there is a clear distinction between the saturated and unsaturated zones of the karst, while such a distinction is more problematic in other basins. As a result, the lack of discrimination of other unsaturated zones is perhaps artificial, due to weak distinction between the unsaturated and saturated zones in basins B1, B3 and B4 (Bocq, Ourthe and Amblève rivers).

The results of DCOA analyses suggest that a random sampling strategy may give comparable results. Namely a distinction between either basins, or karst (**K**) and porous (**P**) strata, or basins and KP strata, or unsaturated karst (**us**), saturated karst (**s**), phreatic (**p**) and hyporheic (**h**) zones (**usshp**) is not supported by the results. However a more careful examination of data suggests that a distinction should be made between at least the phreatic zone of the Bocq basin (B1), the unsaturated zone of the Lesse Basin (B2), the hyporheic zone of the Ourthe basin (B3) and all other zones. Moreover, a stratification pattern could emerge as a result of an increased sampling effort. For these reasons, the stratified sampling strategy considered in this study may be retained, distributing an equal number of sampling sites in each stratum.





**Figure 5:** Species richness accumulation curves for the karst and porous strata in the Walloon region. Each point represents the mean of 100 randomizations without replacement. Error bars are the corresponding standard deviations.



**Figure 6 :** Results of the between-basin DCOA for the Walloon region. Upper panel: position of hierarchical units on the F1x2 factorial plane. Lower panel: position of species on the F1x2 factorial plane. The most contributing species ( $p < 0.001$ ) are shown in red.

### **1.3.3 Optimisation of the field sampling strategy for the Jura Region (France) :**

The number of occurrences for stygobiotic species was highest in the Jura region (Figure 1): 50 % of the species occurred in more than 10% of the sites. The two most frequent species (*Niphargus rhenorhodanensis* and *Diacyclops cf. belgicus*) were collected within 95% and 60% of the sites, respectively; 12 species were collected within more than 30% of the sites and 9 species within 1% only. Stygobionts are completely absent in 4 sites only.

On a total of 61 species observed in the Jura Region, **43 and 45** species were present in the karst and porous strata, respectively, and 15 to 18 species were exclusive to each of the two strata (Figure 2). The contribution of the four different zones (**us, s, h, p**) to the species richness is equivalent reaching about 35 species (Figure 3). Regarding the Species Richness Accumulation Curves (SAC, Figure 7), and despite the fact that curves did not showed a perfect plateau, the 80% of species observed in the karst could be estimated by sampling 30 and the 93% sampling 60 sites. In the porous stratum, the 80 % of species observed could be estimated sampling 35 sites and the 93 % sampling 55 sites.

The results of Decentred COrrespondence Analysis (DCOA) for the Meridional Jura showed significant differences in the species composition between Karst and Porous strata (KP), between Basins and Karst/Porous relationships (Bas\_KP, Table 1) and between unsaturated (**us**), saturated (**s**), phreatic (**p**) and hyporheic (**h**) zones, (**usshp** all together). Differences in the species composition between Basins were not significant. Taking into account that the effect between Bas\_KP (63 % of total inertia) was higher than the explained by the KP effect (19% of total inertia.) and the usshp effect (33 % of total inertia.), for the meridional Jura, a stratified sampling taking into account the Karst and Porous strata and the effect of Basin together is retained.

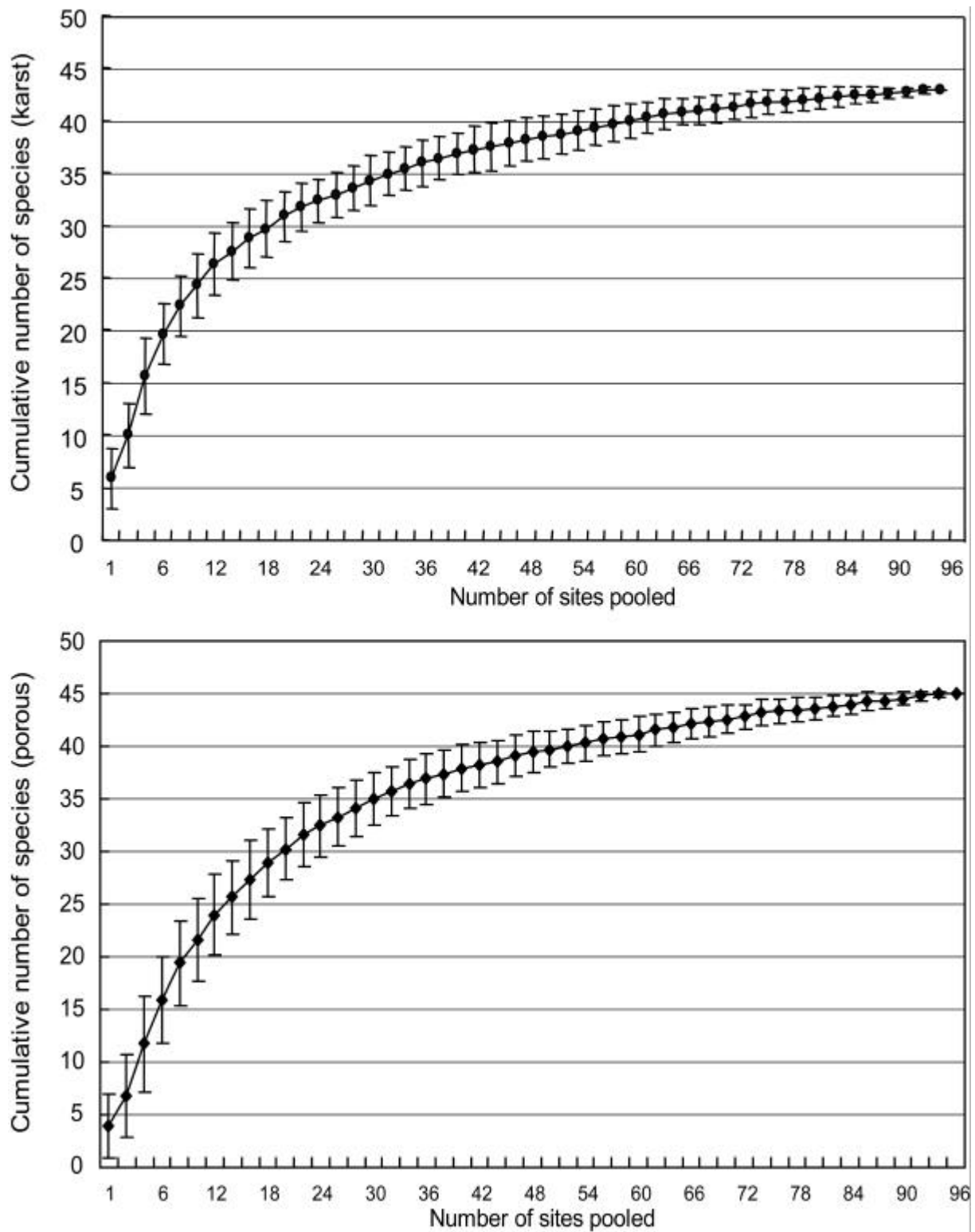
The first axis (F1) of the DCOA for the Bas\_KP effect evidenciates the KP effect and the second axis (F2) the Basin effect (Figure 8A). The F1 axis separates clearly sites belonging to the Porous strata (negative values) from those belonging to the Karst strata (positive values).

Regarding the negative dimension of the F1 axis, two groups can be identified: one, grouping the porous strata from the Suran (B1), Oignin (B3) and Valouse (B4) basins and the other grouping the porous zones of the Albarine basin (B2). The species distribution observed in the Figure 8B shows that the porous strata of Albarine basin includes a stygobiotic fauna which is different from the other basins. In fact, the presence of *Microcharon reginae* (MiRe), *Schellencondona triquetra* (ScTr), *Salentinella juberthieae* (SaJu) and *Niphargus fontanus* (NpFo) within this fauna, suggests a colonization carried out by species (allochtonal fauna)

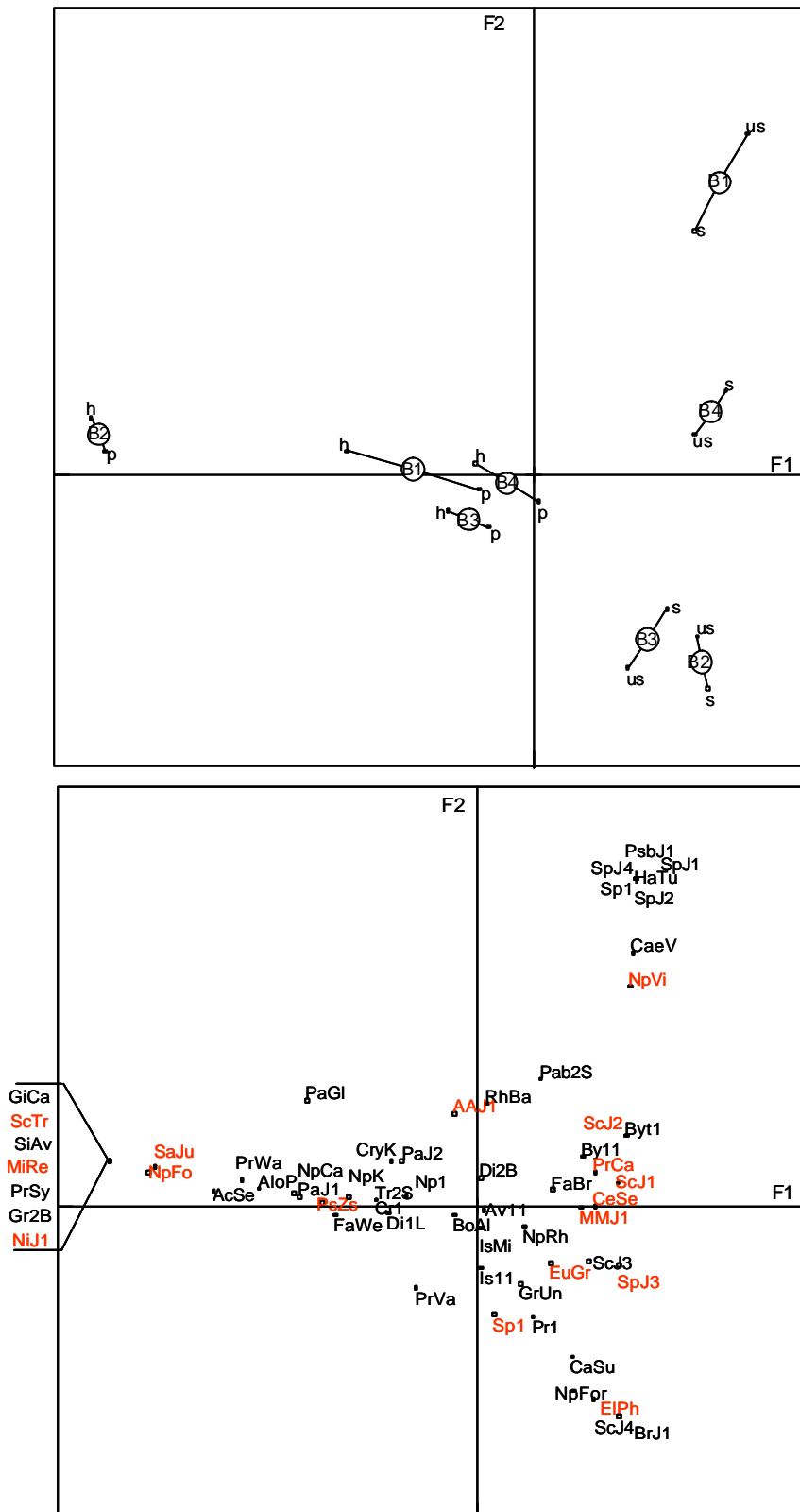
coming from the Mediterranean fluvial corridor of the Ain basin which is connected to the Albarine basin. Thus, two units should be used in the Porous strata, one including the Albarine basin (B2) and the other including the Suran, Valouse and Oignin basins (B1, B3 and B4).

In relation to the positive dimension of the F1 axis, which show the Karst group, the four basins represent an unique group. Nevertheless a separation in three clusters can be observed regarding the F2 axis: a first containing the karst strata from the Suran, a second with the Valouse and finally, the third, clustering the Oignin and the Albarine basin. The position of species along the axis indicates that stygobiotic fauna of the karst is the result of other effect than the geology, which in this analysis we named "Basin". In fact, species such as *Niphargus virei* (NpVi), *Schellencandona insueta* (ScJ2), *Proasellus cavaticus* (PrCa) and *Ceuthonectes serbicus* (CeSe) seems to be associated with a gradient defined by the altitude and the distance to the Würm glacier (cf. section 2). On the other hand, species like *Elaphoidella phreatica* (ElPh), *Speocyclops* sp. (Sp1, SpJ3) and *Eucyclops graeteri* (EuGr) seem to be associated to the Würm and permeability (Karst) effect.

Taking into account that the effect between Bas\_KP (63% of total inertia) was higher than the one explained by the KP effect (19% of total inertia) and the usshp effect (33% of total inertia), for the meridional Jura a stratified sampling strategy taking into account the Karst and Porous strata and the effect of Basin together should give the best results. Two sampling units can be distinguished within the karst: one including the Suran and Valouse basins (B1 and B4) and the other including the Oignin and Albarine basins (B3 and B2).



**Figure 7:** Species richness accumulation curves for the karst and porous strata in the Jura region. Each point represents the mean of 100 randomizations without replacement. Error bars are the corresponding standard deviations.



**Figure 8 :** Results of the between-basin KP DCOA for the Jura region. Upper panel: position of hierarchical units on the F1x2 factorial plane. Lower panel: position of species on the F1x2 factorial plane. The most contributing species ( $p < 0.001$ ) are shown in red.

#### **1.3.4 Optimisation of the field sampling strategy for the Cantabrica Region (Spain) :**

As for the Walloon region, the number of occurrences for stygobiont species is exceedingly low in the Cantabrica region (Figure 1). The most frequent species (*Diacyclops sp languidoide* - group) was collected within 32% of the sites only., about 70 % of the total number of species occurred in less than 4% of the sites. On a total of 67 species, 58 were collected within less than 10% of the sites and 25 occurred in 1% only. In addition, stygobionts are completely absent in 78 sites (on a total of 189)

The number of species observed was higher in the karst (**52** species), than in the porous strata (**38** species), with **28** and **15** species exclusive to each of the two strata (Figure 2). The contribution of the four different zones (**us, s, h, p** Figure 3), to the species richness varies from 19 (**p**) to 45 (**us**). Whatever the stratification considered (karst, porous, karst and porous), the Species Richness Accumulation Curve (SACs, Figure 9) for observed species still increased with the number of samples and does not reach the saturation (Figures 4 & 9). The 80% of the observed species richness in both the karst and porous strata may be obtained by sampling more than 70 sites in each strata. The total number of stations sampled during this study (189) is not satisfactory as regards the best estimate of species richness.

The results of the DCOA for the Cantabrica showed significant differences in the species composition between Basins, between KP and between Bas\_KP. Not significant differences were found between usshp. The Bas\_KP effect (59 % of total inertia) was higher than the one explained by the Basins effect (27 % of total inertia) and the effect KP (11 % of total inertia). The first axis (F1) of the DCOA for the Bas\_KP effect evidenciates the KP effect and the second axis (F2) the Basin effect (Figure 10A).

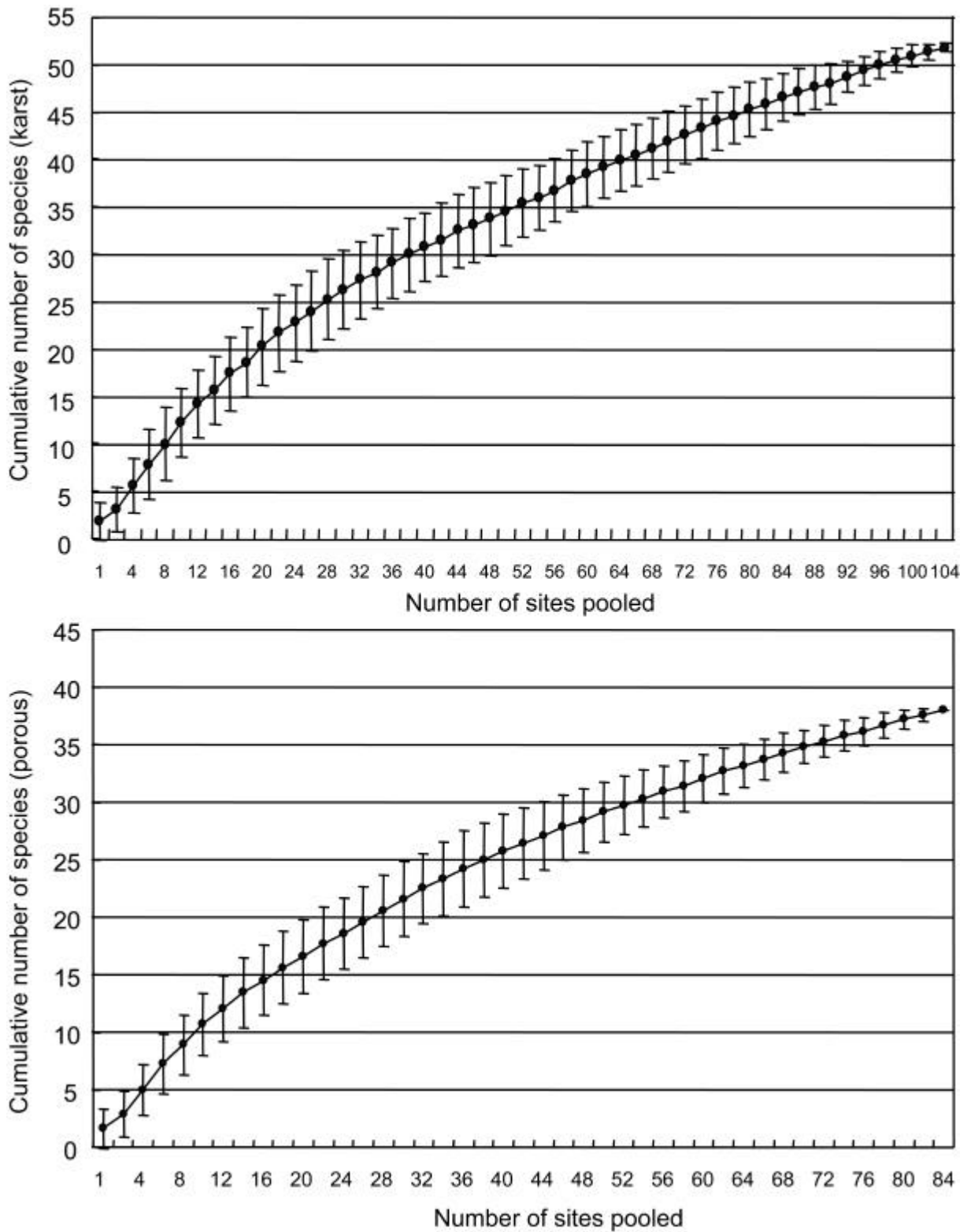
The F1 axis separates sites belonging to the Porous strata (negative values) from those belonging to the Karst strata (positive values) for all basins except for the B4 which presents the Karst strata on the negative dimension too. A cluster of points, including Basin 2 and Basin 3 with their karstic and porous strata, are close to the origins of F1 and F2, indicating low explanatory power in these two first axes and low discrimination between basins and strata.

Regarding the positive dimension of the F1 axis, two groups can be identified: one, clustering the karst belonging to the Ason basin (B1) and the other grouping the Matienzo (B2) and the Ojo Guareña karst (B3). The species distribution observed in the Figure 10B shows that the inertia on B1 is mainly due to endemic species of Amphipoda and Oligochaeta (*Phallodrillinae, Pseudoniphargus*).

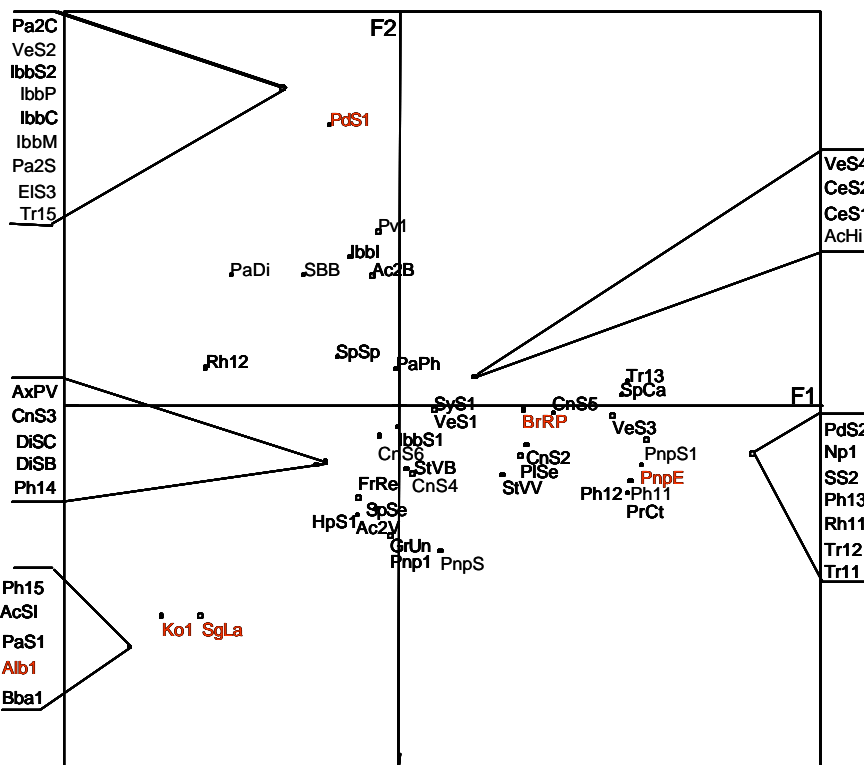
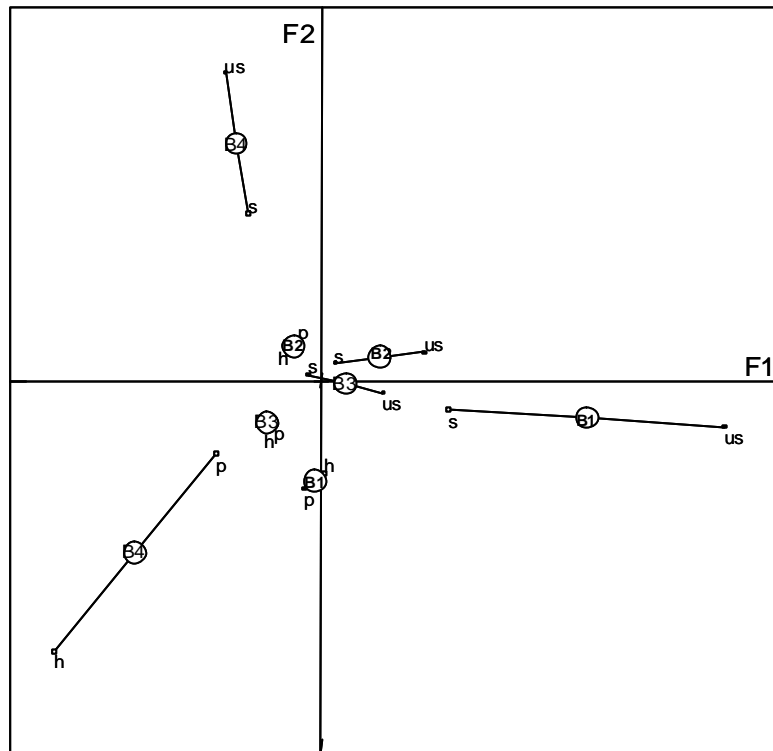
The negative dimension of the F1 axis show the porous and the karstic strata from the Basin 4. At the same time, taking into account the inertia on the F2 axis, three groups are distinguished: the first, clustering the porous strata of the B1, B2 and B3 basins; the second grouping the porous zones of B4 and thirdly the karstic zones of B4. In fact the Basin 4 is particularly rich in endemic species belonging to the Syncarida and Harpacticoida in the karst (genera *Parastenocaris*, *Iberobathynella*, *Vejdovskybathynella*, *Paradoxiclamousella*, *Elaphoidella*) and to the Hydrachnidia (genera *Axonopsis*, *Albaxona*, *Kongsbergia*, *Stygomomonina*) in the porous environment.

For the Cantabrica region, regarding the SACs, a larger sampling effort is strongly suggested. A stratified sampling strategy taking into account the Karst and Porous strata and the effect of Basin together (Table 1) is highly recommended.





**Figure 9:** Species richness accumulation curves for the karst and porous strata in the Cantabrica region. Each point represents the mean of 100 randomizations without replacement. Error bars are the corresponding standard deviations



**Figure 10:** Results of the between-basin KP DCOA for the Cantabrica region. Upper panel: position of hierarchical units on the F1x2 factorial plane. Lower panel: position of species on the F1x2 factorial plane. The most contributing species ( $p < 0.001$ ) are shown in red.

### **1.3.5 Optimisation of the field sampling strategy for the Lessinian Region (Italy) :**

The number of occurrences for stygobiotic species is generally low in the Lessinian region (Figure 1): 78 % of the species occurred in less than 10% of the sites. The two most frequent species (*Speocyclops* cf. *infernus* and *Nitocrella psammophila*) were collected within 54% of the sites; 27 species were collected within 1% of sites only. Stygobionts are completely absent in 29 sites on a total of 197.

On a total of 89 species observed in the Lessinian Region, **63 and 55** species were present in the karst and porous strata, respectively, and 34 and 27 species were exclusive to each of the two strata (Figure 2). The contribution of the four different zones (**us, s, h, p**) to the species richness is less important for the phreatic than for the three other strata (Figure 3). The SACs, (Figure 4) for observed species still increased with the number of samples and does not reach an asymptote. In the karst, 80% of the observed species could be estimated by sampling 60 sites. In the porous stratum, 80 % of the observed species could be estimated by sampling 52 sites (Figure 11).

The results of the DCOA for the Lessinian region showed no significant differences in the species richness between Basins, between KP and between Bas\_KP. Significant differences were found between **usshp** zones (33% of total inertia, Table 1). The F1 axis separates sites belonging to the unsaturated zone (negative values) from those belonging to the saturated, hyporheic and phreatic zones (positive values). This result is corroborated by the OMI analysis (section 2). The F2 axis isolates sites belonging to the hyporheic zone (Figure 12A). The proximity of **p** and **s** habitats could be related to the difficulty to distinguish these two habitats. In this region, the saturated zone of the karst is often accessible through boreholes or wells and on the other hand phreatic wells are very deep and may reach layers situated below the alluvial floor (i.e. in the karst) leading to a possible “confusion” overlap of these two habitats.

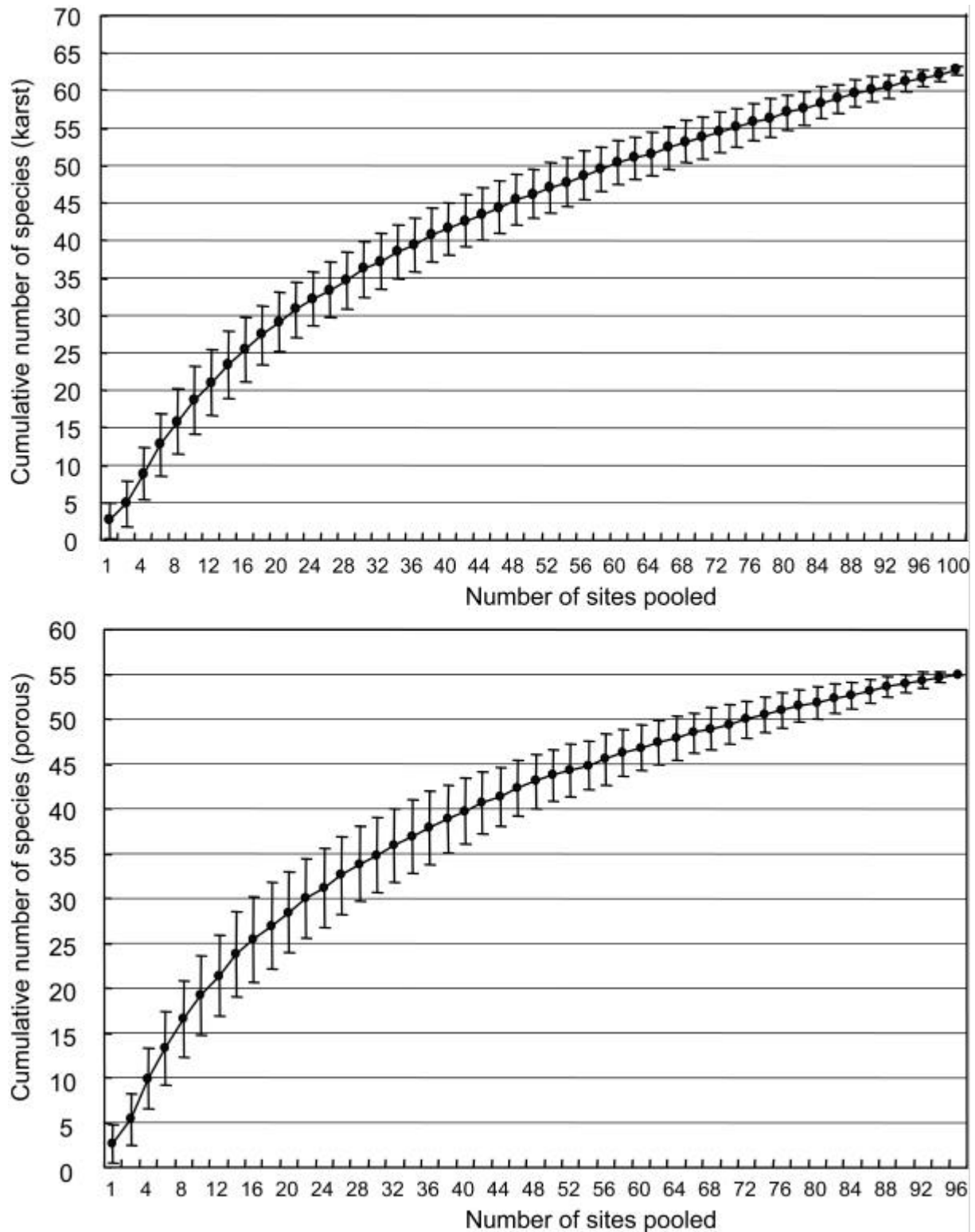
The four most contributive species for the definition of the **us**-group, *Niphargus* cf. *costozzae* (Np2C), *Moraria* sp.11 (MM1) *Lessinocamptus pivai* (LePi) and *Niphargus lessiniensis* (NpLe) are **us**-exclusive or more frequent in the **us** zone, *Speocyclops* cf. *infernus* (Sp2I) occupies an intermediate position with occurrences distributed in the **us, s** and **h** zones. The other species of this group are often endemic species.

Three copepods give a high contribution to the definition of the **s-p** group situated on the positive part of the F1. *Diacyclops ruffoi* (DiRu) is exclusive to the phreatic zone,

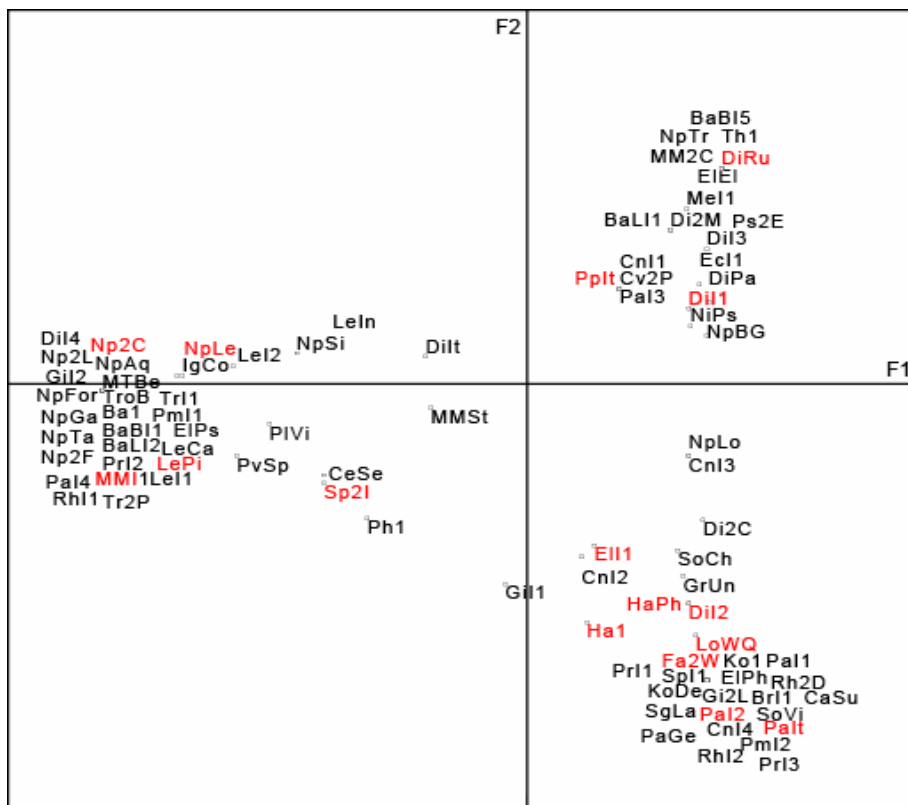
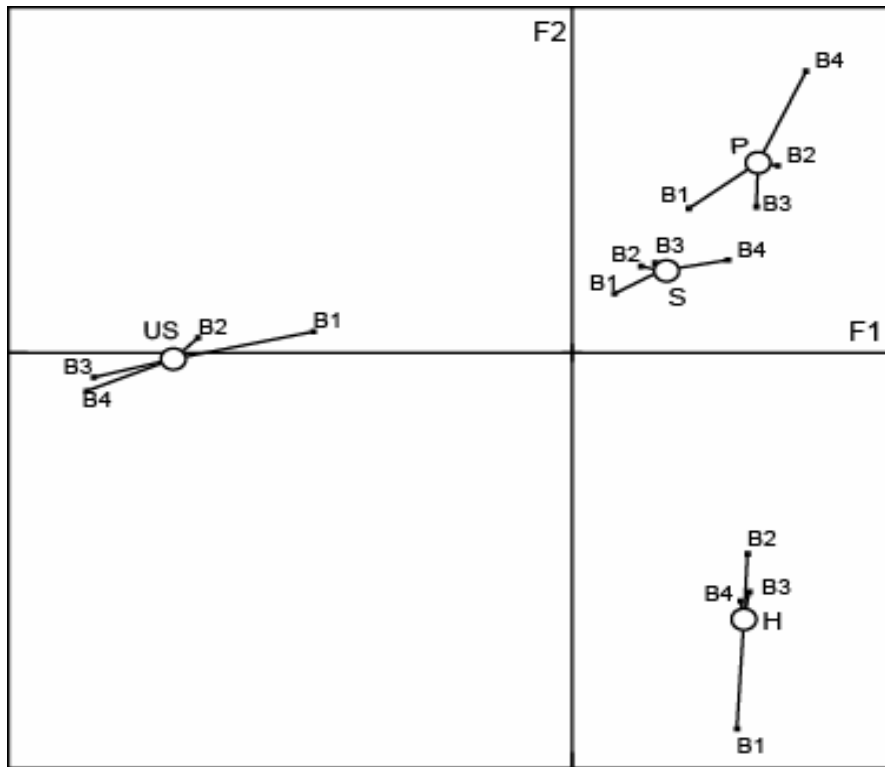
*Paraspeudoleptomesocra italica* (PpIt) is exclusive to the karst saturated zone and *Diacyclops* sp.I1 (DiI1) is collected everywhere, except in the unsaturated zone.

Most of the species determinant for the **h**-group (F2) are exclusive to the hyporheic zone: *Parastenocaris italica* (PaIt), *Parastenocaris* sp.I2 (PaI2), *Fabaeformiscandona* cf. *wegelini* (Fa2W), *Haber* indet.(Ha1), *Lobohalacarus weberi*.(LoWQ), *Diacyclops* sp.I2 (DiI2) and *Halacarellus phreaticus* .(HaPh) were preferentially collected in the hyporheic, but with few occurrences in the saturated zone of the karst. *Elaphoidella* sp.I1 (EII1) occupies a more intermediate position with occurrences distributed in the three **us**, **s** and **h** zones.

To summarize, the stygobiotic communities of the Lessinian region is characterized by a high number of rare species, most of them being exclusive to one particular habitat. The DCOA analysis delineates the existence of three groups of species, which are more or less exclusive to the **us** zone of the karst, the hyporheic zone, and the two **s-p** zones together, suggesting as optimal a stratified sampling strategy (Table 1). The direct analysis of the exclusive species richness between **usshp** strata also shows that **p** strata has a lower contribution to the total species richness than the other strata (Figure 3). Regarding the SACs, a larger sampling effort is suggested.



**Figure 11:** Species richness accumulation curves for the karst and porous strata in the Lessinian region. Each point represents the mean of 100 randomizations without replacement. Error bars are the corresponding standard deviations.



**Figure 12:** Results of the between- usshp DCOA for the Lessinian region. Upper panel: position of hierarchical units on the F1x2 factorial plane. Lower panel: position of species on the F1x2 factorial plane. The most contributing species ( $p < 0.001$ ) are shown in red.

### **1.3.6 Optimisation of the field sampling strategy for the Krim Region (Slovenia) :**

The number of species is highest in the Krim region (105 species), but the number of occurrences for stygobiont species is generally low (Figure 1). The two most frequent species (*Acanthocyclops sambugarae* and *Diacyclops* cf. *clandestinus*) were collected within 44% and 43% of the sites respectively. The absolute frequency of occurrence is less than, or equal to, 3% of the sites for 52 % of the total number of species. More than one third of the species occurred in 1% of sites only. Stygobionts are completely absent in 13 sites only.

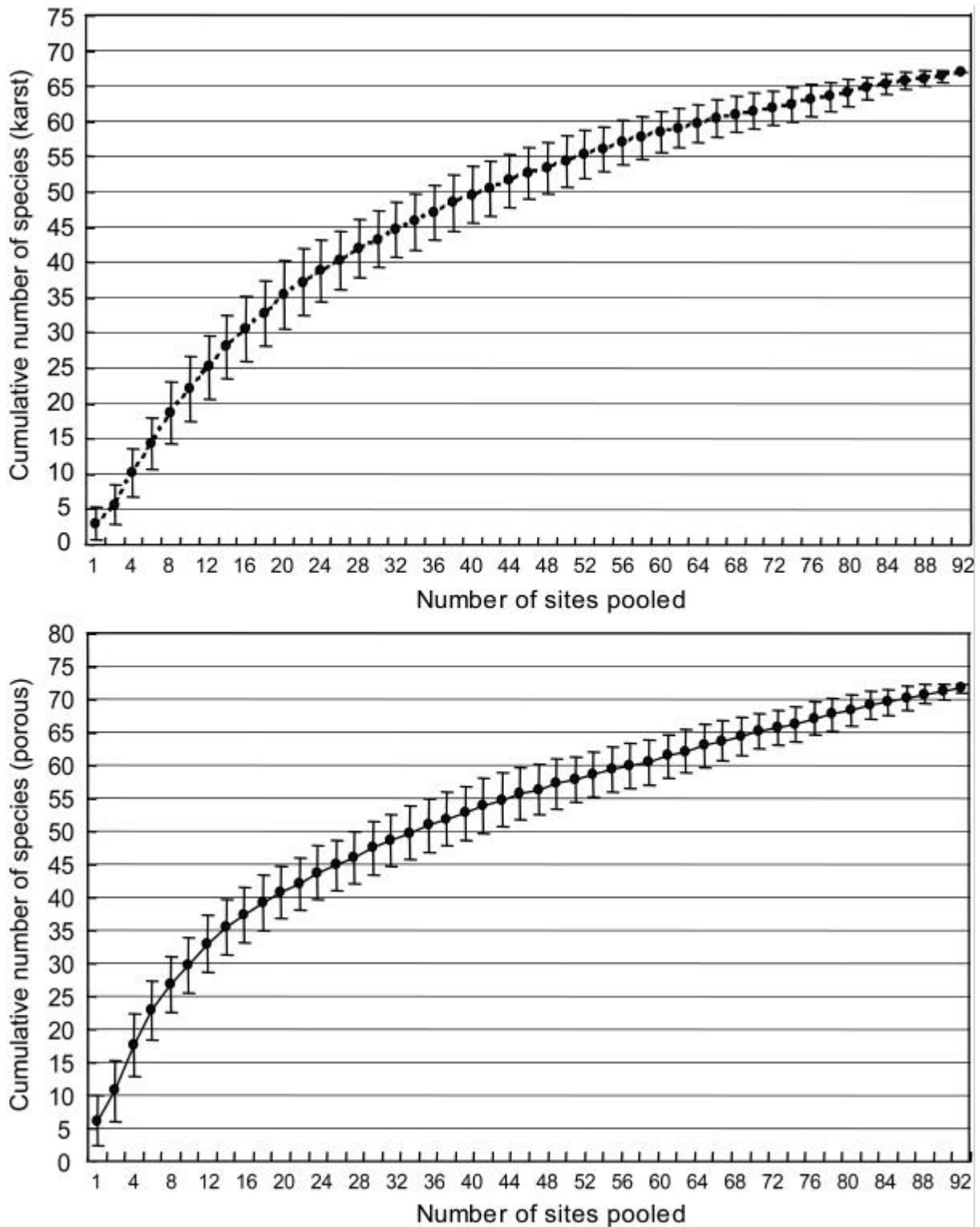
The number of species collected were similar in the karst and in the porous zones (Figure 2A), with high number of species exclusive of the two strata (Figure 2B). The contribution of the four different zones (**us**, **s**, **h**, **p**, Figure 3) to the species richness varies from 33 (**us**) to 58 (**p**). Whatever the stratification considered (karst, porous, karst and porous), the SACs for observed species still increased in parallel with the number of samples and does not reach an asymptote (Figures 4 & 13). 80% of the species observed in the karst could be estimated sampling 45 sites. In the porous stratum, the 80 % of species observed could be estimated sampling 50 sites.

The results of the DCOA for the Krim region showed no significant differences between Basins and between Bas\_KP. Significant differences were found in the species composition between KP and **usshp** zones. Although the total inertia in the analysis is low, the between usshp effect (35 % of total inertia.) was higher than the between KP effect (19% of total inertia). The F1 axis separates sites belonging to the Porous strata (negative values) from those belonging to the Karst strata (positive values) and the F2 axis separates sites belonging to the saturated zone (positive values) from those belonging to the unsaturated zone (negative values, Figure 14). The species distribution observed in the Figure 14 evidenced three groups of species. The first one (negative values on F1) corresponds to species exclusive to the porous habitats, such as the copepods *Elaphoidella charon* (ElCh), *Nitocrella hirta* (NiJ1), *Acanthocyclops sambugarae* (AcSa) and *Diacyclops* cf. *zschokkei* (Di2Z), and the hydrachnidians *Lethaxona cavifrons* (LetC) and *Frontipodopsis reticulafrons* (FrRe). Three other prevalent species are very frequent in the porous but are also present in the **s** zone (*Proasellus vulgaris*-PrVu- and *Tubificiade* gen sp.K1-TubK1-) or in the **us** zone of the karst (*Elaphoidella* sp.K1 -ElK1-). The most representative species for the second group of sites (positive values on F1 and F2), the amphipoda *Niphargus rejici* (NpRe) and the oligochaete *Embolocephalus* sp.K1 (EmK1), are exclusive to this **s** zone. A set of species, situated between these two groups were collected both in the porous and in the saturated zone of the

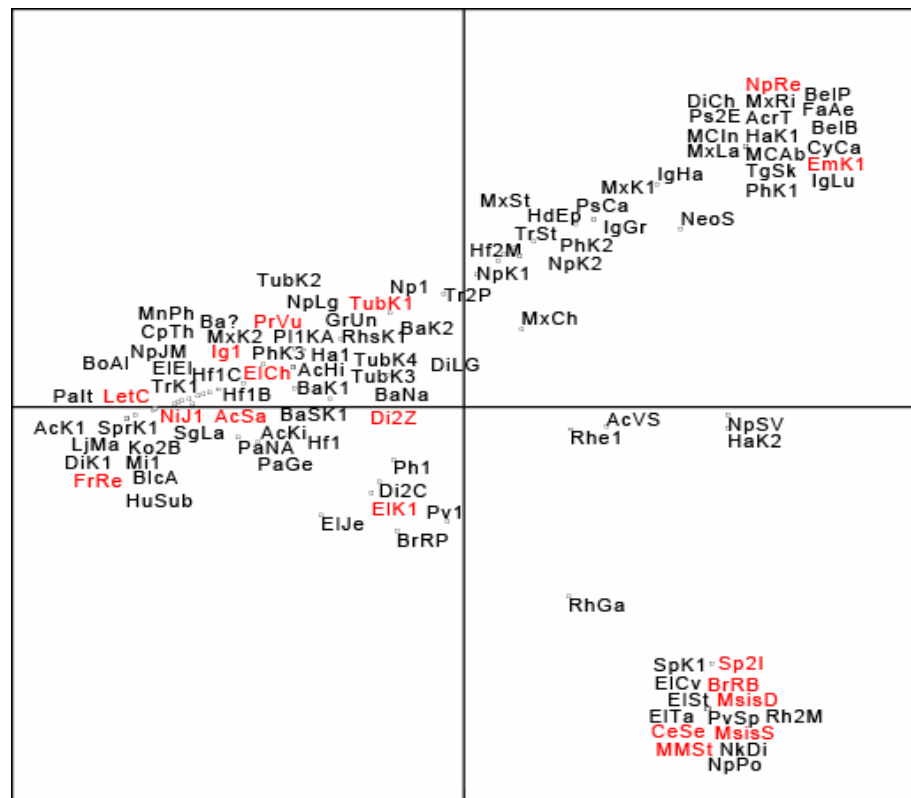
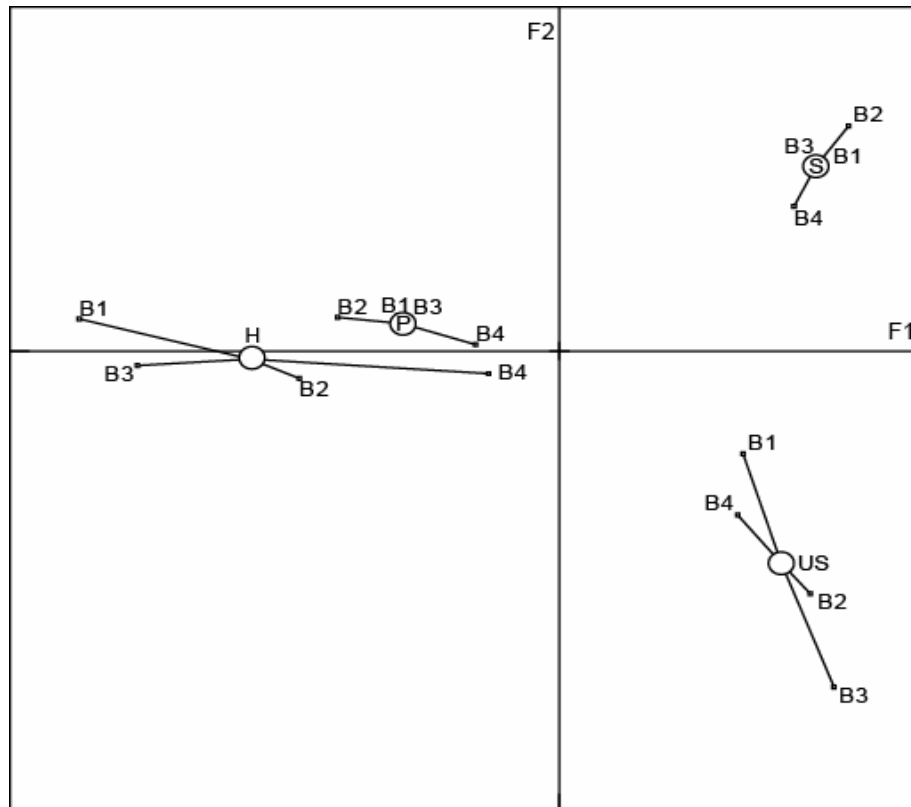
karst. All the species of the third group are copepods **us**-exclusive: *Speocyclops infernus* (Sp2I), *Bryocamptus balcanicus* (BrRB) *Morariopsis dumonti* (MsisD), *Morariopsis scotenophila* (MsisS), *Moraria stankovitchi* (MMSt) and *Ceuthonectes serbicus* (CeSe).

Karst and P strata are well characterized by different sets of exclusive species. The low frequency of species and their distribution in the sites suggest that a stratified sampling strategy is appropriate, taking into account the porous strata (**h** and **p** together) and the karst strata (**us** and **s** separately). The Krim region gathers a great number of rare and/or endemic species, regarding the SACs; for this reason, a larger sampling effort is strongly recommended.





**Figure 13:** Species richness accumulation curves for the karst and porous strata in the Krim region. Each point represents the mean of 100 randomizations without replacement. Error bars are the corresponding standard deviations.



**Figure 14:** Results of the between- **usshp** DCOA for the Krim region. Upper panel: position of hierarchical units on the F1x2 factorial plane. Lower panel: position of species on the F1x2 factorial plane. The most contributing species ( $p < 0.001$ ) are shown in red.

#### **1.4 DISCUSSION :**

After examination of SACs and DCOA results, several propositions to optimize the field sampling strategy were proposed herein and adapted to each region. The results clearly indicate that, in any region which will be sampled in future studies to assess groundwater species richness, the following operational strategy is recommended:

- The implementation of the suggested sampling strategy requires for most cases a higher number of sites than planned in the PASCALIS protocol (192 sites per region). This conclusion results from two aspects: the low occurrence of species (rarity) and the presence of a large number of species with limited distribution, mainly strict endemics. The number of endemic species is higher in the Southern regions.
- As a general rule, a common field sampling strategy for the PASCALIS countries based on the same number of sampling stations in the different hierarchical units cannot be recommended. The protocol may be improved.
- The implementation of a strategy common to all regions may be taken with caution as species distribution within the various hierarchical units differs from one region to another. In any case however, the porous/karst stratification was preponderant in all regions and should remain whatever strategy is proposed. Differences in species composition between basins were less or not statistically significant.
- Areas influenced by Quaternary glaciations (such as the Walloon region) are species-poor, mainly colonized by post-glacial, opportunist invaders, and the stratified sampling scheme, even the porous/karst stratification, seems to be a less compelling strategy; more complex areas, with a longer history marked by a large number of endemics, should be more carefully investigated.

Consequently, it is recommended to search for a sampling strategy adapted to each region in taking into account endemism, which is much more significant in Southern regions (Cantabria, Lessinia, Krim) than in Northern regions (Walloon and part of Jura), as well as rarity. Thus it is essential in the regions where the endemism is high to sample more intensively the lower hierarchical levels of the protocol. Environmental parameters, including historical ones (see statistical analyses reported in the following section 2), may play an important role in explaining species distribution and should be taken in account to finely tune the sampling strategy and adapt the stratified sampling scheme to the environmental complexity of the study area, . The recommended sampling strategy will be refined by the

results which will be reported in sections 2 and 3 (relations to environmental parameters, partition of biodiversity).

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## 1.6 APPENDIX 1 : CODES OF TAXA USED IN THE SECTION 1 AND 2 ANALYSES :

Region	Group	Species	Code
Meridional Jura (France)	Oligochaeta	Trichodrilus cf. serei	Tr2S
Meridional Jura (France)	Oligochaeta	Gianus cavealis	GiCa
Meridional Jura (France)	Oligochaeta	Haber turquini	HaTu
Meridional Jura (France)	Oligochaeta	Rhyacodrilus balmensis	RhBa
Meridional Jura (France)	Gastropoda	Spiralix sp.	Sp1
Meridional Jura (France)	Gastropoda	Bythiospeum sp. pl.	By11
Meridional Jura (France)	Gastropoda	Islamia minuta	IsMi
Meridional Jura (France)	Gastropoda	Islamia sp. pl.	Is11
Meridional Jura (France)	Gastropoda	Avenionia sp. pl.	Av11
Meridional Jura (France)	Gastropoda	Bythinella sp.	Byt1
Meridional Jura (France)	Cladocera	Alona phreatica	AloP
Meridional Jura (France)	Cyclopoida	Eucyclops graeteri	EuGr
Meridional Jura (France)	Cyclopoida	Acanthocyclops sensitivus	AcSe
Meridional Jura (France)	Cyclopoida	Diacyclops sp. group languidoides	Di1L
Meridional Jura (France)	Cyclopoida	Diacyclops cf. belgicus	Di2B
Meridional Jura (France)	Cyclopoida	Speocyclops sp.J1	SpJ1
Meridional Jura (France)	Cyclopoida	Speocyclops sp.J2	SpJ2
Meridional Jura (France)	Cyclopoida	Speocyclops sp.J.3	SpJ3
Meridional Jura (France)	Cyclopoida	Speocyclops sp.J4	SpJ4
Meridional Jura (France)	Cyclopoida	Speocyclops indet.	Sp1
Meridional Jura (France)	Cyclopoida	Graeteriella (Graeteriella) unisetigera	GrUn
Meridional Jura (France)	Cyclopoida	Graeteriella cf. boui	Gr2B
Meridional Jura (France)	Harpacticoida	Nitocrella gr. hirta sp. J1	NiJ1
Meridional Jura (France)	Harpacticoida	Attheyella (A) sp.J1	AAJ1
Meridional Jura (France)	Harpacticoida	Moraria (M.) sp.J1	MMJ1
Meridional Jura (France)	Harpacticoida	Bryocamptus sp. J1	BrJ1
Meridional Jura (France)	Harpacticoida	Elaphoidella phreatica	ElPh
Meridional Jura (France)	Harpacticoida	Ceuthonectes serbicus	CeSe
Meridional Jura (France)	Harpacticoida	Parastenocaris sp J1	PaJ1
Meridional Jura (France)	Harpacticoida	Parastenocaris glareola	PaGl
Meridional Jura (France)	Harpacticoida	Parastenocaris sp. J2	PaJ2
Meridional Jura (France)	Ostracoda	Pseudocandona zschokkei	PsZs
Meridional Jura (France)	Ostracoda	Cryptocandona kieferi	CryK
Meridional Jura (France)	Ostracoda	Fabaeformiscandona wegelinei	FaWe
Meridional Jura (France)	Ostracoda	Schellencandona triquetra	ScTr
Meridional Jura (France)	Ostracoda	Schellencandona spJ1 schellenbergi	ScJ1
Meridional Jura (France)	Ostracoda	Schellencandona spJ2 insueta	ScJ2
Meridional Jura (France)	Ostracoda	Schellencandona spJ 3	ScJ3
Meridional Jura (France)	Ostracoda	Schellencandona spJ4	ScJ4
Meridional Jura (France)	Ostracoda	Fabaeformiscandona breuili	FaBr
Meridional Jura (France)	Ostracoda	Cavernocypris subterranea	CaSu
Meridional Jura (France)	Isopoda	Proasellus cavaticus	PrCa
Meridional Jura (France)	Isopoda	Proasellus "non walteri"	Pr1
Meridional Jura (France)	Isopoda	Proasellus walteri	PrWa
Meridional Jura (France)	Isopoda	Proasellus valdensis	PrVa
Meridional Jura (France)	Isopoda	Proasellus synaselloides	PrSy
Meridional Jura (France)	Isopoda	Caecosphaeroma virei	CaeV
Meridional Jura (France)	Isopoda	Microcharon reginae	MiRe
Meridional Jura (France)	Amphipoda	Niphargus indet.	Np1
Meridional Jura (France)	Amphipoda	Niphargus kochianus	NpK

Meridional Jura (France)	Amphipoda	<i>Niphargus rhenorhodanensis</i>	NpRh
Meridional Jura (France)	Amphipoda	<i>Niphargus virei</i>	NpVi
Meridional Jura (France)	Amphipoda	<i>Niphargus fontanus</i>	NpFo
Meridional Jura (France)	Amphipoda	<i>Niphargus forelii</i>	NpFor
Meridional Jura (France)	Amphipoda	<i>Niphargopsis casparyi</i>	NpCa
Meridional Jura (France)	Amphipoda	<i>Crangonyx</i> indet.	Cr1
Meridional Jura (France)	Amphipoda	<i>Bogidiella albertimagni</i>	BoAl
Meridional Jura (France)	Amphipoda	<i>Salentinella juberthieae</i>	SaJu
Meridional Jura (France)	Bathynellacea	<i>Parabathynella</i> cf. <i>stygia</i>	Pab2S
Meridional Jura (France)	Bathynellacea	<i>Pseudobathynella</i> sp.J1	PsbJ1
Meridional Jura (France)	Coleoptera	<i>Siettitia avenionensis</i>	SiAv
Cantabrica (Spain)	Oligochaeta	<i>Trichodrilus</i> indet.1	Tr11
Cantabrica (Spain)	Oligochaeta	<i>Trichodrilus</i> indet.2	Tr12
Cantabrica (Spain)	Oligochaeta	<i>Trichodrilus</i> indet.3	Tr13
Cantabrica (Spain)	Oligochaeta	<i>Trichodrilus</i> indet.5	Tr15
Cantabrica (Spain)	Oligochaeta	<i>Rhyacodrilus</i> indet.1	Rh11
Cantabrica (Spain)	Oligochaeta	<i>Rhyacodrilus</i> indet.2	Rh12
Cantabrica (Spain)	Oligochaeta	Phallodrilinae indet.1	Ph11
Cantabrica (Spain)	Oligochaeta	Phallodrilinae indet.2	Ph12
Cantabrica (Spain)	Oligochaeta	Phallodrilinae indet.3	Ph13
Cantabrica (Spain)	Oligochaeta	Phallodrilinae indet.4	Ph14
Cantabrica (Spain)	Oligochaeta	Phallodrilinae indet.5	Ph15
Cantabrica (Spain)	Oligochaeta	Parvidrilidae indet.	Pv1
Cantabrica (Spain)	Gastropoda	<i>Paladilhiosis(?) septentrionalis</i>	PlSe
Cantabrica (Spain)	Gastropoda	<i>Spiralix (Burgosia) burgensis</i>	SBB
Cantabrica (Spain)	Cyclopoida	<i>Acanthocyclops</i> cf. <i>biarticulatus</i>	Ac2B
Cantabrica (Spain)	Cyclopoida	<i>Acanthocyclops</i> cf. <i>venustus</i>	Ac2V
Cantabrica (Spain)	Cyclopoida	<i>Acanthocyclops hispanicus</i>	AcHi
Cantabrica (Spain)	Cyclopoida	<i>Acanthocyclops</i> sp. S1	AcSI
Cantabrica (Spain)	Cyclopoida	<i>Diacyclops</i> sp. SB group <i>languidoides</i>	DiSB
Cantabrica (Spain)	Cyclopoida	<i>Diacyclops</i> sp. SC group <i>languidoides</i>	DiSC
Cantabrica (Spain)	Cyclopoida	<i>Graeteriella (Graeteriella) unisetigera</i>	GrUn
Cantabrica (Spain)	Cyclopoida	<i>Speocyclops cantabricus</i>	SpCa
Cantabrica (Spain)	Cyclopoida	<i>Speocyclops sebastianus</i>	SpSe
Cantabrica (Spain)	Cyclopoida	<i>Speocyclops spelaeus</i>	SpSp
Cantabrica (Spain)	Harpacticoida	<i>Bryocamptus (R.) pyrenaicus</i>	BrRP
Cantabrica (Spain)	Harpacticoida	<i>Ceuthonectes</i> sp. S1	CeS1
Cantabrica (Spain)	Harpacticoida	<i>Ceuthonectes</i> sp. S2	CeS2
Cantabrica (Spain)	Harpacticoida	<i>Elaphoidella</i> sp. S3	ElS3
Cantabrica (Spain)	Harpacticoida	<i>Parastenocaris</i> cf. <i>cantabrica</i>	Pa2C
Cantabrica (Spain)	Harpacticoida	<i>Parastenocaris</i> cf. <i>stammeri</i>	Pa2S
Cantabrica (Spain)	Harpacticoida	<i>Parastenocaris diana</i>	PaDi
Cantabrica (Spain)	Harpacticoida	<i>Parastenocaris phyllura</i>	PaPh
Cantabrica (Spain)	Harpacticoida	<i>Parastenocaris</i> sp. S1	PaS1
Cantabrica (Spain)	Harpacticoida	<i>Spelaecamptus</i> sp. S2	SS2
Cantabrica (Spain)	Ostracoda	<i>Candoninae</i> gen. sp.S2 Trapezoid	CnS2
Cantabrica (Spain)	Ostracoda	<i>Candoninae</i> gen. sp.S3 Trapezoid	CnS3
Cantabrica (Spain)	Ostracoda	<i>Candoninae</i> gen. sp. S4Triangular	CnS4
Cantabrica (Spain)	Ostracoda	<i>Candoninae</i> gen. sp. S5Triangular	CnS5
Cantabrica (Spain)	Ostracoda	<i>Candoninae</i> gen. sp.S6 Triangular	CnS6
Cantabrica (Spain)	Isopoda	<i>Stenasellus virei buchneri</i>	StVB
Cantabrica (Spain)	Isopoda	<i>Stenasellus virei virei</i>	StVV
Cantabrica (Spain)	Isopoda	<i>Proasellus cantabricus</i>	PrCt
Cantabrica (Spain)	Amphipoda	<i>Pseudoniphargus elongatus</i>	PnpE

Cantabrica (Spain)	Amphipoda	<i>Pseudoniphargus semielongatus</i>	PnpS
Cantabrica (Spain)	Amphipoda	<i>Pseudoniphargus</i> sp.S1	PnpS1
Cantabrica (Spain)	Amphipoda	<i>Pseudoniphargus</i> indet.	Pnp1
Cantabrica (Spain)	Amphipoda	<i>Niphargus</i> Indet.	Np1
Cantabrica (Spain)	Amphipoda	<i>Haploginglymus</i> sp.S1	HpS1
Cantabrica (Spain)	Bathynellacea	<i>Iberobathynella imuniensis</i>	IbbI
Cantabrica (Spain)	Bathynellacea	<i>Iberobathynella magna</i>	IbbM
Cantabrica (Spain)	Bathynellacea	<i>Iberobathynella cantabriensis</i>	IbbC
Cantabrica (Spain)	Bathynellacea	<i>Iberobathynella parasturiensis</i>	IbbP
Cantabrica (Spain)	Bathynellacea	<i>Iberobathynella</i> sp.S1	IbbS1
Cantabrica (Spain)	Bathynellacea	<i>Iberobathynella</i> sp.S2	IbbS2
Cantabrica (Spain)	Bathynellacea	<i>Paradoxicalamousella fideli</i> sp. S1	PdS1
Cantabrica (Spain)	Bathynellacea	<i>Paradoxicalamousella</i> sp.S2	PdS2
Cantabrica (Spain)	Bathynellacea	<i>Vejdovskybathynella edelweiss</i> sp.S1	VeS1
Cantabrica (Spain)	Bathynellacea	<i>Vejdovskybathynella</i> sp.S 2	VeS2
Cantabrica (Spain)	Bathynellacea	<i>Vejdovskybathynella</i> sp.S3	VeS3
Cantabrica (Spain)	Bathynellacea	<i>Vejdovskybathynella</i> sp.S4	VeS4
Cantabrica (Spain)	Bathynellacea	<i>Syncarida</i> gen. S1 sp. S1	SyS1
Cantabrica (Spain)	Acari	<i>Stygomonomia latipes</i>	SgLa
Cantabrica (Spain)	Acari	<i>Frontipodopsis reticulatifrons</i>	FrRe
Cantabrica (Spain)	Acari	<i>Axonopsis (Paraxonopsis) vietsi</i>	AxPV
Cantabrica (Spain)	Acari	<i>Albaxona</i> indet.	Alb1
Cantabrica (Spain)	Acari	<i>Kongsbergia</i> indet.	Ko1
Cantabrica (Spain)	Acari	<i>Barbaxonella</i> indet.	Bba1
Krim massif (Slovenia)	Oligochaeta	<i>Embolocephalus</i> sp.K1	EmK1
Krim massif (Slovenia)	Oligochaeta	<i>Haber</i> sp. K1	HaK1
Krim massif (Slovenia)	Oligochaeta	<i>Haber</i> sp. K2	HaK2
Krim massif (Slovenia)	Oligochaeta	<i>Haber</i> indet.	Ha1
Krim massif (Slovenia)	Oligochaeta	<i>Parvidrillidae</i> indet.	Pv1
Krim massif (Slovenia)	Oligochaeta	<i>Parvidrilus spelaeus</i>	PvSp
Krim massif (Slovenia)	Oligochaeta	<i>Phallodrilinae</i> indet.	Ph1
Krim massif (Slovenia)	Oligochaeta	<i>Phallodrilinae</i> gen. sp. K1	PhK1
Krim massif (Slovenia)	Oligochaeta	<i>Phallodrilinae</i> gen. sp. K2	PhK2
Krim massif (Slovenia)	Oligochaeta	<i>Phallodrilinae</i> sp. K3	PhsK3
Krim massif (Slovenia)	Oligochaeta	<i>Spiridion</i> sp. K1	SprK1
Krim massif (Slovenia)	Oligochaeta	<i>Rhyacodrilinae</i> indet.	Rhe1
Krim massif (Slovenia)	Oligochaeta	<i>Rhyacodrilus gasparoi</i>	RhGa
Krim massif (Slovenia)	Oligochaeta	<i>Rhyacodrilus</i> cf <i>maculatus</i>	Rh2M
Krim massif (Slovenia)	Oligochaeta	<i>Rhyacodriloides</i> sp.K1	RhsK1
Krim massif (Slovenia)	Oligochaeta	<i>Trichodrilus pragensis</i>	Tr2P
Krim massif (Slovenia)	Oligochaeta	<i>Trichodrilus strandi</i>	TrSt
Krim massif (Slovenia)	Oligochaeta	<i>Trichodrilus</i> sp. K1	TrK1
Krim massif (Slovenia)	Oligochaeta	<i>Tubificidae</i> gen.sp. K 1	TubK1
Krim massif (Slovenia)	Oligochaeta	<i>Tubificidae</i> gen. sp. K2	TubK2
Krim massif (Slovenia)	Oligochaeta	<i>Tubificidae</i> gen. sp. K3	TubK3
Krim massif (Slovenia)	Oligochaeta	<i>Tubificidae</i> gen. sp. K4	TubK4
Krim massif (Slovenia)	Gastropoda	<i>Iglica hauffeni</i>	IgHa
Krim massif (Slovenia)	Gastropoda	<i>Iglica gracilis</i>	IgGr
Krim massif (Slovenia)	Gastropoda	<i>Iglica luxurians</i>	IgLu
Krim massif (Slovenia)	Gastropoda	<i>Iglica</i> indet. AB (wide)	Ig1
Krim massif (Slovenia)	Gastropoda	<i>Hadziella ephippiostoma</i>	HdEp
Krim massif (Slovenia)	Gastropoda	<i>Hauffenia</i> indet.	Hf1
Krim massif (Slovenia)	Gastropoda	<i>Hauffenia</i> cf <i>michleri</i>	Hf2M
Krim massif (Slovenia)	Gastropoda	<i>Hauffenia</i> indet. B (flattened)	Hf1B

Krim massif (Slovenia)	Gastropoda	Hauffenia indet. C (flat)	Hf1C
Krim massif (Slovenia)	Gastropoda	Neohoratia subpiscinalis	NeoS
Krim massif (Slovenia)	Gastropoda	Belgrandiella superior	BelP
Krim massif (Slovenia)	Gastropoda	Belgrandiella substricta	BelB
Krim massif (Slovenia)	Gastropoda	Paladalihiopsis(?) indet. KA	Pl1KA
Krim massif (Slovenia)	Gastropoda	Acroloxus tetensi	AcrT
Krim massif (Slovenia)	Calanoida	Troglodiptomus sketi	TgSk
Krim massif (Slovenia)	Cyclopoida	Acanthocyclops hispanicus	AcHi
Krim massif (Slovenia)	Cyclopoida	Acanthocyclops kiefferi	AcKi
Krim massif (Slovenia)	Cyclopoida	Acanthocyclops sp. K1	AcK1
Krim massif (Slovenia)	Cyclopoida	Acanthocyclops sambugarea	AcSa
Krim massif (Slovenia)	Cyclopoida	Acanthocyclops venustus stammeri	AcVS
Krim massif (Slovenia)	Cyclopoida	Diacyclops charon	DiCh
Krim massif (Slovenia)	Cyclopoida	Diacyclops cf. clandestinus	Di2C
Krim massif (Slovenia)	Cyclopoida	Diacyclops languidoides goticus	DiLG
Krim massif (Slovenia)	Cyclopoida	Diacyclops cfr. zschokkei	Di2Z
Krim massif (Slovenia)	Cyclopoida	Diacyclops sp.K1	DiK1
Krim massif (Slovenia)	Cyclopoida	Graeteriella (Graeteriella) unisetigera	GrUn
Krim massif (Slovenia)	Cyclopoida	Speocyclops infernus	Sp2I
Krim massif (Slovenia)	Cyclopoida	Speocyclops n.sp.	SpK1
Krim massif (Slovenia)	Harpacticoida	Bryocamptus (R.) balcanicus	BrRB
Krim massif (Slovenia)	Harpacticoida	Bryocamptus (B.) pyrenaicus	BrRP
Krim massif (Slovenia)	Harpacticoida	Ceuthonectes serbicus	CeSe
Krim massif (Slovenia)	Harpacticoida	Elaphoidella charon	EiCh
Krim massif (Slovenia)	Harpacticoida	Elaphoidella cvetkae	EiCv
Krim massif (Slovenia)	Harpacticoida	Elaphoidella elaphoides	EiEI
Krim massif (Slovenia)	Harpacticoida	Elaphoidella jeanneli	EiJe
Krim massif (Slovenia)	Harpacticoida	Elaphoidella sp. K1	EiK1
Krim massif (Slovenia)	Harpacticoida	Elaphoidella stammeri	EiSt
Krim massif (Slovenia)	Harpacticoida	Elaphoidella tarmani	EiTt
Krim massif (Slovenia)	Harpacticoida	Moraria (M.) stankovitshi	MMSt
Krim massif (Slovenia)	Harpacticoida	Morariopsis dumonti	MsisD
Krim massif (Slovenia)	Harpacticoida	Morariopsis scotenophila	MsisS
Krim massif (Slovenia)	Harpacticoida	Nitocrella hirta	NiJ1
Krim massif (Slovenia)	Harpacticoida	Nitokra divaricata	NkDi
Krim massif (Slovenia)	Harpacticoida	Parastenocaris gertrudae	PaGe
Krim massif (Slovenia)	Harpacticoida	Parastenocaris italica	PaIt
Krim massif (Slovenia)	Harpacticoida	Parastenocaris noll alpina	PaNA
Krim massif (Slovenia)	Isopoda	Monolistra caeca intermedia	MCIn
Krim massif (Slovenia)	Isopoda	Monolistra caeca absoloni	MCAb
Krim massif (Slovenia)	Isopoda	Microcharon indet.	Mi1
Krim massif (Slovenia)	Amphipoda	Niphargus indet.	Np1
Krim massif (Slovenia)	Amphipoda	Niphargus giovanovici multipennatus	NpJM
Krim massif (Slovenia)	Amphipoda	Niphargus longidactylus	NpLg
Krim massif (Slovenia)	Amphipoda	Niphargus sp. K1	NpK1
Krim massif (Slovenia)	Amphipoda	Niphargus podpecanus	NpPo
Krim massif (Slovenia)	Amphipoda	Niphargus rejici	NpRe
Krim massif (Slovenia)	Amphipoda	Bogidiella albertimagni	BoAl
Krim massif (Slovenia)	Amphipoda	Niphargus sp. K2	NpK2
Krim massif (Slovenia)	Amphipoda	Niphargus stygius+valvasori	NpSV
Krim massif (Slovenia)	Bathynellacea	Bathynella ?	Ba?
Krim massif (Slovenia)	Bathynellacea	Bathynella natans	BaNa
Krim massif (Slovenia)	Bathynellacea	Bathynella sp .K1	BaK1
Krim massif (Slovenia)	Bathynellacea	Bathynella sp. K2	BaK2



Krim massif (Slovenia)	Bathynellacea	Bathynella slovenica sp. K1	BaSK1
Krim massif (Slovenia)	Bathynellacea	Proasellus vulgaris	PrVu
Krim massif (Slovenia)	Acari	Balcanohydracarus alveolatus	BlcA
Krim massif (Slovenia)	Acari	Chappuisides thienemanni	CpTh
Krim massif (Slovenia)	Acari	Frontipodopsis reticulatifrons	FrRe
Krim massif (Slovenia)	Acari	Hungarohydracarus subterraneus	HuSub
Krim massif (Slovenia)	Acari	Lethaxona cavifrons	LetC
Krim massif (Slovenia)	Acari	Ljanina cf. macilenta	LjMa
Krim massif (Slovenia)	Acari	Momonisia phreatica	MnPh
Krim massif (Slovenia)	Acari	Stygomomonina latipes	SgLa
Lessinian Mount. (Italy)	Polychaeta	Troglochaetus beranecki	TroB
Lessinian Mount. (Italy)	Oligochaeta	Cernovitoviella cf. parviseta	Cv2P
Lessinian Mount. (Italy)	Oligochaeta	Gianius sp. I1	GiI1
Lessinian Mount. (Italy)	Oligochaeta	Gianius sp. I2	GiI2
Lessinian Mount. (Italy)	Oligochaeta	Gianius cf. labouchensis	Gi2L
Lessinian Mount. (Italy)	Oligochaeta	Haber indet.	Ha1
Lessinian Mount. (Italy)	Oligochaeta	Parvidrilus spelaeus	PvSp
Lessinian Mount. (Italy)	Oligochaeta	Phalodrilinae indet.	Ph1
Lessinian Mount. (Italy)	Oligochaeta	Pristina sp. I1	PrI1
Lessinian Mount. (Italy)	Oligochaeta	Pristina sp. I2	PrI2
Lessinian Mount. (Italy)	Oligochaeta	Pristina sp. I3	PrI3
Lessinian Mount. (Italy)	Oligochaeta	Rhyacodrilus cf. dolci	Rh2D
Lessinian Mount. (Italy)	Oligochaeta	Rhyacodrilus sp. I2	RhI2
Lessinian Mount. (Italy)	Oligochaeta	Rhyacodrilus sp. I1	RhI1
Lessinian Mount. (Italy)	Oligochaeta	Trichodrilus sp. I1	TrI1
Lessinian Mount. (Italy)	Oligochaeta	Trichodrilus cf. pragensis	Tr2P
Lessinian Mount. (Italy)	Gastropoda	Iglica concii	IgCo
Lessinian Mount. (Italy)	Gastropoda	Paladilhliopsis virei	PIVi
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops cf. maggii	Di2M
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops cf. clandestinus	Di2C
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops italianus	DiIt
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops paolae	DiPa
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops ruffoi	DiRu
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops sp. I1	DiI1
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops sp. I2	DiI2
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops sp. I3	DiI3
Lessinian Mount. (Italy)	Cyclopoida	Diacyclops sp. I4	DiI4
Lessinian Mount. (Italy)	Cyclopoida	Graeteriella (G.) unisetigera	GrUn
Lessinian Mount. (Italy)	Cyclopoida	Speocyclops cf. infernus	Sp2I
Lessinian Mount. (Italy)	Cyclopoida	Speocyclops sp. I1	SpI1
Lessinian Mount. (Italy)	Harpacticoida	Bryocamptus sp. I1	BrI1
Lessinian Mount. (Italy)	Harpacticoida	Ceuthonectes serbicus	CeSe
Lessinian Mount. (Italy)	Harpacticoida	Ectinosomatidae gen. I1 sp. I1	EcI1
Lessinian Mount. (Italy)	Harpacticoida	Elaphoidella elaphoides	EIEI
Lessinian Mount. (Italy)	Harpacticoida	Elaphoidella phreatica	EIPh
Lessinian Mount. (Italy)	Harpacticoida	Elaphoidella pseudophreatica	EIPs
Lessinian Mount. (Italy)	Harpacticoida	Elaphoidella sp. I1	EII1
Lessinian Mount. (Italy)	Harpacticoida	Lessinocamptus caoduroi	LeCa
Lessinian Mount. (Italy)	Harpacticoida	Lessinocamptus insoletus	LeIn
Lessinian Mount. (Italy)	Harpacticoida	Lessinocamptus pivai	LePi
Lessinian Mount. (Italy)	Harpacticoida	Lessinocamptus sp. I1	LeI1
Lessinian Mount. (Italy)	Harpacticoida	Lessinocamptus sp. I2	LeI2
Lessinian Mount. (Italy)	Harpacticoida	Moraria (M.) sp. I1	MMI1
Lessinian Mount. (Italy)	Harpacticoida	Moraria (M.) stankovitchi	MMSt

Lessinian Mount. (Italy)	Harpacticoida	Nitocrella psammophila	NiPs
Lessinian Mount. (Italy)	Harpacticoida	Parapseudoleptomesochra italica	PpIt
Lessinian Mount. (Italy)	Harpacticoida	Paramorariopsis sp. I1	PmI1
Lessinian Mount. (Italy)	Harpacticoida	Paramorariopsis sp. I2	PmI2
Lessinian Mount. (Italy)	Harpacticoida	Parastenocaris gertrudae	PaGe
Lessinian Mount. (Italy)	Harpacticoida	Parastenocaris italica	PaIt
Lessinian Mount. (Italy)	Harpacticoida	Parastenocaris sp. I1	PaI1
Lessinian Mount. (Italy)	Harpacticoida	Parastenocaris sp. I2	PaI2
Lessinian Mount. (Italy)	Harpacticoida	Parastenocaris sp. I3	PaI3
Lessinian Mount. (Italy)	Harpacticoida	Parastenocaris sp. I4	PaI4
Lessinian Mount. (Italy)	Ostracoda	Candoninae gen. sp. (rectangular) I1	CnI1
Lessinian Mount. (Italy)	Ostracoda	Candoninae gen. sp. (rectangular) I2	CnI2
Lessinian Mount. (Italy)	Ostracoda	Candoninae gen. sp. (trapez) I1	CnI3
Lessinian Mount. (Italy)	Ostracoda	Candoninae gen. sp. (trapez) I2	CnI4
Lessinian Mount. (Italy)	Ostracoda	Cavernocypris subterranea	CaSu
Lessinian Mount. (Italy)	Ostracoda	Fabaeformiscandona cf. wegelinei	Fa2W
Lessinian Mount. (Italy)	Ostracoda	Pseudocandona cf. eremita	Ps2E
Lessinian Mount. (Italy)	Isopoda	Monolistra (Typhlosphaeroma) berica	MTBe
Lessinian Mount. (Italy)	Isopoda	Monolistra (Monolistra) cf. coeca	MM2C
Lessinian Mount. (Italy)	Amphipoda	Niphargus aquilex	NpAq
Lessinian Mount. (Italy)	Amphipoda	Niphargus bajuvaricus grandii	NpBG
Lessinian Mount. (Italy)	Amphipoda	Niphargus cf. costozzae	Np2C
Lessinian Mount. (Italy)	Amphipoda	Niphargus cf. forelii	Np2F
Lessinian Mount. (Italy)	Amphipoda	Niphargus cf. lessiniensis	Np2L
Lessinian Mount. (Italy)	Amphipoda	Niphargus forelii	NpFor
Lessinian Mount. (Italy)	Amphipoda	Niphargus galvagnii	NpGa
Lessinian Mount. (Italy)	Amphipoda	Niphargus lessiniensis	NpLe
Lessinian Mount. (Italy)	Amphipoda	Niphargus longidactylus	NpLo
Lessinian Mount. (Italy)	Amphipoda	Niphargus similis	NpSi
Lessinian Mount. (Italy)	Amphipoda	Niphargus tamaninii	NpTa
Lessinian Mount. (Italy)	Amphipoda	Niphargus transitivus	NpTr
Lessinian Mount. (Italy)	Thermosbaenacea	Thermosbaenacea indet.	Th1
Lessinian Mount. (Italy)	Bathynellacea	Bathynella indet.	Ba1
Lessinian Mount. (Italy)	Bathynellacea	Bathynella (Bathynella) sp. I1	BaBI1
Lessinian Mount. (Italy)	Bathynellacea	Bathynella (Bathynella) sp. I5	BaBI5
Lessinian Mount. (Italy)	Bathynellacea	Bathynella (Lombardobathynella) sp. I1	BaLI1
Lessinian Mount. (Italy)	Bathynellacea	Bathynella (Lombardobathynella) sp. I2	BaLI2
Lessinian Mount. (Italy)	Bathynellacea	Meridiobathynella sp. I1	MeI1
Lessinian Mount. (Italy)	Acari	Stygomomonina latipes	SgLa
Lessinian Mount. (Italy)	Acari	Kongsbergia dentata	KoDe
Lessinian Mount. (Italy)	Acari	Kongsbergia indet.	Ko1
Lessinian Mount. (Italy)	Acari	Lobohalacarus weberi quadriporus	LoWQ
Lessinian Mount. (Italy)	Acari	Soldanellonyx visurgis	SoVi
Lessinian Mount. (Italy)	Acari	Soldanellonyx chappuisi	SoCh
Lessinian Mount. (Italy)	Acari	Halacarellus phreaticus	HaPh
Walloon karst (Belgium)	Gastropoda	Avenionia indet.	Av1
Walloon karst (Belgium)	Oligochaeta	Rhyacodrilus subterraneus	RhSu
Walloon karst (Belgium)	Oligochaeta	Trichodrilus cernovitovi	TrCe
Walloon karst (Belgium)	Oligochaeta	Trichodrilus indet.	Tr1
Walloon karst (Belgium)	Cladocera	Alona phreatica	AloP
Walloon karst (Belgium)	Cyclopoida	Acanthocyclops "venustus"	AcVe
Walloon karst (Belgium)	Cyclopoida	Acanthocyclops sensitivus	AcSe
Walloon karst (Belgium)	Cyclopoida	Diacyclops belgicus	DiBe
Walloon karst (Belgium)	Cyclopoida	Diacyclops "clandestinus"-group	Di1C

Walloon karst (Belgium)	Cyclopoida	Diacyclops"languidoides"-group	Di1L
Walloon karst (Belgium)	Cyclopoida	Graeteriella unisetigera	GrUn
Walloon karst (Belgium)	Cyclopoida	Speocyclops indet.	Sp1
Walloon karst (Belgium)	Ostracoda	Cavernocypris subterranea	CaSu
Walloon karst (Belgium)	Ostracoda	Fabaeformiscandona wegelini	FaWe
Walloon karst (Belgium)	Ostracoda	Pseudocandona zschokkei	PsZs
Walloon karst (Belgium)	Ostracoda	Schellencandona belgica	ScBe
Walloon karst (Belgium)	Ostracoda	Schellencandona triquetra	ScTr
Walloon karst (Belgium)	Isopoda	Proasellus cavaticus	PrCa
Walloon karst (Belgium)	Isopoda	Proasellus hermallensis	PrHe
Walloon karst (Belgium)	Isopoda	Proasellus cf. hermallensis	Pr2H
Walloon karst (Belgium)	Amphipoda	Crangonyx subterraneus	CrSu
Walloon karst (Belgium)	Amphipoda	Niphargus aquilex	NpAq
Walloon karst (Belgium)	Amphipoda	Niphargus fontanus	NpFo
Walloon karst (Belgium)	Amphipoda	Niphargus kochianus ssp. indet.	NpK1
Walloon karst (Belgium)	Amphipoda	Niphargus kochianus dimorphopus	NpKD
Walloon karst (Belgium)	Amphipoda	Niphargus kochianus kochianus	NpKK
Walloon karst (Belgium)	Amphipoda	Niphargus schellenbergi	NpSc
Walloon karst (Belgium)	Amphipoda	Niphargus virei	NpVi
Walloon karst (Belgium)	Amphipoda	Niphargus indet.	NpI
Walloon karst (Belgium)	Acari	Lobohalacarus weberi	LoWe
Walloon karst (Belgium)	Acari	Neoacarus hibernicus	NeHi
Walloon karst (Belgium)	Acari	Soldanellonyx chappuisi	SoCh
Walloon karst (Belgium)	Acari	Soldanellonyx visurgis	SoVi
Walloon karst (Belgium)	Acari	Stygomomonina latipes	SgLa
Roussillon (France)	Oligochaeta	Cookidrilus indet.	Coin
Roussillon (France)	Oligochaeta	Trichodrilus longipenis	Trlo
Roussillon (France)	Oligochaeta	Trichodrilus capilliformis	Trca
Roussillon (France)	Oligochaeta	Trichodrilus cf. leruthi	Trle
Roussillon (France)	Oligochaeta	Aktedrilus indet.	Akin
Roussillon (France)	Oligochaeta	Krenedrilus indet.	Krin
Roussillon (France)	Oligochaeta	Rhyacodrilus lindbergi	Rhli
Roussillon (France)	Oligochaeta	Rhyacodrilus cf. lindbergi	Rhcfli
Roussillon (France)	Gastropoda	Moitessiera simoniana	Mosi
Roussillon (France)	Gastropoda	Moitessiera massoti	Moma
Roussillon (France)	Gastropoda	Moitessiera sp. R1	MoR1
Roussillon (France)	Gastropoda	Moitessiera indet.	Moin
Roussillon (France)	Gastropoda	Islamia indet.	Isin
Roussillon (France)	Cladocera	Alona phreatica	Alph
Roussillon (France)	Ostracoda	Mixtacandona sp. R1	MiR1
Roussillon (France)	Ostracoda	Fabaeformiscandona breuili	Fabr
Roussillon (France)	Ostracoda	Fabaeformiscandona wegelini	Fawe
Roussillon (France)	Ostracoda	Dolekiella europaea	Doeu
Roussillon (France)	Isopoda	Faucheria indet.	Fain
Roussillon (France)	Isopoda	Microcharon indet.	Miin
Roussillon (France)	Isopoda	Microcharon sp. R1	MiR1
Roussillon (France)	Isopoda	Microcharon sp. R2	MiR2
Roussillon (France)	Isopoda	Microcharon sp. R3	MiR3
Roussillon (France)	Isopoda	Microcharon sp. R4	MiR4
Roussillon (France)	Isopoda	Microcharon angelieri	Mian
Roussillon (France)	Isopoda	Stenasellidae indet.	Stin
Roussillon (France)	Isopoda	Stenasellus buili	Stbu
Roussillon (France)	Isopoda	Stenasellus virei angelieri	Stvian
Roussillon (France)	Amphipoda	Niphargidae indet.	Niin

Roussillon (France)	Amphipoda	Niphargus angelieri	Nian
Roussillon (France)	Amphipoda	Niphargus delamarei	Nide
Roussillon (France)	Amphipoda	Niphargus gallicus	Niga
Roussillon (France)	Amphipoda	Niphargus thienemanni	Nith
Roussillon (France)	Amphipoda	Niphargus indet.	Niain
Roussillon (France)	Amphipoda	Salentinella delamarei	Sade
Roussillon (France)	Amphipoda	Salentinella sp. R1	SaR1
Roussillon (France)	Amphipoda	Salentinella petiti	Sape
Roussillon (France)	Bathynellacea	Paraiberobathynella (P.) fagei	PaPfa
Roussillon (France)	Bathynellacea	Paraiberobathynella cf. fagei	Pafa
Roussillon (France)	Bathynellacea	Gallobathynella sp.R.1	GaR.1
Roussillon (France)	Bathynellacea	Gallobathynella sp.R.2	GaR.2
Roussillon (France)	Bathynellacea	Gallobathynella sp.R.3	GaR.3
Roussillon (France)	Bathynellacea	Gallobathynella indet. 1	Gain1
Roussillon (France)	Bathynellacea	Gallobathynella indet. 2	Gain2

## **2 SECTION 2 :BIODIVERSITY AND ENVIRONMENT RELATIONSHIPS :**

### **SUMMARY :**

Observational approaches exploring the relationships between the structure of obligate-groundwater invertebrate (i.e. stygobiotic) assemblages and environmental gradients at a regional scale are scarce. The present study is the first attempt to identify the main factors driving the composition of stygobiotic assemblages in multiple regions. Biological (i.e. presence / absence of species) and environmental data (11 variables) collected following a standardized sampling procedure in 6 regions (192 sampling sites per region) distributed in southern Europe were used to examine the responses of multiple species to environmental and palaeogeographic factors.

Multivariate analysis (OMI: Outlying Mean Index) was used to determine the most important factors driving stygobiotic assemblage structure and to identify the ecological preferences of taxa along environmental gradients. In all regions, the overall distribution of species differed significantly from a uniform distribution along the environmental gradient. The habitat breadth of species colonizing the most northern region (i.e. the Walloon karst) was distinctly higher than that of species in southern regions. This corresponds to the view that the most northern regions were recolonized by expansive and ubiquitous species following the eradication of their fauna during the Quaternary glaciations. Between-region comparisons indicated that the geological attributes of sites and to a lesser extent elevation were the main factors driving the structure of stygobiont assemblages. However, geology, elevation, palaeogeographic factors and human activities interacted in a complex way to produce dissimilar patterns of species distribution among regions.

Hierarchical diversity and hierarchical habitat units were used to explore the possibility of using higher taxa species richness as a surrogate of species diversity to explore general biodiversity patterns. PCA (Principal Component Analysis) and RDA (Redundancy Analysis) were applied to taxa x sites biodiversity matrices. The results of the analyses performed at the regional and at the European scale confirm that taxonomic units above the species level can be used efficiently in describing patterns of species richness within regions. Moreover, water chemistry and pollution, geographical location (e.g. history), and the vertical structure of habitats are the main factors driving overall species richness patterns throughout PASCALIS countries. The taxa which account for most of overall variance are the harpacticoids, followed by gastropods, ostracods and amphipods, which may be considered as potential indicators of biodiversity patterns and need to be included in any monitoring study.

## **2.1 INTRODUCTION :**

Observational approaches exploring the relationships between stygobiont community structure and environmental gradients at a regional scale are scarce (Gibert et al. 1994). Moreover, we are unaware of any published work reporting on the main environmental factors driving the composition of stygobiont assemblages in multiple regions. The present chapter is based on environmental and biological data collected in 6 regions within the framework of the European project PASCALIS. The objectives of the present study are as follow:

- 1) to determine the main environmental factors driving assemblage structure and biodiversity patterns in the study regions
- 2) to identify the ecological preferences of species and study their distributional ecology
- 3) to identify species assemblages which preferentially occur together in similar habitats
- 4) to explore the relationship between patterns of hierarchical diversity (species, genus, family or higher taxa level) testing the possibility of using higher taxa as surrogates of species in biodiversity studies.

## **2.2 MATERIALS AND METHODS :**

### ***2.2.1 Data sets and statistical methods used in the analysis of species-environment relationships :***

Data were collected following a standardized sampling procedure in 6 regions distributed in southern Europe (WP4: Sampling design; Malard et al. 2002): the Walloon karst (Belgium), the meridional Jura (Eastern France), the Roussillon region (France), the Cantabria (Spain), the Lessinian mountains (Italy), and the Krim massif (Slovenia). In each region, the sampling strategy involved the collection of stygobiont species and the measurement of environmental variables at 192 sites, which were evenly distributed among 4 habitats (1-unsaturated zone of karst aquifers; 2-saturated zone of karst aquifer; 3-hyporheic zone, 4-ground water in unconsolidated sediments) of 4 hydrogeographic basins. The species data set provided by WP6A contained for each site the presence (1) or absence (0) of species. The number of sites and species used for analysis in each region is provided in Table 1.

Region	Number of sites	Number of taxa
Meridional Jura (France)	192	61
Cantabrica (Spain)	189	67
Krim Massif (Slovenia)	187	94
Lessinian Mountains (Italy)	197	89
Walloon karst (Belgium)	201	34
Roussillon (France)	187	44

***Table 1:*** Number of sites and species in each region

The environmental data set provided by WP5 leader (WP5: field data collection, Brancelj 2004) contained for each site the values of the following environmental variables: elevation (m above sea level), hydrogeological variables (i.e. geology and hydrological connectivity), physico-chemical variables (i.e. temperature, pH, specific conductance, dissolved oxygen, calcium, magnesium, nitrates, phosphates), land cover, and distance from the Würmian glacier borders. Table 2 shows the environmental variables used for analysis in each region. We explain below the way some variables were introduced into the analysis; details concerning the measurement of variables can be found in Brancelj 2004.

Geology: In each region, we assigned a score to distinct geological formations. This score increased with decreasing permeability and pore size. The number of scores varied from 2 (i.e. 1: karst aquifer and 2: alluvium in the Cantabria and Krim Massif) to 5 (i.e. 1: karst aquifer; 2: coarse alluvium and glacio-fluvial deposits; 3: medium-size alluvium; 4: fine alluvium, glacial till and arena; and 5=clay in the meridional Jura) depending on the degree of geological information available in a region.

Hydrological connectivity: This variable was used to assess the strength of hydrologic linkages with the surface environment. Indeed, the degree of hydrological connection with the surface strongly influences the amounts of organic matter and nutrients that reach ground water. A score ranging from 3 (low connectivity) to 8 (high connectivity) was assigned to each site. In the meridional Jura, the transit time (expressed as hours) of water from the soil surface to the groundwater table was estimated for each site.

Distance to the glacier: Four of six regions were partially covered by the glaciers during the Quaternary. Because Quaternary glaciations probably affected the distribution of stygobionts, we measured the distance between the sampling sites and the border of the nearest Quaternary glacier.

Land cover: In four of the six regions, a scoring system ranging from 1 to 5 was used to indicate the dominant land use in the “catchment area” of each sampling site (see Brancelj

2004 for the delineation of the catchment area of each sampling site). The score value increased with decreasing human pressure at the land surface in the Roussillon region (France), the Lessinian mountains (Italy), and the Krim massif (Slovenia) and increased with decreasing vegetation cover in the Cantabria (Spain). In the Walloon karst (Belgium) and the meridional Jura (Eastern France), the proportions of different land uses in the catchment area of each sampling site (arcsine transformed data) were introduced as quantitative variables in the analysis.

	Jura	Cantabrica	Krim	Lessinian	Walloon	Roussillon
Number of environmental variables	16	11	10	13	18	12
Elevation (m)	yes	yes	yes	yes	yes	yes
Geology	1 to 5	1 to 2	1 to 2	1 to 4	1 to 4	1 to 3
Distance to the glacier (km)	yes	no	yes	yes	yes	no
Hydrological connectivity	hour	3 to 8	3 to 8	3 to 8	3 to 8	3 to 8
Temperature (°C)	no	yes	no	yes	yes	yes
pH	yes	yes	yes	yes	yes	yes
Specific conductance (µS/cm)	yes	yes	yes	yes	yes	yes
Dissolved oxygen (mg/L)	yes	yes	yes	yes	yes	yes
Calcium (mg/L)	yes	yes	no	yes	yes	yes
Magnesium (mg/L)	yes	yes	yes	yes	yes	yes
Nitrates (mg/L)	yes	yes	yes	yes	yes	yes
Phosphates (mg/L)	yes	no (< DL)	no (<DL)	yes	yes	yes
Land cover	see below	1 to 4	1 to 5	1 to 5	see below	1 to 5
Artificial land (%)	yes				yes (2)	
Intensive agriculture (%)	yes				yes	
Extensive agriculture (%)	yes				yes (2)	
Deciduous forest (%)	yes				yes	
Mixed and coniferous forest (%)	yes					

**Table 2:** Environmental variables in each region (DL: detection level)

The OMI analysis (Dolédéc et al. 2000) was used to determine the most important factors driving stygobiont community structure and to identify the ecological preferences of taxa. This two-table ordination method provides an integrated description of species-environment relationships by separating habitat preferences of taxa along an environmental gradient. This multivariate method decomposes the variance into three components. The OMI (Outlying Mean Index), or taxon marginality, measures the distance between the mean habitat conditions used by a taxon (taxon centroid) and the mean habitat conditions in ground water of a region. The tolerance which corresponds to the dispersion of sampling sites containing a taxon along the environmental gradient represents a measurement of habitat breadth. The residual tolerance represents the proportion of variability in the habitat of a taxon that is not accounted for by measured environmental variables. The OMI analysis was used to place taxa



along the environmental gradient based on a maximization of their average marginality. A Monte-Carlo permutation test was used to check the statistical significance of the marginality for each taxon as well as the average marginality of all taxa.

The OMI analysis and graphical displays were performed separately for each region using ADE-4 software (Thioulouse et al. 1997). We provide below a brief synthesis of the results for all regions including two figures (axes 1 and 2 of the analysis) that summarize the results of the OMI analysis. All figures are produced using an identical one-axis representation which successively shows from left to right:

Left panel. Canonical weights of environmental variables. The most important environmental variables driving community structure occur at the upper and lower ends of the axis.

Middle panel. Factorial scores of sampling sites. Except for the Lessinian Mountains, labels along axis 1 show the different geological formations. For the Lessinian Mountains, labels along axis 1 indicate the elevation of the sampling sites which appears to be the main environmental variable influencing community structure.

Right panel. Distribution of taxa along the environmental gradient as a function of their weighted average position along site scores. The sizes of black circles are proportional to the total frequencies of taxa. Grey circles represent sites in which a taxon occurs. Vertical lines correspond to standard deviations. Asterisks indicate taxa whose distribution deviates significantly from a uniform distribution along the environmental gradient. Codes of taxa are provided in Appendix 1 (section 1).

### ***2.2.2 Data sets and statistical methods used in the analysis of biodiversity patterns in habitat units :***

Data sets (sites x species matrices) were re-arranged using Excel software and the habitat hierarchical scheme was considered for data analysis following PASCALIS protocol. The following tables were built for each region:

a) Habitat x species matrix. Habitats were distinguished for each basin in Ku (1: karstic unconsolidated), Ks (2: karstic consolidated), Ph (3: porous hyporheic), Ps (4: porous phreatic); the higher hierarchical level included in the analysis deals with karstic (K) and unconsolidated sediments (P). Considering that the basin effect on sampling effort is low (chapter 1: results of DCOA), each basin may be considered as a replicate for the estimation of habitat biodiversity in each region.

b) Habitat x genera, habitat x families and habitat x higher taxa matrices. Cell values include the number of species of each taxonomical unit. Higher taxa of stygobionts were defined as follows in Table 3.

Higher taxa	Code	Cantabria	Jura	Walloon	Lessinia	Krim
Annelida	ANN	*	*	*	* (1)	*
Gastropoda	GAS	*	*	*	*	*
Cladocera	CLA		* (2)	* (2)		
Calanoida	CAL					* (2)
Cyclopoida	CYC	*	*	*	*	*
Harpacticoida	HAR	*	*		*	*
Ostracoda	OST	*	*	*	*	*
Isopoda	ISO	*	*	*	*	*
Amphipoda	AMP	*	*	*	*	*
Bathynellacea	BAT	*	*		*	*
Thermosbaenacea	THE				* (2)	
Acari	ACA	*	* (3)	*	*	* (3)
Coleoptera	COL		* (2)			

**Table 3:** higher taxa codes and their presence in the 5 selected regions

(1) Including Oligochaeta and Polychaeta

(2) Not considered in the analyses at European level because of their low rate of occurrence, but included in total biodiversity

(3) Not identified at species level in Slovenia; not identified at all in Jura; not considered in the analyses at European level, but included in total biodiversity

Considering that dominant taxa are cyclopoid and harpacticoid copepods, the lack of identification at species level of these orders prevented us to include Roussillon data set in this analysis.

c) Habitat x environmental parameters. The following parameters were selected being measured in a homogeneous way throughout Europe:

Geography		Chemistry		Habitat		
Parameter	Code	Parameter	Code	Parameter	Code	Values
Latitude	Lat	pH	pH	Karstic	Karst	1 = Ku
Longitude	Lon	Conductivity	Cond			2 = Ks
Elevation	Z	Dissolved oxygen	DO	Porous	Por	1 = Ph
		Calcium	Ca			2 = Ps
		Magnesium	Mg			
		Nitrates	NO3			

**Table 4:** Environmental parameters selected to study biodiversity patterns

Species richness (code: TOT) was added to the matrix. Mean values of the environmental parameters in each habitat were considered for data analysis.

Multivariate statistical analyses were performed at various hierarchical levels to test the choice of the habitat level for biodiversity analysis. The following multivariate statistical analyses were performed:

- a) Principal Component Analysis (PCA) with (environmental parameters) or without (biodiversity values) standardization, after inspecting the linear relationships between parameters
- b) Redundancy Analysis (RDA) using environmental parameters, biodiversity of higher taxa and habitats
- c) simple regression analyses between biodiversity, PCA/RDA axes and environmental parameters.

PCA was performed using MVSP version 3.0. RDA was performed using CANOCO software (Ter Braak and Smilauer, 2002). Regression analyses and graphics were implemented in Excel datasheets and NCSS software.

## **2.3 RESULTS AND DISCUSSION :**

### **2.3.1 Factors driving community structure :**

The two first axes of the OMI analysis accounted in average for 66 % of the marginality of all taxa (Table 5). In 5 regions out of 6, geology (or a surrogate of geology) was the main variable responsible for the formation of axis 1. Elevation appeared as one the most contributing variables along axis 1 in 2 regions (Lessinian Mountains and Roussillon) and along axis 2 in 4 regions (meridional Jura, Cantabria, Krim and Walloon). However, geology and elevation were correlated in 3 regions out of 6 (i.e. Krim, Lessinia, and Roussillon) because karst aquifers and alluvial aquifers occurred preferentially at high and low elevation, respectively. Elevation was correlated with factors such as distance to the glacier in the meridional Jura and Lessinian mountains and land use in the Lessinian Mountains, the Krim Massif and to a lesser extent in the Walloon (artificialized areas and intensive agriculture mostly occurred at low elevation).

### **2.3.2 Differential habitat preferences of species :**

The average marginality of all taxa was highly significant ( $P < 0.0001$ , global Monte-Carlo permutation test) in 5 regions out of 6 indicating a significant influence of environmental and palaeogeographic variables on the distribution of taxa. In the Walloon karst, the average

marginality of all taxa was close to the non significance threshold ( $p=0.05$ ) indicating that most taxa have large habitat breadth. Except in the Walloon karst, at least one third of the taxa (i.e. 34% in Cantabria and Roussillon to 50 % in Lessinia) showed a significant deviation ( $p<0.1$ ) of their habitat preference from a uniform distribution. However, the ecological preferences of taxa with respect to the main environmental variables differed among regions. In the Meridional Jura, Cantabria, and Walloon, a majority of taxa had their centroid displaced towards the most permeable formations (i.e. karst aquifers) whereas a majority of taxa had their centroid displaced towards less permeable formations in the Roussillon and Krim massif (i.e. groundwater in unconsolidated sediments). Taxa occurred preferentially at low elevation in the meridional Jura, Cantabria, and Roussillon, whereas taxa were either distributed all along the altitudinal gradient in the Lessinian Mountains or occurred preferentially at intermediate elevation in the Krim Massif and “high” elevation in the Walloon karst.

	Number of sites	Number of taxa	Number of environmental variables	Number of taxa showing a deviation ( $p<0,10$ ) of their ecological preference	Test on the average marginality of all taxa	Proportion of variability accounted by axis 1 (%)	Most contributing variables along axis 1 (CA)*	Proportion of variability accounted by axis 2 (%)	Most contributing variables along axis 2 (CA)*
Meridional Jura (France)	192	61	16	28	$p<0.0001$	40.6	Geology (28) Dissolved oxygen (22)	22.3	Distance to the glacier (33) Elevation (31)
Cantabrica (Spain)	189	67	11	23	$p<0.0001$	35.4	Geology (27) Land cover (21)	22.4	Elevation (21) Temperature (21) Nitrate (18) Calcium (17)
Krim Massif (Slovenia)	187	94	10	42	$p<0.0001$	58.5	Hydrological connectivity (35), Geology (33)	12.7	Land cover (27), elevation (20)
Lessinian Mountains (Italy)	197	89	13	45	$p<0.0001$	64.2	Elevation (19), distance to the glacier (16)	16.0	pH (24), Calcium (21), geology (19)
(Belgium)	201	34	18	6	$p=0.03$	36.0	Ca (26), specific conductance (19), geology (13)	19.4	Elevation (27), hydrological connectivity (24), temperature (23)
Roussillon (France)	187	44	12	15	$p<0.0001$	42.2	Geology (26), elevation (20), hydrological connectivity (18)	24.8	Specific conductance (34), Calcium (31), Magnesium (15)

**Table 5:** Summary statistics for the OMI analysis (CA: absolute contribution of variables)

### 2.3.3 Habitat-specific assemblages of taxa :

All regions comprised taxa that appeared exclusively either in karst aquifers or ground water in unconsolidated sediments. However, the proportion of karst-exclusive, interstitial-exclusive and ambivalent taxa varied greatly among regions (see Chapter 1 of this deliverable devoted

to sampling strategy). Moreover, in most regions, habitat-specific assemblages of taxa varied among basins.

### **2.3.4 Species-environment relationships in the meridional Jura :**

#### **2.3.4.1 Factors driving community structure :**

The first axis of the OMI analysis, which represented 40.6 % of the explained variance, arranged the sampling sites along a gradient of decreasing permeability and pore size. Highly permeable karst aquifers showing high dissolved oxygen concentration were clearly separated from weakly permeable and dysoxic aquifers forming in fine alluvium, glacial till, colluvium, and clay. The second axis of the analysis (explained variance: 22.3 %) arranged the sampling sites along a gradient of decreasing distance from the Würm glacier and increasing elevation. Sampling sites belonging to high-elevation areas covered by the Würm glaciers (i.e. Oignin basins and parts of the Valouse and Albarine basins) were separated from sampling sites belonging to low-elevation areas that were free of ice during the Quaternary.

#### **2.3.4.2 Differential habitat preferences of species :**

The average marginality of all taxa was highly significant ( $P < 0.0001$ , global Monte-Carlo permutation test) indicating a significant influence of environmental and palaeogeographic variables on the distribution of taxa. Twenty eight of 61 taxa showed a significant deviation ( $p < 0.1$ ) of their habitat preference from a uniform distribution. Almost all taxa were typically more frequent in highly permeable formations than in poorly-permeable formations (i.e. medium/fine alluvium, glacial till, and clay). Indeed, almost all taxa had their centroids (i.e. habitat preference) displaced towards the most permeable geological formations (i.e. 1-karst aquifers and 2-coarse alluvium). Most taxa also had their centroids displaced towards the negative side of axis 2 indicating that they occurred preferentially in low-elevation areas that were free of ice during the Quaternary. The ordination of taxa along axis 2 of the OMI analysis indicated that taxa were successively added to the stygobiont community with increasing distance from the Würm glacier terminus. Indeed, most taxa colonizing high-elevation and formerly glaciated areas had broader habitats (high tolerance), because they also occurred in low-elevation and non formerly glaciated areas.

#### 2.3.4.3 Habitat-specific assemblages of taxa :

We distinguished between 2 distinct taxon assemblages in the meridional Jura. The first group includes *Niphargus* gr. *jovanovici* (Amphipoda), *Salentinella juberthieae* (Amphipoda), *Siettitia avenionensis* (Coleoptera), *Graeteriella* cf. *boui* (Cyclopoida), *Nitocrella* gr. *hirta* sp. J1 (Harpacticoida), and *Microcharon reginae* (Isopoda). In the Jura, these taxa reach their northern distribution limit: they are restricted to permeable alluvial deposits in the southern foot-hills of the meridional Jura (i.e. glacio-fluvial aquifer of the Ain and Albarine River). They are typical components of coarse alluvial and fluvio-glacial aquifers of the Rhône River and also probably of its major tributaries (i.e. Drome River, Isère River, Durance River). These are species-rich aquifers (e.g. Albarine aquifer: 31 species; Rhône River aquifer upstream of Lyon: 38 species), most representatives of which are probably unable to colonize groundwaters of the Jura, Pre-Alps, and Alps.

The second group of taxa, the indicative value of which is restricted to the Jura, includes the Amphipoda *Niphargus virei* and the Isopoda *Caecosphaeroma virei virei*. These karst-exclusive macrocrustaceans preferentially occurs in karst aquifers of the western side of the Jura, which were free of ice during the Quaternary. These low-elevation karst aquifers, which contain more species than high-elevation karst aquifers affected by the Würm glaciers are preferentially colonized by a set of species among which several species of *Speocyclops* (Cyclopoida), *Schellencondona* (Ostracoda), *Bythiospeum* (Mollusca), the Syncarida *Parabathynella* cf. *stygia*, and the Isopoda *Proasellus cavaticus*.







#### **2.3.5.2 Differential habitat preferences of species :**

The average marginality of all taxa was highly significant ( $P < 0.0001$ , global Monte-Carlo permutation test) indicating a significant influence of environmental and palaeogeographic variables on the distribution of taxa. Twenty three of 67 taxa showed a significant deviation ( $p < 0.1$ ) of their habitat preference from a uniform distribution. Almost all taxa were typically more frequent in highly permeable formations, principally limestone than in poorly-permeable formations. Indeed, almost all taxa had their centroids (i.e. habitat preference) displaced towards the most permeable geological formations (i.e. 1-karst aquifers). Most taxa also had their centroids displaced towards the negative side of axis 2 indicating that they occurred preferentially at high temperatures and low concentrations of  $\text{NO}_3$  and Ca, in low-elevation areas near the sea. The ordination of taxa along axis 2 of the OMI analysis indicated that taxa were successively added to the stygobiont community with decreasing elevation.

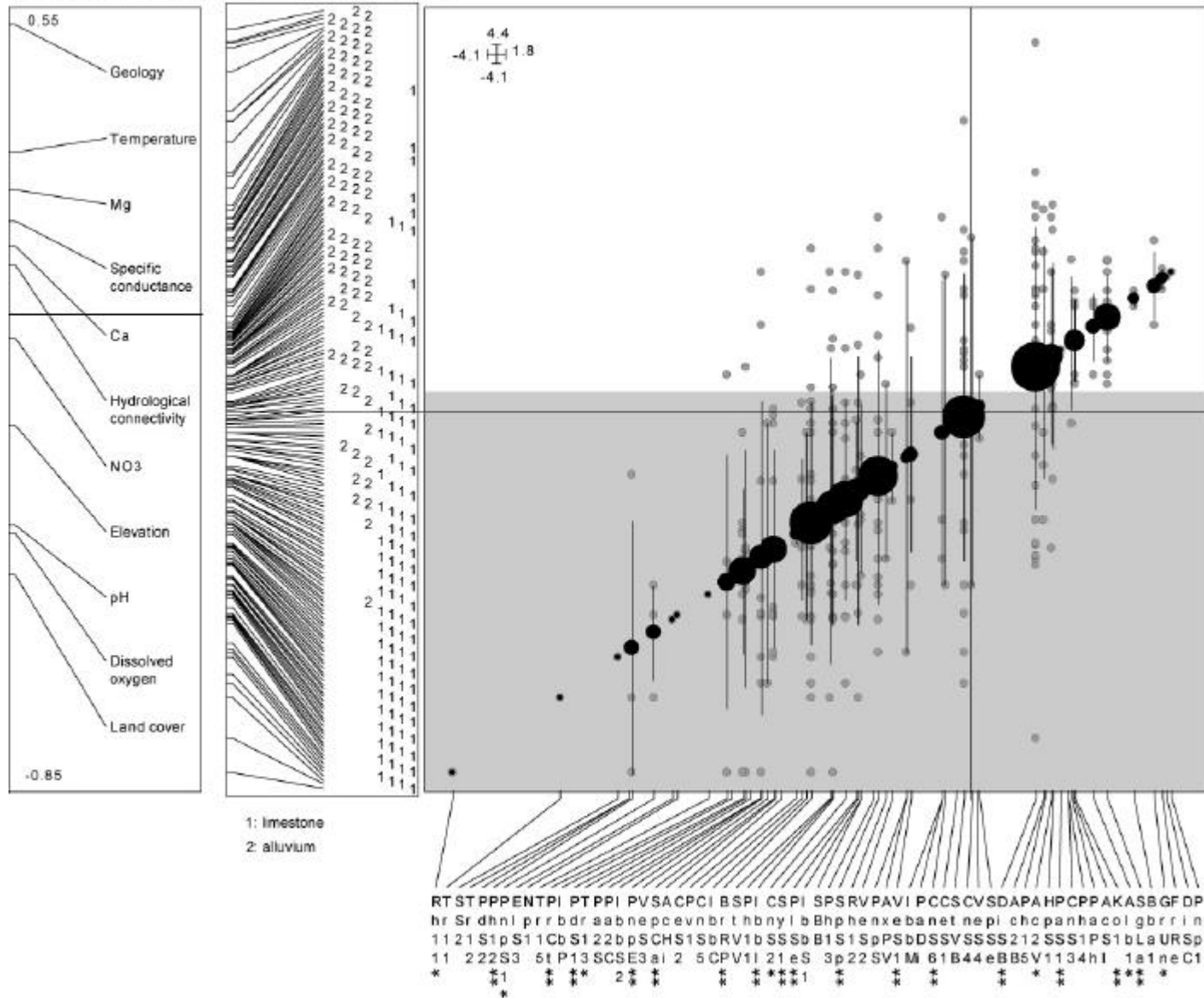
#### **2.3.5.3 Habitat-specific assemblages of taxa :**

The species are distributed differentially through habitat. A group of species with the water mites *Sygomomonia latipes*, *Albaxona* sp. (quite probably *A. minuta*) (only in basin D, Sierra de la Collada) and *Kongsbergia* sp. (Sierra de la Collada and Ojo Guareña basins) all themselves with *Phalodrilus* sp.5 *Acanthocyclops* sp. SI, *Parastenocaris* sp. S1 and *Barbaxonella* sp. This set of species is almost exclusively found in porous aquifer of, and point to a strong stygobiotic tendency, despite water mites are not strictly considered stygobionts.

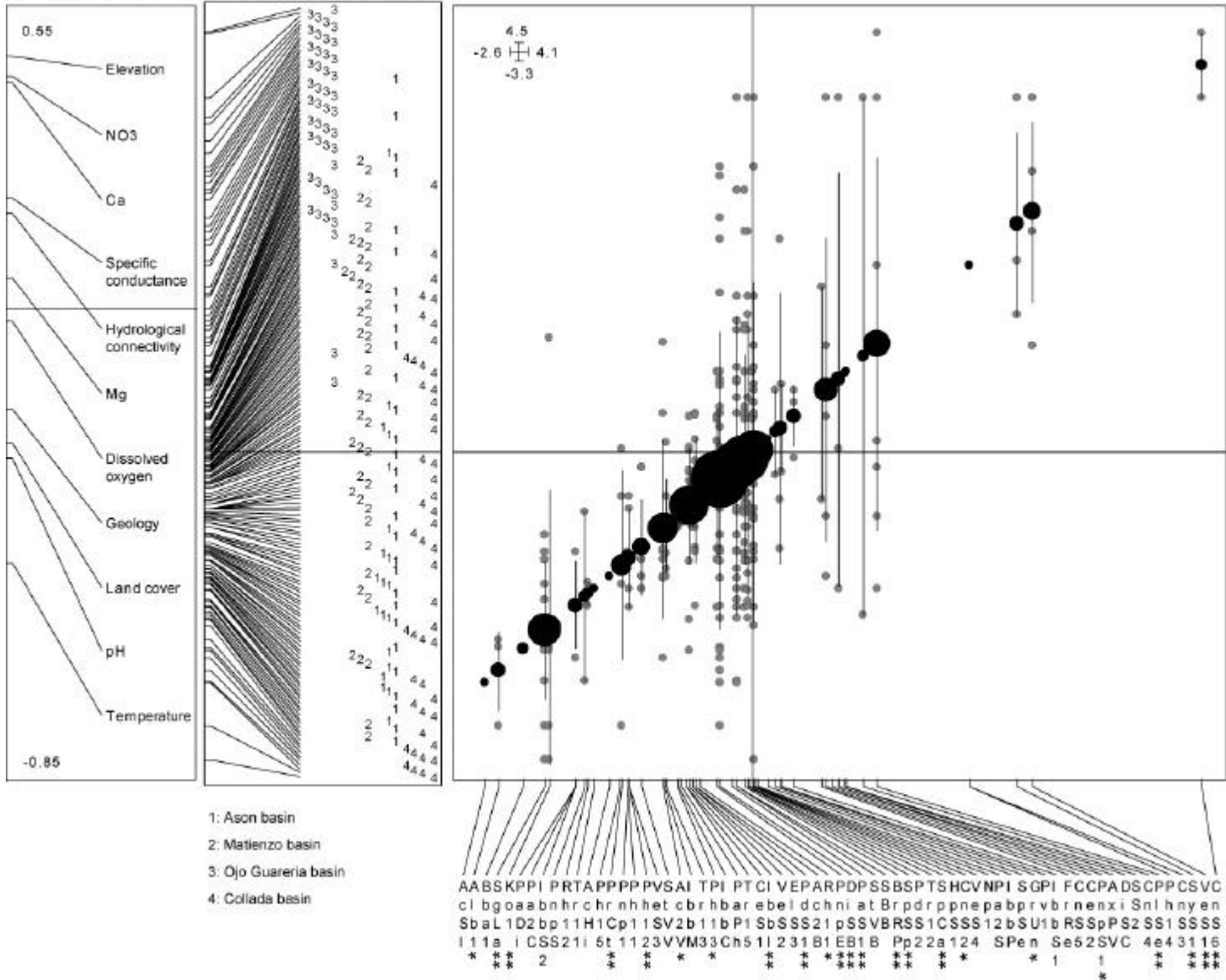
Another species, *Paradoxiclamourella* n. sp. locates itself in the opposite side of the previous set of species. It is exclusively found in caves and one spring (in two basins, Sierra de la Collada and Ason). Whether the distribution of this species is primarily due to biogeographic restrictions is beyond the discrimination of this analysis.

Two additional species, *Bryocamptus* (R.) *pyrenaicus* and *Pseudoniphargus elongatus* point to a somewhat complex determinant. The first is mainly restricted to caves and springs, but is widely distributed in the four basins. *Pseudoniphargus* has been found in the porous aquifer, several caves and one spring (only in two basins, Ason and Matienzo). It can be said that the first species is of wide geographic distribution and the second of wide habitat distribution.

### Cantabrica (Spain) OMI analysis - Axis 1



Cantabrica (Spain) OMI analysis - Axis 2



### **2.3.6 Species-environment relationships in the Krim massif :**

#### **2.3.6.1 Factors driving community structure :**

The first axis of the OMI analysis, which represents 58.5% of the explained variability, arranged the sampling sites along a gradient of decreasing permeability and pore size. Highly permeable karst aquifers developed in limestone are clearly separated from alluvium, which is less permeable. The second axis of the analysis (explained variability: 12.7 %) arranges the sampling sites along a gradient of decreasing intensity of land use reflecting in land cover. In Iška and Borovnišćica basins coniferous and mixed forests prevail, while in Podlipšćica basin both intensive and extensive agriculture land use prevails. Both types of land covers are formally directed by elevation, where “low-elevation” areas (i.e. plain or valleys) are used by agriculture.

#### **2.3.6.2 Differential habitat preferences of species :**

The average marginality of all taxa was highly significant ( $P < 0.0001$ , global Monte-Carlo permutation test) indicating a significant influence of environmental and palaeogeographic variables on the distribution of taxa. Forty-two of 94 taxa showed a significant deviation ( $p < 0.1$ ) of their habitat preference from a uniform distribution. Most of taxa (58/94) were typically more frequent in less permeable formations (alluvium) and more than half of them (33/58) have their centroids displaced exclusively in alluvium in opposite to only 14 /36 in limestone. Most taxa also had their centroids displaced around the centre and in the positive side of axis 2 indicating that they occurred preferentially in forested areas and extensive agriculture. Only few species persist in the plain with intensive agriculture, where river-bed modifications and eutrophication are common. On the opposite side of scale also only few taxa could be found in the most elevated areas, where water bodies are restricted to epikarst zone (pools and small springs).

#### **2.3.6.3 Habitat-specific assemblages of taxa :**

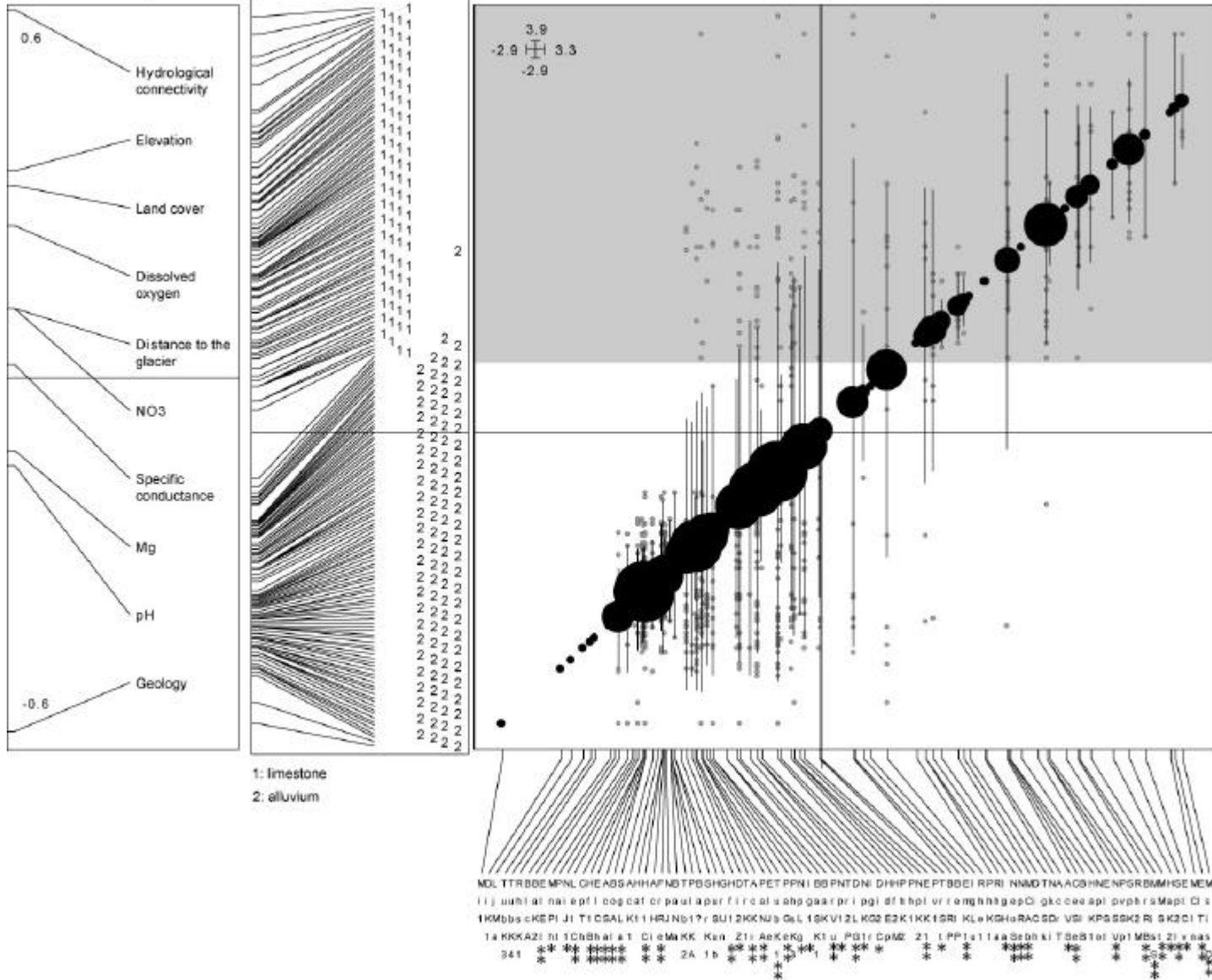
Karst waters are in the area in a close contact and directly feeding the interstitial environment. A number of common cave species are therefore present in the interstitial, blurring differences between both habitat groups and enhancing locally the interstitial biodiversity. This might be

the case with most Gastropoda. Incidentally, a typical cave species could have been found in the interstitial only (like *Neohoratia subpiscinalis*).

The typical interstitial species group consists of a copepod assortment (*Bryocamptus pygmaeus*, *Elaphoidella charon*, *E. elaphoides*, *Parastenocaris gertrudae*, *P. nolli alpina*, *Acanthocyclops hispanicus*, *A. kieferi*), all *Bathynella* spp., the tiny isopods and amphipods (*Microcharon* sp.n.; *Bogidiella albertimagni*, *Niphargus giovanovici multipennatus*), all *Hydracarina*, probably some *Hauffenia* spp. (Gastropoda) and some new taxa of Tubificidae (Oligochaeta). Most interstitial species are present along the Sava valley (where the Krim area hydrographically belongs to), some also outside Slovenia.

Another copepod assemblage characterises karst waters, mainly from unsaturated fissure systems (which was only exceptionally observable in the study localities): *Bryocamptus balcanicus*, *Elaphoidella cvetkae*, *E. stammeri*, *E. tarmani*, *Moraria stankovitchi*, *Morariopsis dumonti*, *Diacyclops charon*, *Speocyclops* spp., *Troglodiptomus sketi*. Explicitly cavernicolous are bigger isopods and amphipods: *Monolistra caeca* (2 subspecies in area), *Niphargus stygius*, *N. podpecanus*, *N. rejici*. Distribution areas of both *Monolistra* ssp. are reaching very little outside parts of the Krim area. *Niphargus rejici rejici* seems to be endemic in a part of it (with *N. r. jadranko* in a Kvarner island), as is also *Elaphoidella millenii*. Some other species are widely spread through the Dinaric karst of Slovenia, some also in neighbouring Croatia or/and NE Italy. *Troglodiptomus* is a holodinaric inhabitant of the saturated karst layer. Since the Krim massif is at the N Dinaric karst border, most Dinaric species are here at their distribution limits.

Krim Massif (Slovenia) OMI analysis - Axis 1









### **2.3.7 Species-environment relationships in the Lessinian Mountains (Italy):**

#### **2.3.7.1 Factors driving community structure :**

The first axis of the OMI analysis, which represented 64.16% of the explained variance, arranged the sampling sites along a gradient of decreasing elevation (19%) and increasing distance to the glacier (16%). The land cover is the third variable along axis 1 contributing 12% to the formation of the axis and is related to different human activities along the altitudinal gradient, the most man-perturbed areas being located along the alluvial plain of the River Adige (southern Lessinian sector) and the less-perturbed ones located at higher elevation areas of the Lessinian Mountains. The second axis of the analysis (explained variance: 16.04%) arranged the sampling sites along a gradient of increasing pH (24%) and decreasing concentration of calcium (21%). The geology (19%) is the third variable of axis 2, showing low-permeable porous aquifers (alluvial aquifers) separated from high-permeable karst aquifers (limestone aquifers). The highest values of pH were detected in unsaturated porous (hyporheos), being more affected by man-induced perturbations (especially agriculture, industries).

#### **2.3.7.2 Differential habitat preferences of species :**

The average marginality of all taxa was highly significant ( $P < 0.001$ , global Monte-Carlo permutation test) indicating a significant influence of environmental and palaeogeographic variables on the occurrence of taxa. Forty five out of 89 taxa showed a significant deviation ( $p < 0.1$ ) of their habitat preference from a uniform distribution. Nevertheless, the stygobiont communities as a whole did not show a clear *preferendum* along the altitudinal gradient and the distance to the front of Quaternary glaciers. As displayed by the plot (axis 1), almost all taxa had their centroids (i.e. habitat preference) distributed along the whole gradient. Several taxa with the highest frequency of occurrence are located at an intermediate level along the geological gradient, showing weak preferences for limestone aquifers. A few number of taxa, with low frequency of occurrence, have been recorded from porous aquifers, defined also by high pH, and low calcium concentration, suggesting some kind of tolerance for basic values of pH (as a reflection of man-induced perturbation) and for porous aquifers (characterized by lower calcium concentration). As regards the palaeogeographic factors, the ordination of taxa along axis 1 of the OMI analysis indicated also that high-altitude karstic habitats harbor low numbers of taxa, with low frequency of occurrence. Most of these species are endemic to

single microhabitats of the epikarstic and vadose zones of the Lessinian karstic aquifer; these habitats are concentrated in the northern sector of the Lessinian mountains. It is likely that vicariance events in different microhabitats, characterized also by high heterogeneity, and some degree of spatial isolation, may have led to the high speciation rate observed in these environments.

### 2.3.7.3 Habitat-specific assemblages of taxa :

We distinguished between 2 distinct groups of taxa in the Lessinian Mountains. The first group includes *Lessinocamptus caoduroi*, *Bathynella* (*Bathynella*) sp. I1, *Bathynella* (*Lombardobathynella*) sp. I2, *Parastenocaris* sp. I4, *Niphargus forelii*. These taxa are exclusively located in epikarstic and vadose zones of the karstic aquifer (the Lessinian caves), in sampling sites distributed in the northern sector of the Lessinian Mountains. They are also characterized by a high degree of endemism. This cenotype is well defined by a biogeographical point of view, being all members derived by freshwater ancestors. Species of *Lessinocamptus* are also rare species, found in general with low abundances. The second community is defined by those taxa which predominantly occur in alluvial aquifers, and in particular in habitat of high pH and low values of calcium. This cenotype is composed by *Parastenocaris* sp.I1, *Parastenocaris* sp. I2, *Parastenocaris italica*, *Parastenocaris gertrudae*, *Soldanellonyx visurgis*, *Fabaeformiscandona* cf. *wegelini*. This cenotype is predominantly linked to the unsaturated porous (hyporheos). The genus *Parastenocaris* appears to be the most diversified taxon in this cenotype, as expected for the high preference of the genus as a whole for the unconsolidated sediments.

There is a group of species which show a non-uniform distribution, namely, Ectinosomatidae gen.I1, sp.I1 (Copepoda Harpacticoida), Thermosbenacea gen. sp., and *Monolistra* cf. *coeca* (Isopoda). The most striking factor assembling these species is their ancient marine origin. They entered groundwater presumably in the Tertiary, and the deep porous habitats operated as refugial zones, where these phylogenetic relicts survived. The new genus of Ectinosomatidae has been also sporadically recorded in two different sampling sites along the alluvial plain of the River Adige, showing the high frequency of occurrence in the Alpone-Tramigna basin, as Thermosbenacea and *Monolistra* cf. *coeca*. The distribution of these species is probably not explained by the variables which strongly describe both axis 1 and axis 2 of OMI. The paleogeography of the area may better explain some enigmatic distributions of these phylogenetic and distributional relicts.





### **2.3.8 Species-environment relationships in the Walloon karst :**

#### **2.3.8.1 Factors driving community structure :**

The first axis of the OMI analysis, which represents 36.03 % of the explained variance, arranged the sampling sites along a gradient of decreasing permeability, calcium concentration and specific conductance. Highly permeable karst aquifers, whose waters are naturally highly conductive, mostly due to high calcium carbonate content, were clearly separated from weakly permeable aquifers. The second axis of the analysis (explained variance: 19.35 %) arranged the sampling sites along a gradient of decreasing elevation and increasing temperature and hydrological connectivity. A closer examination of data suggests that the second axis mostly separates hyporheic sites from the others. If hyporheic sites have indeed a higher hydrological connectivity, high temperatures generally associated to these sites could be a sampling artifact (sites preferentially sampled during summer months). An examination of habitat preferences of species gives more support to this hypothesis.

#### **2.3.8.2 Differential habitat preferences of species :**

The average marginality of all taxa was significant ( $P=0.03$ , global Monte-Carlo permutation test) indicating a significant, while moderate, influence of environmental and palaeogeographic variables on the distribution of taxa. Six of a total of 34 taxa showed a significant deviation ( $p<0.1$ ) of their habitat preference from a uniform distribution. Most taxa were typically more frequent in highly permeable formations, with hard waters (high conductance and high calcium content), characterized by moderate to low hydrological connectivity. The mean habitat conditions used by species (species centroid) are indeed displaced towards the most permeable geological formations (karst aquifers and phreatic zones in unconsolidated sediments). A group of species, mainly constituted by hydrachnidians, is distinctly separated from the bulk of taxa. This group seems to prefer a habitat characterized by low permeability, specific conductance calcium content and elevation, and high temperature and hydrological connectivity, typical of sites located in the hyporheic zone. While the deviation of ecological preferences of most of these taxa is not statistically significant, these results are meaningful as they are in good accordance with the known biology of hydrachnidians (hyporheobiont species). Following this interpretation, the arrangement of sites along a gradient of increasing temperature (second axis) probably results

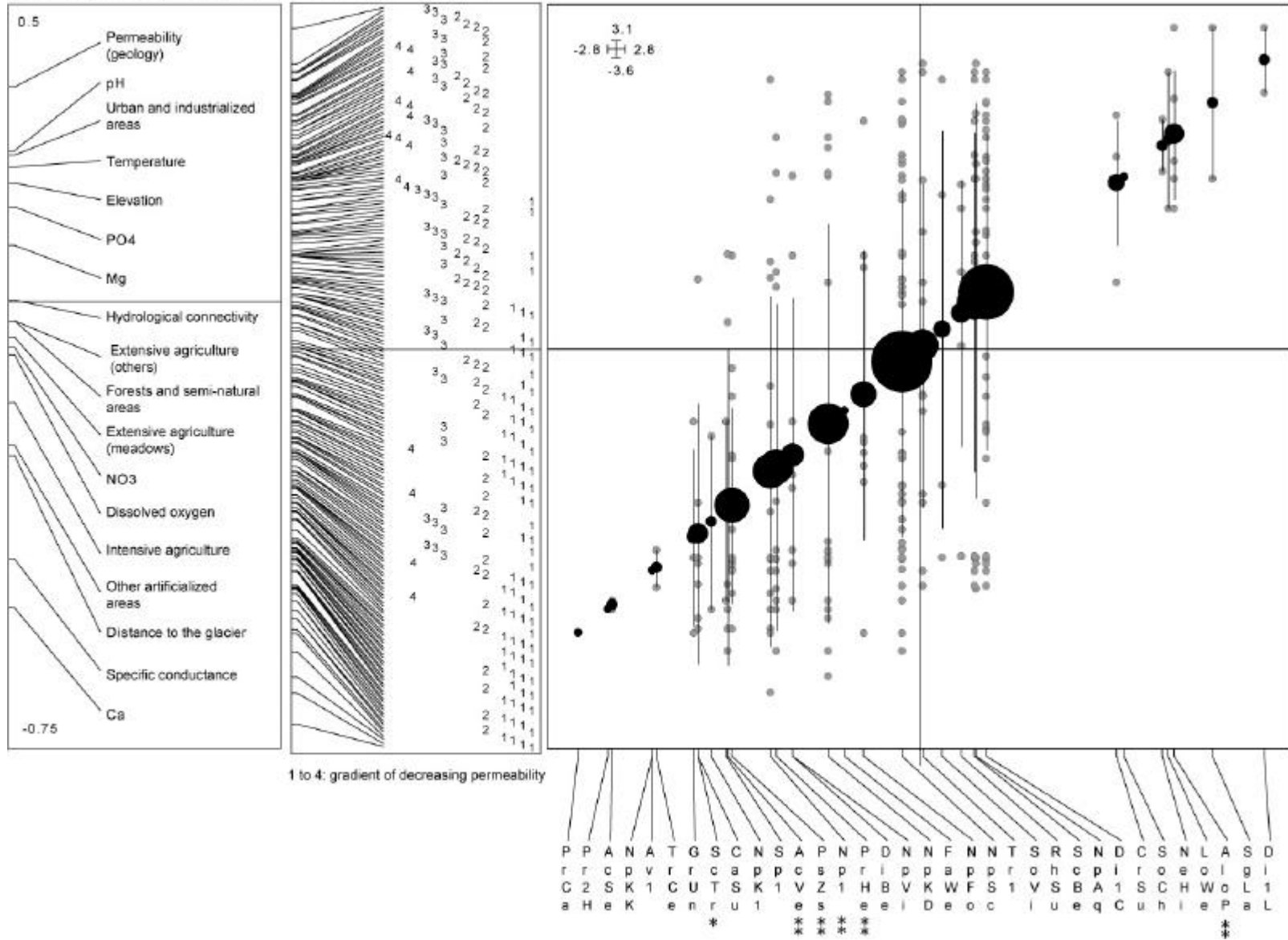
from a sampling artifact (artifactual positive correlation between temperature and hyporheic sites; see above).

The bulk of taxa can be considered as ubiquitous species which seem to have wide ranges of tolerance for most factors. These ecological dispositions probably facilitated recolonization of the Walloon karst, following the eradication of its fauna during the Quaternary glaciations. Even if the maximum ice extension never reached the Walloon area, the permafrost penetrated several dozen of meters below the surface.

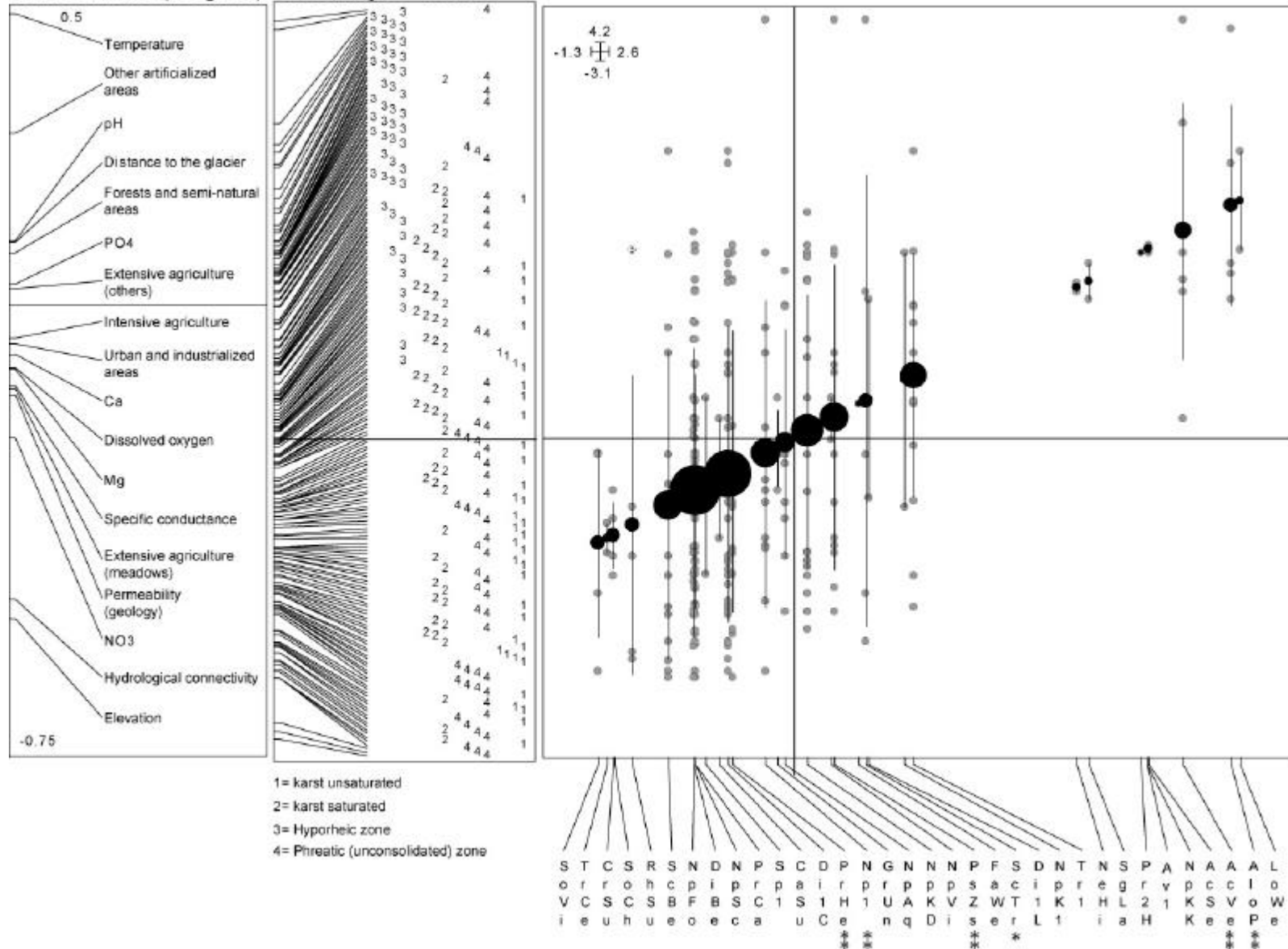
#### **2.3.8.3 Habitat-specific assemblages of taxa :**

The OMI analysis is primarily designed to address the issue of niche separation and niche breadth. Its use in the framework of indicators can be considered as a side effect which has to be interpreted cautiously. In the Walloon karst, the OMI analysis separates two distinct groups of species. One of them is characterized by hydrachnidian species which are indicative of habitat conditions (the hyporheos) rather than a preferential association of species. For instance, while *Lobohalacarus weberi* is grouped together with *Stygomomonium latipes*, both species occur in different basins (the Lesse and the Ourthe rivers, respectively).

Walloon karst (Belgium) OMI analysis - Axis 1



# Walloon karst (Belgium) OMI analysis - Axis 2





### **2.3.9 Species-environment relationships in the Roussillon (France):**

#### **2.3.9.1 Factors driving community structure :**

The first axis of the OMI analysis, which extracted 42.24 % of the explained variance, arranged the sampling sites along a gradient of geology. Karstic aquifers in limestone at moderate elevation, characterized by high hydrological connectivity, were clearly separated from flood plain porous systems (small pore size) at low elevation with lower hydrological connectivity and ground water containing much more nitrates in agricultural areas. The second axis accounted for 24.83 % of the explained variability. It arranged the sampling sites along a decreasing gradient of mineralization and an increasing gradient of dissolved oxygen. Mountains of the axial part of the Pyrénées represent the essential of the Tech and Têt basins when the floodplains are restricted to the last quarter downstream. The sites from these basins, mainly those of the lowlands, were separated from the sites from the Agly and Aude basins where rivers, their tributaries and ground waters are flowing across soluble limestone of the Corbières region.

#### **2.3.9.2 Differential habitat preferences of species :**

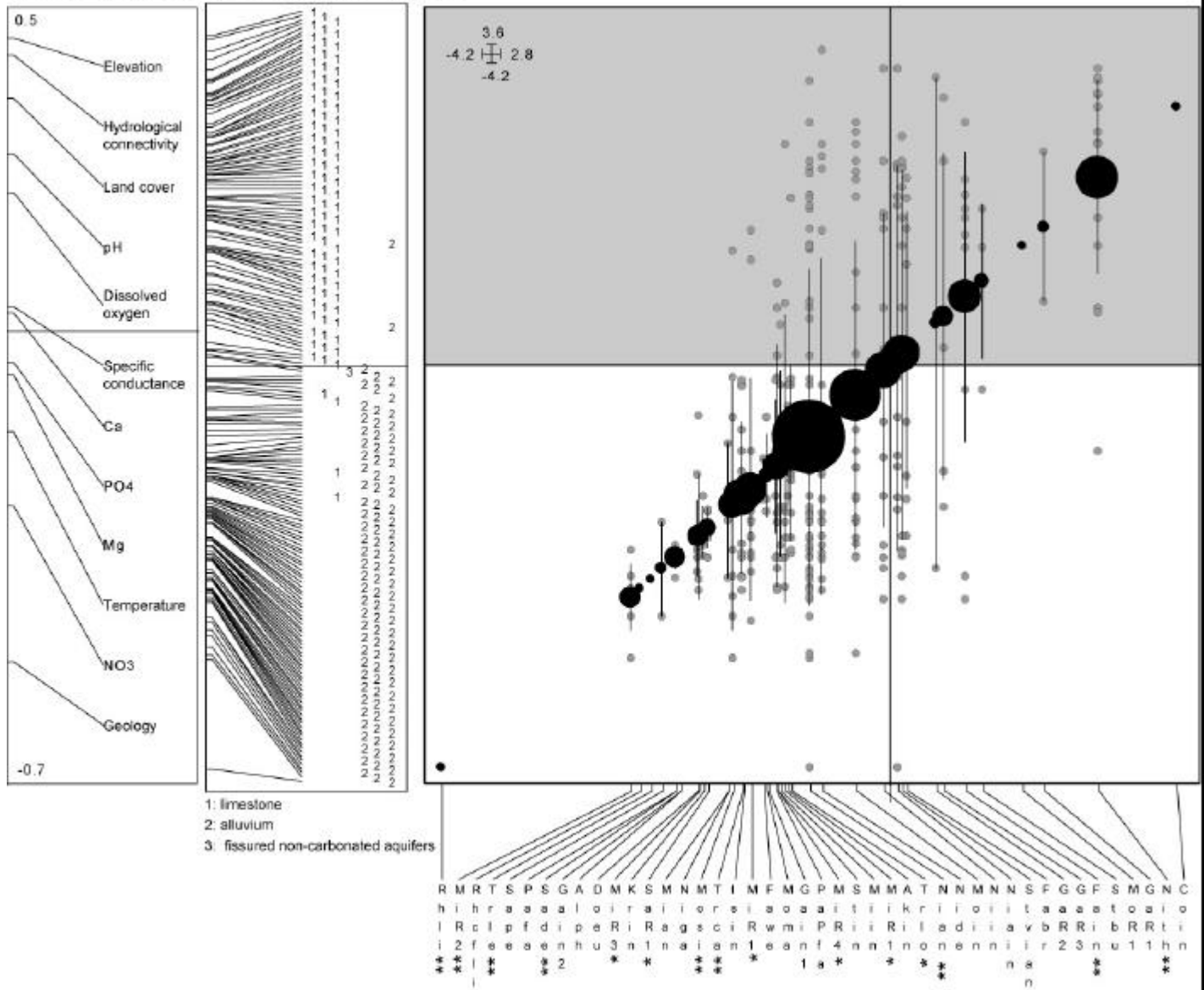
The average marginality of all taxa was highly significant ( $P < 0.0001$ , global Monte Carlo permutation test) indicating a significant influence of environmental variables on the distribution of taxa. Fifteen of 44 taxa showed a significant deviation ( $p < 0.1$ ) of their habitat preference from a uniform distribution. Most of the taxa benefited from formations exhibiting a good permeability both in karsts and coarse alluvium. Alluvium of lowland areas were preferably inhabited by stygobionts, in contrast to karstic areas, as shown by most of the taxa centroids which were located within the negative part of axis 1. Among these taxa were strict interstitial stygobionts (mainly crustaceans) of ancient marine origin. Other more ubiquitous taxa occurred in both karstic and porous aquifers in the four basins (along axis 2).

#### **2.3.9.3 Habitat-specific assemblages of taxa :**

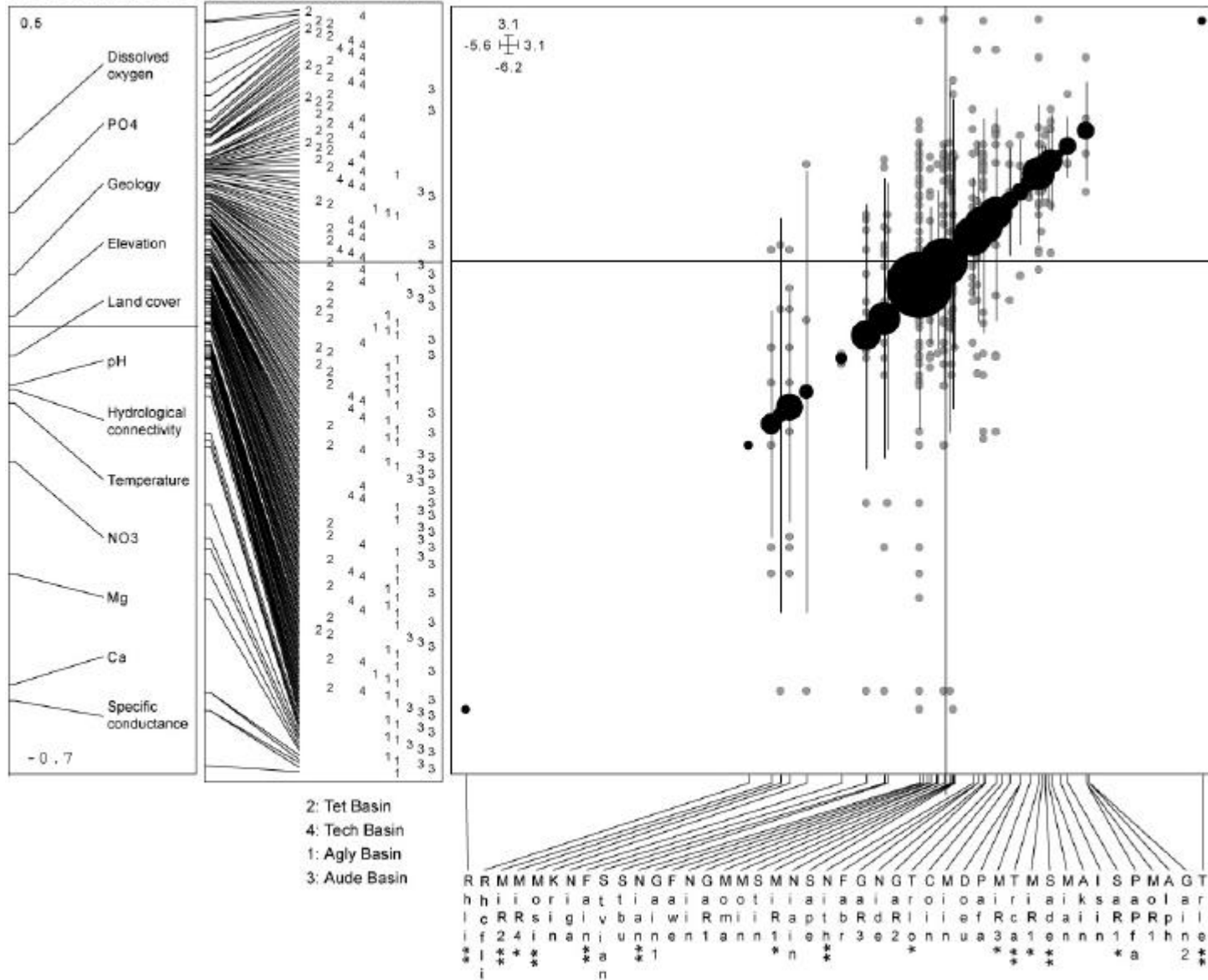
Two groups of taxa were distinguished along the first axis. The first group included *Cookidrilus* (Oligochaeta), *Niphargus thienemanni* (Amphipoda), *Gallobathynella* sp.1 (Syncarida), *Moistesseria* sp.1 (Gastropoda), the Isopoda *Stenasellus buili* and *Faucheria* sp. These species were restricted to karstic areas, mainly in the northern part of the Roussillon.

The second group of taxa was composed of the oligochaetes *Rhyacodrilus lindbergi*, *R. cf lindbergi*, *Trichodrilus cf leruthi* and *T. capilliformis*, the isopods *Microcharon* sp. 2, *M. sp.3*, *M. angelieri* and *M. sp.4*, the amphipods *Salentinella petiti* and *S. delamarei*, the syncarids *Paraiberobathynella* and *Gallobathynella* sp.1, the ostracods *Dolekiella* sp. and *Mixtacandona* sp., and the amphipod *Niphargus gallicus* among others. These taxa live exclusively in porous systems, mostly at low elevation. Moreover, some of these crustaceans were endemic from one basin. Some species such as *Faucheria* sp, and *Stenasellus buili* from the Aude and Verdoube basins, or *Atkedrillus*, *Salentinella delamarei* and *Paraiberobathynella* from the Têt and Tech basins were present in two basins only. Such a distribution may result from geographic proximity, or from common ancestral populations together with similar palaeogeographic history. Other species, e.g. *Niphargus angelieri* and *N. delamarei*, *Trichodrilus longipenis*, *Mixtacandona*, *Fabaeformiscandona wegelini* occurred in all four basins.

### Roussillon (France) OMI analysis - Axis 1



Roussillon (France) OMI analysis - Axis 2



### ***2.3.10 Discussion of species-environment relationships :***

OMI analysis showed that environmental and palaeogeographic factors had a significance influence on the overall distribution of species in all regions. In the Walloon karst, i.e. the most northern region in this study, species had distinctly higher ecological tolerances for most environmental factors than species of all other regions. This corresponds to the view that the most northern regions were recolonized by expansive and ubiquitous species following the eradication of their fauna during the Quaternary glaciations. Indeed, the Walloon karst essentially harbors widely distributed taxa that also occur in southern regions.

The comparison of the results of OMI analysis among regions supported the hypothesis according to which the geological attributes of sites would be the main factor driving the distribution of species. However, results did not support the expectation according to which a majority of species would be more frequent in highly permeable formations than in poorly permeable formations in all regions. Indeed, in the Roussillon and Krim massif, a higher number of taxa were more frequent in unconsolidated sediments than in karst aquifers. However, in these 2 regions, the beneficial influence of high permeability and large pore size might have been obscured by other environmental or palaeogeographic factors. In particular, the ecological preference of many species for groundwaters in unconsolidated sediments might reflect the fact that these groundwater systems occurred mostly at low elevations. Similarly, the apparent lack of a common pattern among species responses to elevation might be due to the negative effect of human activity on groundwater fauna at low elevation in several regions (Lessinian Mountains, Krim massif, and Walloon karst). The adverse effect of human perturbations, including river-bed modifications, might restrict the presence of several species at low elevation sites, thereby altering the natural gradient of increasing biodiversity with decreasing elevation.

Overall, between-region comparisons indicated that palaeogeographic factors, geology, elevation, and human activities interacted in a complex way to produce dissimilar patterns of species distribution among regions. These interaction effects have to be considered for implementing conservation measures in each region. Results clearly indicated that the search for common patterns among groundwater species responses to multiple environmental factors (e.g. geology and elevation) require that a rigorous balanced sampling design has to be applied in regions that were spatially homogeneous from a palaeogeographic point of view. Our ability to separate the ecological preferences of multiple species would have also largely benefited from a better description of geological attributes of sites. The dichotomy between

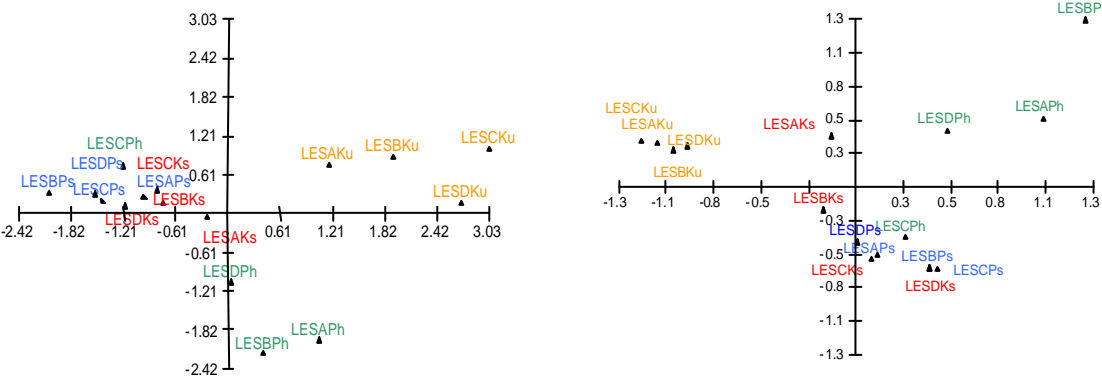
karst aquifers and ground water in unconsolidated sediments was probably too rough for reflecting adequately the diversity of environmental conditions along a gradient of decreasing permeability and pore size.

The OMI analysis was primarily designed to address the issue of habitat separation and habitat breadth along environmental gradients. Its use in the framework of indicators was considered as a side effect which had to be interpreted cautiously. Whereas karst aquifers and ground water in unconsolidated sediments contained a number of exclusive species, assemblages of karst and interstitial exclusive species varied over space in all regions. This indicated that the spatial scale of indicators in groundwater was in most cases relatively small.

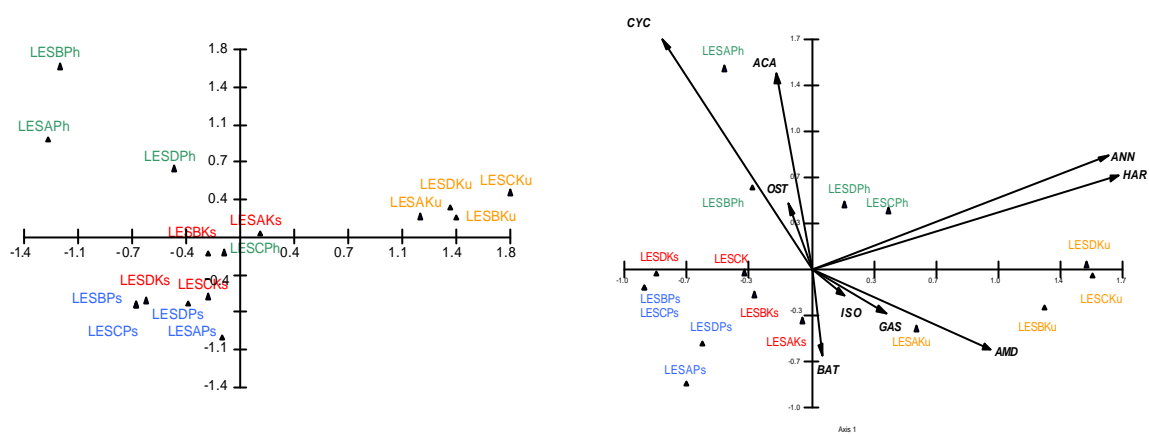
**2.3.11 Factors driving stygobiotic biodiversity trends at a regional scale :**

Species richness patterns and environmental factors driving hierarchical biodiversity were examined in detail within each PASCALIS region using PCA. The results from Lessinia are discussed in detail as an example of hierarchical biodiversity structure analysis.

Species richness (S) of the 16 habitats examined (Ku – unsaturated karst, Ks – saturated karst, Ph - hyporheos, Ps – saturated, phreatic porous, each sampled in four basins, named A, B, C, D: see WP5 tables) may be predicted using the number of genera ( $S = 1.3679 G, r = 0.957, p < 0.0001$ ), families ( $S = 1.9404 F, r = 0.933, p < 0.001$ ) and higher taxa ( $S = 2.7941 HT, r = 0.808, p < 0.001$ ). For this reason, the results of PCA using species (Figure 1 left, cumulative variance explained by the first three axes, cv3: 64.4%), genera (Figure 1 right, cv3: 70.3%), families (Figure 2 left, cv3: 79.39%) and higher taxa (Figure 2 right, cv3: 84.01%) give similar results.



**Figure 1 :** PCA ordination of habitat units in Lessinia using species incidences (left) and species richness within genera (right)

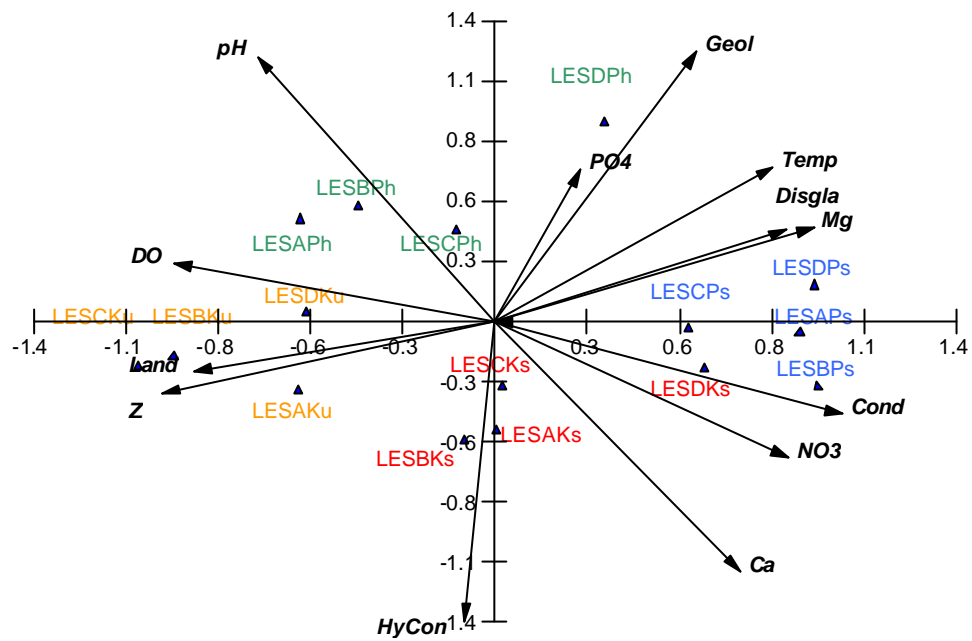


**Figure 2:** PCA ordination of habitat units in Lessinia using species richness within families (left) and higher taxa (right)

In any ordination, the same habitat types are clustered together independently from basin location. The ordination is mainly driven by the species richness within harpacticoids (and annelids), amphipods and cyclopoids (and acari); a good separation of habitats is displayed using higher taxa biodiversity in the biplot of Figure 2 right.

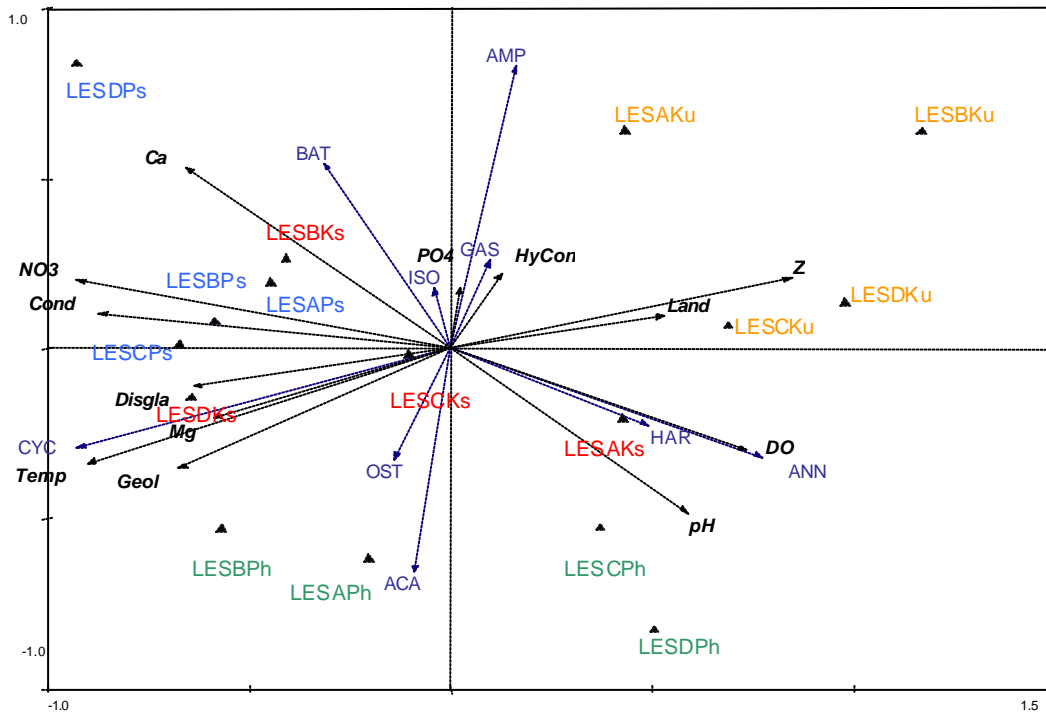
The first axis extracted is an axis of biodiversity; the correlation (Pearson's  $r$ ) between total species richness and PCA axis 1 in Figure 2 right is  $r = 0.720$  ( $p < 0.001$ ). Taxa correlated with PCA axis 1 are potential indicators of biodiversity.

The ordination of habitats using standardized environmental parameters (Figure 3; cv3: 81.78%) displays a similar arrangement of sites, indicating a strong correlation between biotic and abiotic factors (complete data set). In this case, apart from geology, distance from glacier and elevation, a mineralization and anthropization gradient is clearly visible along axis 1 (positively correlated with conductivity and nitrates and negatively correlated with dissolved oxygen and landcover).



**Figure 3:** Biplot of habitat units of Lessinia and mean values of environmental parameters resulting from PCA

The first axis is a biodiversity gradient; correlations between environmental PCA axis 1 and higher taxon PCA axis 1 ( $r = 0.797$ ,  $p < 0.001$ ), OMI axis 1 (habitat baricentres,  $r = 0.991$ ,  $p < 0.0001$ ) and total species richness (logarithmic scale,  $r = 0.780$ ,  $p < 0.001$ ) is very strong.



**Figure 4:** Triplot of habitat units, taxa and environmental parameters resulting from RDA



A more complete picture resulting from Redundancy Analysis (RDA) displays the interrelationships between higher taxa biodiversity, environmental parameters and habitat units (Figure 4). The first three axes biodiversity-environmental parameter correlations are respectively  $r = 0.968$ ,  $0.972$  and  $0.951$ ; the cumulative percentage of explained variance is 75.9% for biodiversity data and 83.8% for biodiversity-environmental parameters correlation. The high percentage of variance explained by the analysis clearly explain the influence of environmental parameters and habitat structure on higher taxa biodiversity.

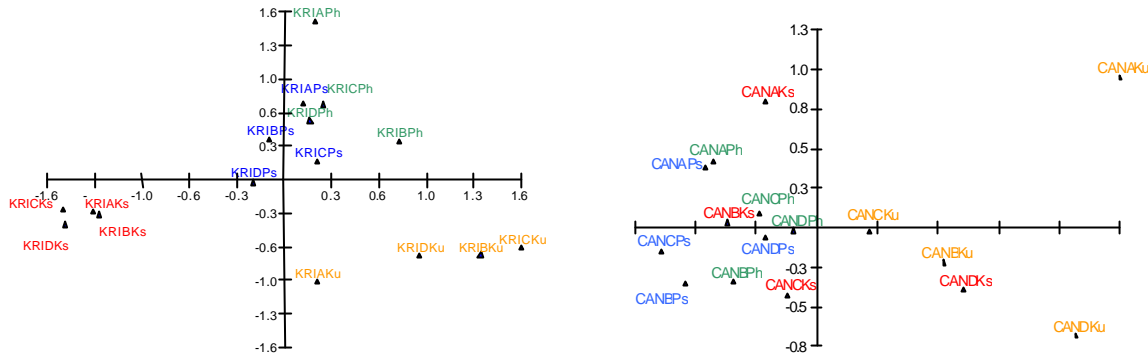
The correlation between higher taxa biodiversity (axis 1) and environmental parameters is summarized in the following table:

	<b>r</b>	<b>p</b>
Z (Elevation)	0.6569	<0.01
Geol (Geology)	-0.5249	<0.05
HyCon (Hydraulic Connectivity)	0.0976	n.s.
pH (pH value)	0.4562	n.s.
Cond (Conductance)	-0.6791	<0.01
Temp (Temperature)	-0.7003	<0.01
DO (Dissolved Oxygen)	0.5708	<0.05
Ca (Calcium)	-0.5122	<0.05
Mg (Magnesium)	-0.4579	n.s.
NO3 (Nitrates)	<b>-0.7210</b>	<0.001
PO4 (Phosphates)	0.0164	n.s.
Land (Corine Landcover)	0.4113	n.s.
Disgla (Distance from glacier)	-0.4965	<0.05

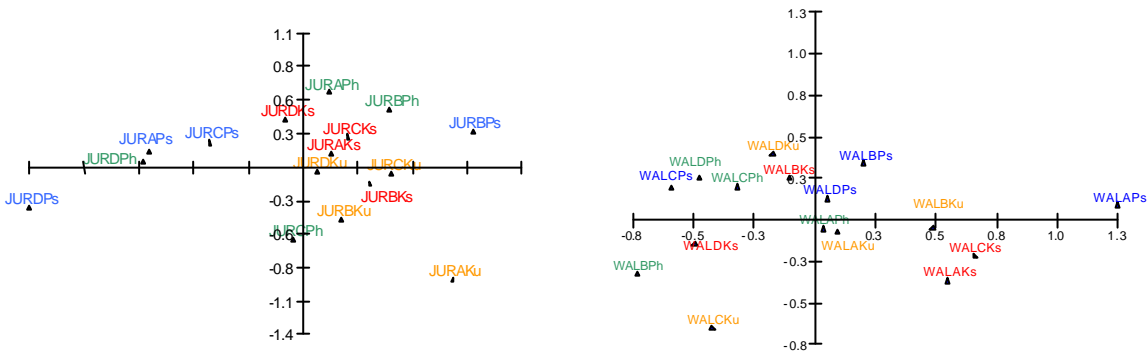
It is clear that the most important parameter which influences biodiversity in the study area is the concentration of nitrates, a strong indicator of pollution; nitrates reach high values (over 30 mg/l) especially in the phreatic zone of alluvial areas. As regards the effect of habitat structure, Aacari reach the highest diversity in the hyporheic zone, Cyclopoida in the saturated zone of karst and porous systems, while Harpacticoida and Annelida are represented by a higher number of species in the subsurface, unsaturated layers of karstic and alluvial systems. A strong, positive correlation between harpacticoid and annelid species number and dissolved oxygen (inversely correlated with nitrates and deeper alluvial sediments), and a strong negative correlation with nitrates and mineralized waters is clearly evidenced by the graph.

The results obtained analysing the other data sets using higher taxa biodiversity are similar for Krim (Figure 5 left); in this regions the effect of pollution is reduced, and higher taxa biodiversity is clearly influenced by habitat structure; habitat typologies are clearly separated in PCA graphs. Less clear-cut results are obtained for Cantabria (Figure 5 right), Jura (Figure

6 left) and Walloon (Figure 6 right). In all the PCA analyses, the explained variance by the first three axes is around 80%.



**Figure 5:** PCA ordination of habitat units in the Krim (left) and Catabrian (right) regions using species richness within higher taxa



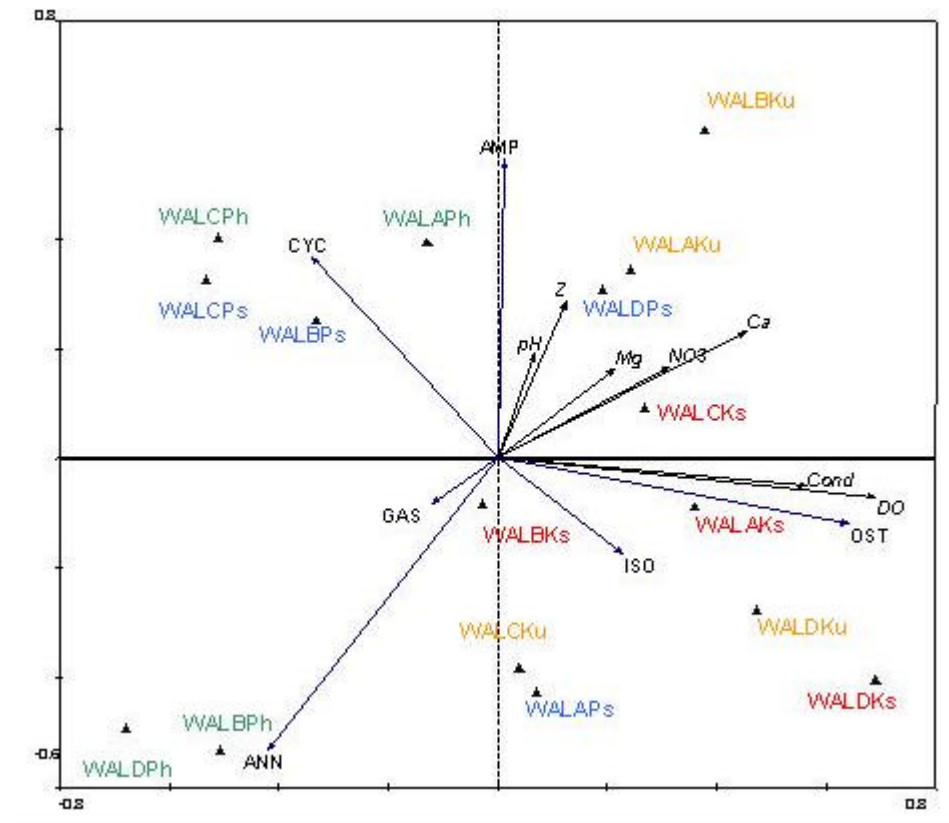
**Figure 6:** PCA ordination of habitat units in the Jura (left) and Walloon (right) regions using species richness within higher taxa

In the Walloon region, which displays a very low species richness probably due to historical factors (the recent colonization of a previously glaciated area), and part of the glaciated Jura, habitat types are intermixed in the graphs; in the Walloon region, stygobiotic Harpacticoida as well as Bathynellacea are completely lacking. A detailed analysis (RDA) is reported for Walloon region in Figure 7. The first three axes biodiversity-environmental parameter correlations are respectively  $r = 0.738$ ,  $0.582$  and  $0.484$ , clearly lower than in the case of the Lessinian region; the cumulative percentage of explained variance is 33.1% for biodiversity data and 95.5% for biodiversity-environmental parameters correlation. Also in this case, a strong correlation between environmental parameters and biodiversity do exists, even if the explained biodiversity variance is quite low. The most important parameters are mineralization (conductivity:  $r = 0.4832$ ,  $p < 0.05$ ) and dissolved oxygen ( $r = 0.5906$ ,  $p < 0.05$ ),

but no one reaches the probability level of 0.01.

The results of the analyses performed at the regional scale confirm that:

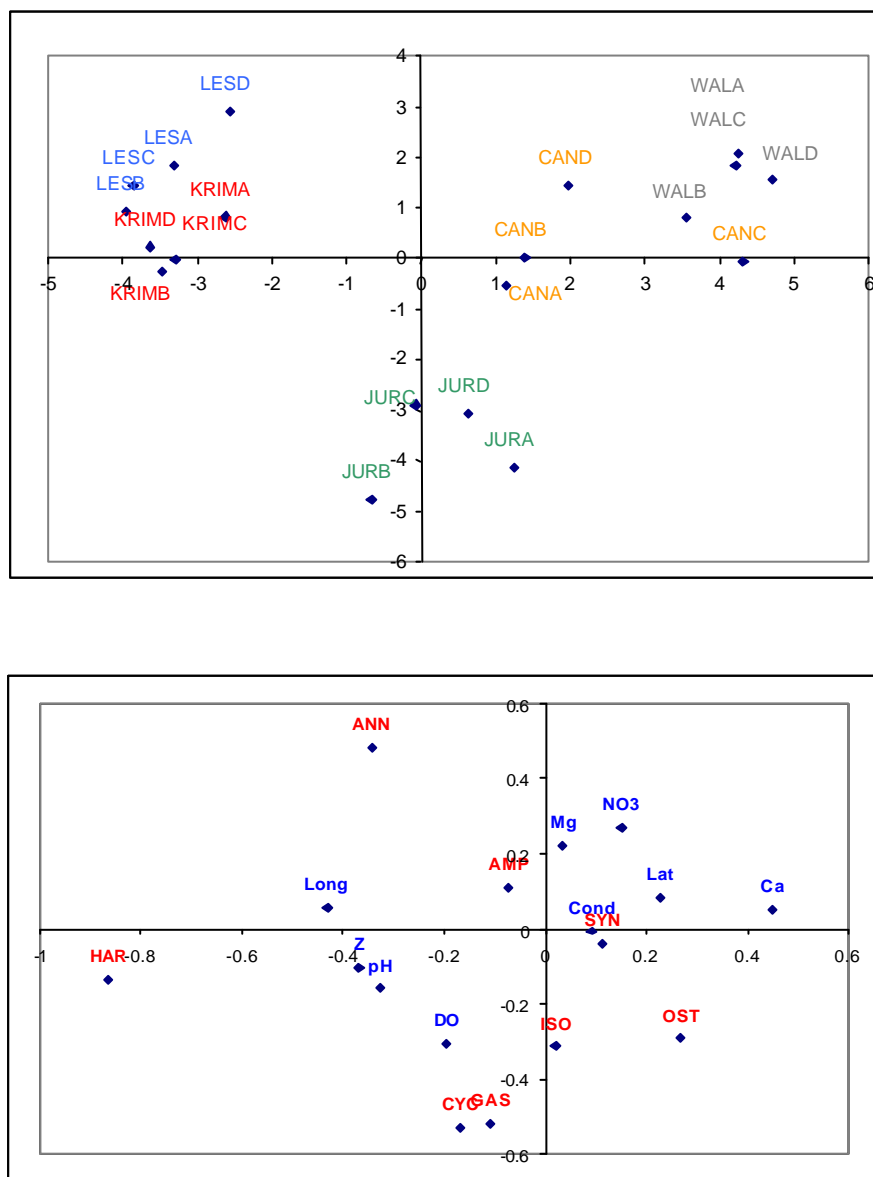
- higher taxa as defined herein can be considered as good “surrogates” in describing patterns of species richness within regions
- habitat structure is one of the main factors driving species richness patterns within regions; cumulating various layers (unsaturated and saturated within karst, hyporheic and phreatic within unconsolidated sediments) in monitoring studies may lead to an important lack of information
- apart from habitat structure, the strong effect of water chemistry and pollution in driving species richness patterns suggests that these factors may be considered as appropriate environmental surrogates when analysing biodiversity at a regional scale.



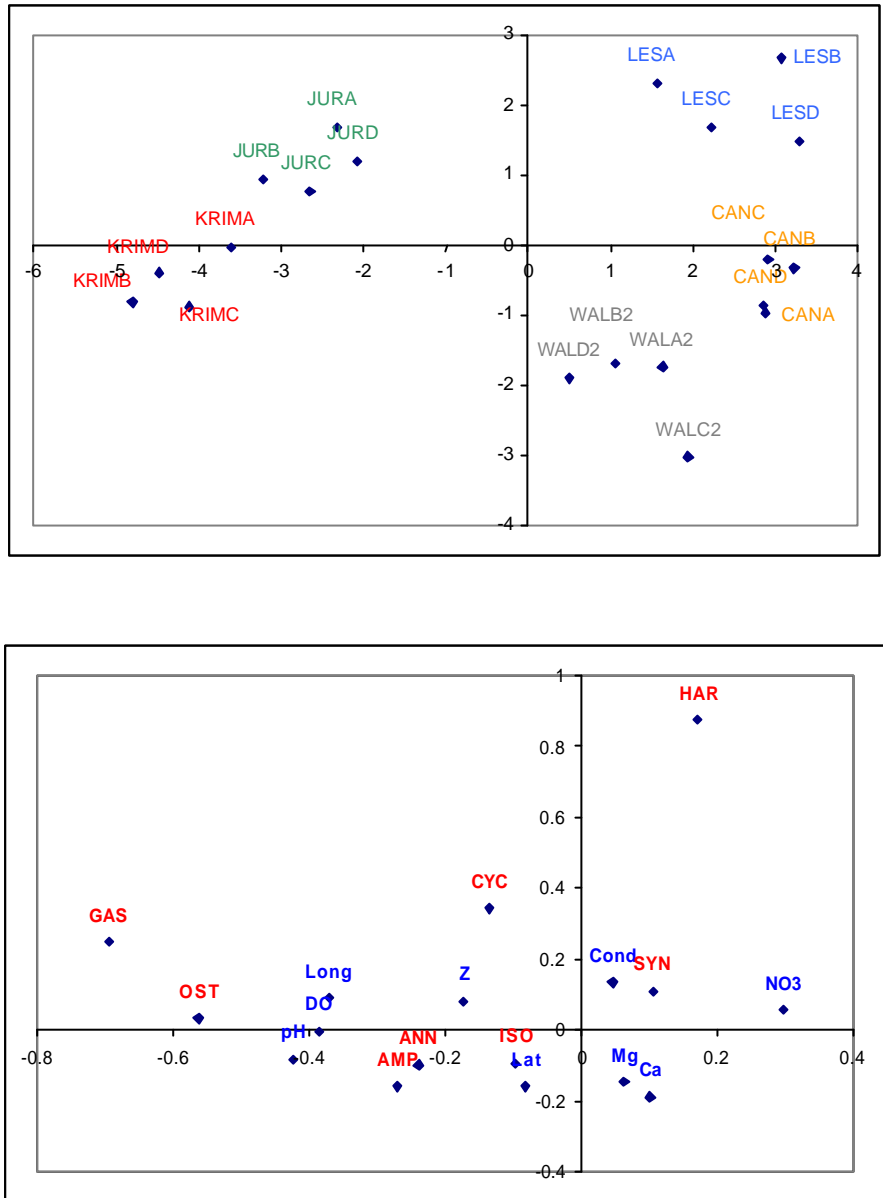
**Figure 7:** Walloon: triplot of habitat units, taxa and environmental parameters resulting from RDA

**2.3.12 *Factors driving stygobiotic biodiversity trends at an European scale :***

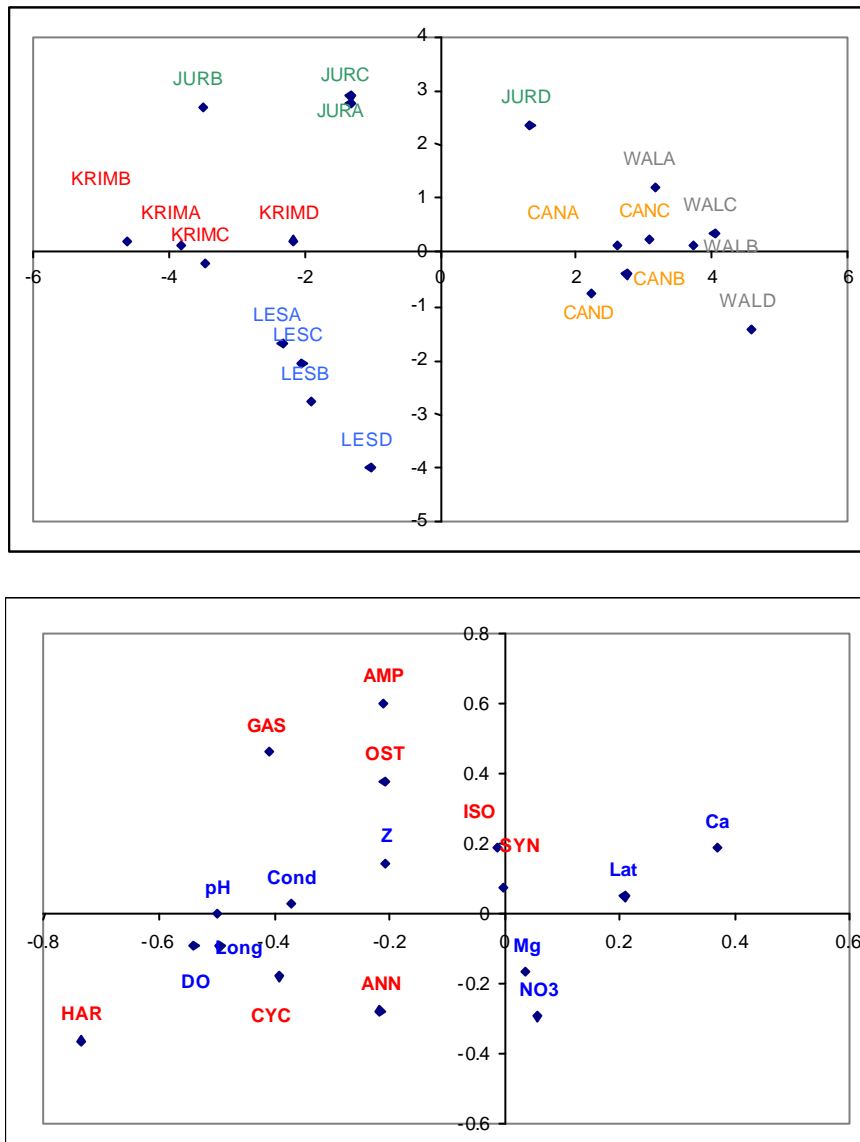
The biodiversity patterns across Europe were explored separately for Ku (unsaturated Karst), Ks (saturated karst), Ph (hyporheic porous) and Ps (saturated porous) habitats using RDA (redundancy analysis). The results are reported in Figs. 8, 9, 10, and 11. Each figure reports the ordination, based on the first two axes of RDA in its linear form, of habitats, higher taxa, and environmental parameters (based on geography and water chemistry); analyses without geographic factors obtained dropping out latitude and longitude (see below) give similar results, indicating that geography and water chemistry are closely correlated at an European scale.



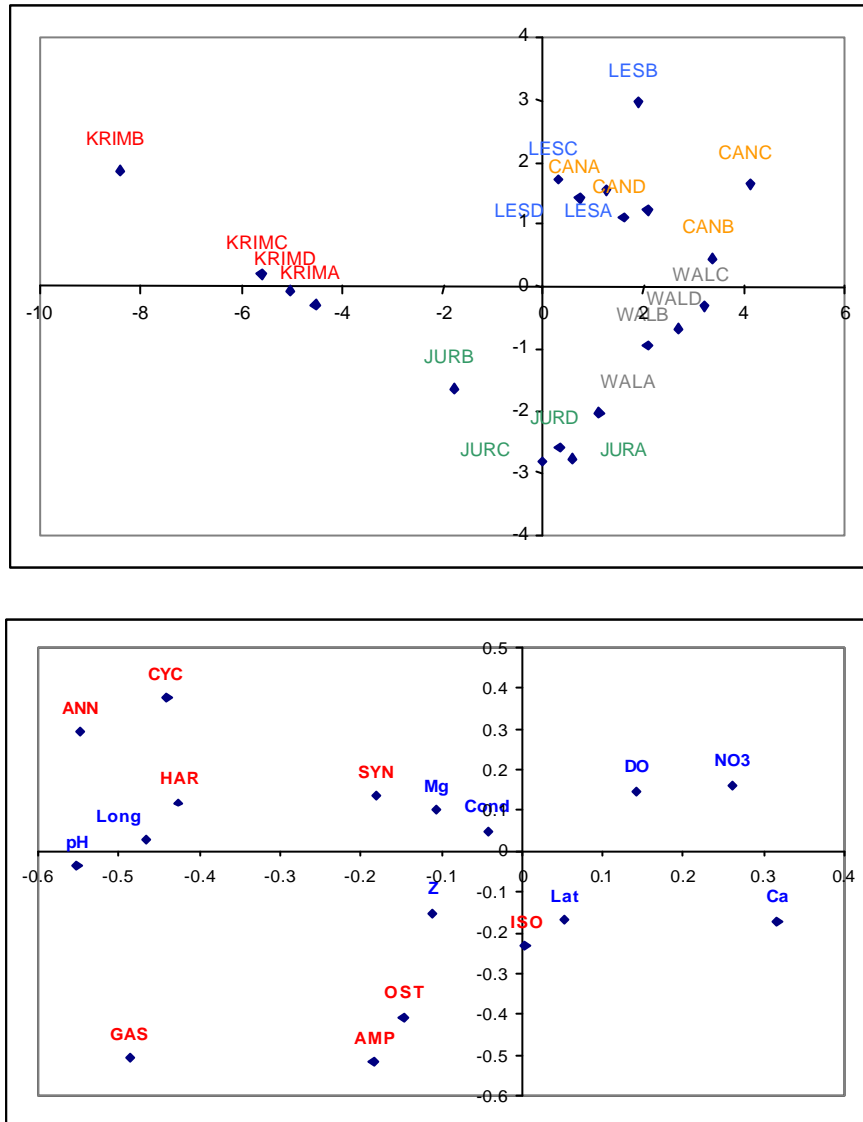
**Figure 8:** RDA ordination of habitat units (above), higher taxa and environmental parameters (below) for unsaturated karst.



**Figure 9:** RDA ordination of habitat units (left), higher taxa and environmental parameters (right) for saturated karst



**Figure 10:** RDA ordination of habitat units (above), higher taxa and environmental parameters (below) for hyporheic sediments



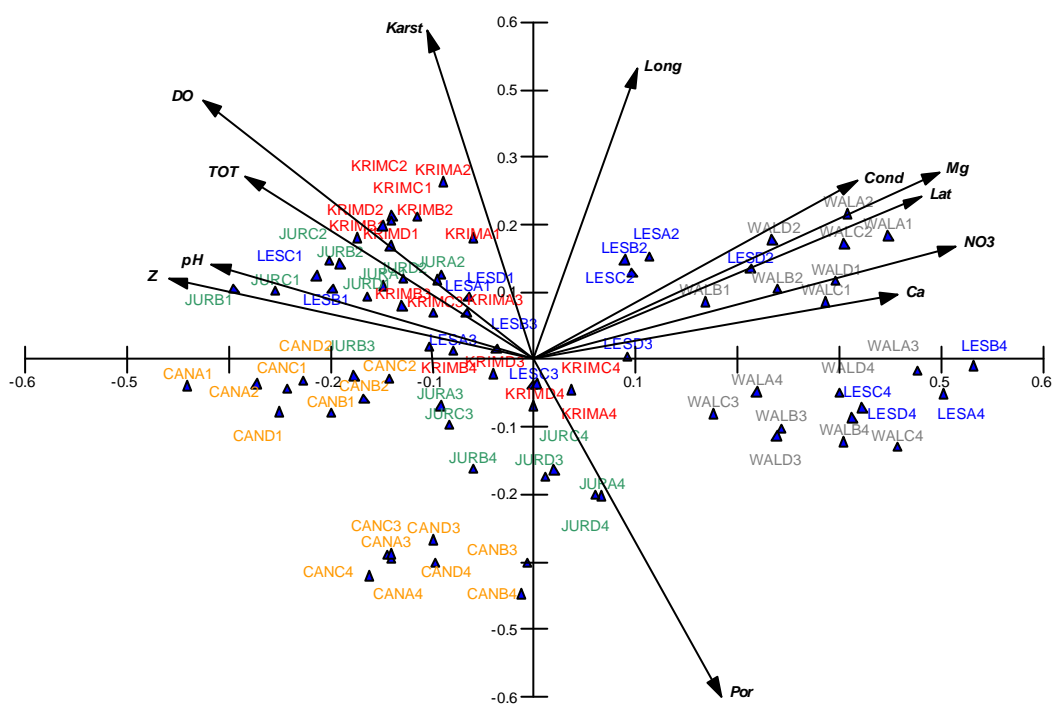
**Figure 11:** RDA ordination of habitat units (left), higher taxa and environmental parameters (right) for saturated (phreatic) unconsolidated sediments

For each analysis, correlation between the first axis of redundancy analysis and total species richness (expressed in logarithmic scale) was calculated as well. The results of the analysis are reported in the following table ( $r^2$ : mean coefficient of multiple determination; %var: percentage of the total variance of higher taxa biodiversity accounted for;  $r_{bio}$ : Pearson's correlation coefficient between total biodiversity (logarithmic scale) and RDA axis 1;  $p$ : probability of  $r_{bio}$ ):

	$r^2$	%var	$r_{bio}$	$p$
Ku	0.712	70.97	0.665	<0.01
Ks	0.603	66.71	0.680	<0.001
Ph	0.709	78.07	0.931	<0.0001
Ps	0.657	73.26	0.815	<0.0001

Each RDA ordination accounts for a large percentage of the variance (around 75%) of the distribution of species richness among higher taxa; moreover, in every habitat type the interaction between geographic location and water chemistry are the main determinants of biodiversity.

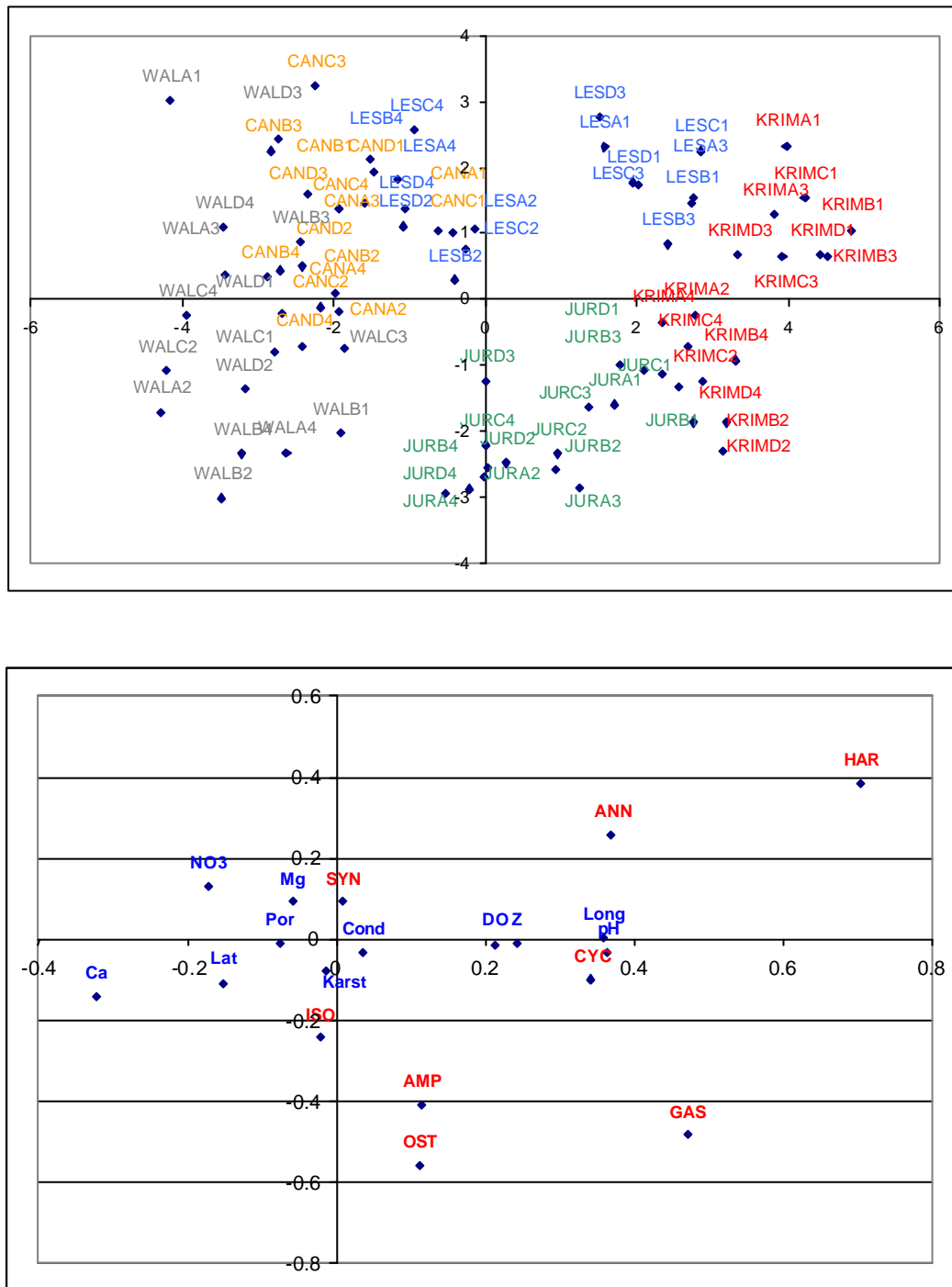
A full, detailed analysis of biodiversity patterns across PASCALIS regions and habitats was performed using PCA and RDA ordinations. The PCA ordination based on environmental parameters (Figure 12: first two axes accounting for 70.27% of variance) clearly shows that the overall environmental structure is driven by two main factors.



**Figure 12:** PCA ordination (biplot) of European habitat units based on environmental parameters; total biodiversity (TOT) is included (habitat units numbers: 1=Ku; 2=Ks; 3=Ph; 4=Ps)

The overall picture is clearly confirmed by Redundancy Analysis (Figure 14, more than 80% of variance explained by the first three axes), which displays the effect of habitat structure, water chemistry and geography on the biodiversity of higher taxa.





**Figure 14:** RDA ordination of habitat units (above), higher taxa and environmental parameters (below) of all PASCALIS regions (habitat units numbers: 1=Ku; 2=Ks; 3=Ph; 4=Ps)

The RDA plot gives additional informations; the inclusion of higher taxa species richness in the analysis clearly allows to display historical determinants. The following results deserve to be listed:

a) *geographical (e.g. historical) effect*: habitat units are more clearly grouped within their regions than in PCA ordination, indicating that geographic location and hence history is the

main responsible of biodiversity structure within PASCALIS countries; its effect taking in account overall biodiversity, without considering its partition within different taxonomic units, was low in PCA analysis (Figures 12, 13)

b) *water chemistry and pollution*: the most important biodiversity determinant is given by the gradient evidenced in PCA; the first RDA axis is clearly negatively correlated with calcium and nitrates content (left side) and positively correlated with dissolved oxygen, pH and elevation; total biodiversity is highly correlated with this gradient ( $r = 0.792$ ,  $p < 0.0001$ )

c) *habitat structure*: within the main clusters defined by geography and water chemistry, habitat units may be grouped together (at least for Lessinia and Krim); as can be observed in the graphs, there is a tendency for the most superficial units (e.g. unsaturated karst and hyporheos) to be clumped together; the vertical structure of habitat seems to be important at a local scale, but may not be considered the most important factor driving biodiversity patterns of different groups, because the correlation of karst and porous structure with the first two RDA axes is low.

The taxa which account for most of overall variance are the Harpacticoida (positively correlated with axis 1), followed by gastropods, ostracods and amphipods. These groups may be considered as potential indicators of biodiversity patterns at an European scale, and need to be included in any monitoring sampling protocol.

## **2.4 REFERENCES:**

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### **3 SECTION 3 : PARTITION OF GROUNDWATER BIODIVERSITY :**

#### **SUMMARY :**

Determining how species diversity is generated over spatial scales is of paramount importance for selecting appropriate strategies for biodiversity assessment and conservation. We examined the partitioning of groundwater species diversity across nested spatial scales – aquifers, basins, and regions – using species-richness data collected in the six European regions of the European project PASCALIS. As expected, the between-region component made by far the highest contribution to the stygobiotic richness of southern Europe. The contribution of between-region diversity to total richness of stygobiotic fauna (i.e. 81 %) was much higher than that observed in any other invertebrate groups. Hierarchical cluster analysis and correspondence analysis corroborated this finding because they identified marked differences in community composition among regions. The contribution of between-diversity increased monotonically with increasing spatial scale. On the other side, parametric and non-parametric analyses of variance revealed little differences in aquifer species richness among regions and basins. These patterns of species composition and richness have strong implications for the assessment and conservation of stygobiotic diversity. Although spatially extensive sampling designs for assessing groundwater community diversity would produce unsaturated accumulation curves, they provide a better assessment of the heterogeneity of species diversity than spatially intensive designs. The most effective way to preserve stygobiotic diversity in southern Europe is to protect multiple aquifers within different regions, thereby maintaining regionally distinctive species-rich assemblages. Such a conservation strategy requires that ecoregions have to be more precisely defined in southern Europe. To this end, scientists should maintain their effort in identifying the stygobiotic communities of multiple aquifers in areas that have so far been poorly investigated. Stygobiotic community studies in selected aquifers belonging to distinct eco-regions would provide more efficient data for defining conservation measures than extensive surveys of specific taxonomic groups.

### **3.1 INTRODUCTION :**

Determining how species diversity is generated over spatial scales is of paramount importance for selecting appropriate strategies for conservation (Wagner et al. 2000, Fournier and Loreau 2001, Gering et al. 2003, Fleishman et al. 2003, Summerville et al. 2003). The diversity of obligate-groundwater species (i.e. stygobionts) assemblages is presumably low at a local scale but diversity is expected to increase markedly with increasing spatial scales because of the importance of groundwater system fragmentation in generating species. This concept is deeply rooted into the mind of groundwater ecologists (Gibert and Deharveng 2002) despite the fact that it has almost never been thoroughly tested using robust data sets. The ensuing material examines the partitioning of groundwater species diversity across nested spatial scales – aquifers, basins, and regions – using species-richness data collected in six European regions. The European environment and associated stygobiotic fauna are partitioned using a three-level nested spatial hierarchy. It is partitioned into regions, each region is partitioned into basins, and each basin is partitioned into several aquifers. An aquifer is a finite and continuous subsurface hydrological system the spatial limits of which can be clearly delineated. In groundwater ecology, the concepts of ecosystem and aquifer are closely related because the aquifer comprises an abiotic component, i.e. the habitat, and a biocenosis. The objective of the present section was threefold:

- 1) to examine variation in the mean species richness of aquifers across the different spatial levels defined in the hierarchy (i.e. basins and regions);
- 2) to partition total richness (i.e. the total number of species collected in all regions) among spatial levels of the hierarchy; and
- 3) to analyze community composition across spatial levels. We expected that the broad-scale effect of regions would have the strongest influence on groundwater fauna richness because of the overriding importance of biogeographical factors in determining community structure.

### **3.2 MATERIALS AND METHODS :**

#### **3.2.1 Data sets :**

A species list as complete as possible was obtained for two aquifers (i.e. a karst aquifer and a porous aquifer) in each of the four basins of the 6 regions investigated within the framework of the European project PASCALIS (Table 1).

Region	Region code	Basin	Basin code	Aquifer name	Aquifer code	Area (km <sup>2</sup> )
Cantabrica	CAN	Asón	CANA	Gandara River	CANAP	60
Cantabrica	CAN	Asón	CANA	Basin Ason River	CANAK	40
Cantabrica	CAN	Matienzo	CANB	Clarion River	CANBP	20
Cantabrica	CAN	Matienzo	CANB	Matienzo Basin	CANBK	40
Cantabrica	CAN	Ojo Guareña	CANC	Trueba River	CANCP	40
Cantabrica	CAN	Ojo Guareña	CANC	Ojo Guareña Basin	CANCK	36
Cantabrica	CAN	Collada	CAND	Deva River	CANDP	50
Cantabrica	CAN	Collada	CAND	Basin Lamason River	CANDK	50
Meridional Jura	JUR	Suran	JURA	Fluvial aquifer of Villereversure	JURAP	10
Meridional Jura	JUR	Suran	JURA	Suran Synclinal Aquifer	JURAK	101
Meridional Jura	JUR	Albarine	JURB	Alluvial aquifer of Albarine	JURBP	9
Meridional Jura	JUR	Albarine	JURB	Dorvan karst aquifer	JURBK	5
Meridional Jura	JUR	Oignin	JURC	Alluvial aquifer of Montréal	JURCP	9
Meridional Jura	JUR	Oignin	JURC	Corberan karst aquifer	JURCK	6
Meridional Jura	JUR	Valouse	JURD	Morainic Arinthod aquifer	JURDP	20
Meridional Jura	JUR	Valouse	JURD	Karst Arinthod aquifer	JURDK	5
Walloon	WAL	Bocq	WALA	Aquifère colluvial de Evrehailles	WALAP	1
Walloon	WAL	Bocq	WALA	Calcaire Carbonifère de Crupet	WALAK	4
Walloon	WAL	Lesse	WALB	Plaine alluviale de la Lesse	WALBP	2
Walloon	WAL	Lesse	WALB	Lhomme souterraine (Rochefort / Gerny)	WALBK	17
Walloon	WAL	Amblève	WALC	PLaine alluviale de L'ourthe et Lembrée	WALCP	3
Walloon	WAL	Amblève	WALC	Aquifère karstique du synclinal de Houmart	WALCK	4
Walloon	WAL	Ourthe	WALD	Nappe Colluviale du plateau de Sprimont	WALDP	15
Walloon	WAL	Ourthe	WALD	Aquifère karstique du Vallon des chantoirs	WALDK	7
Lessinian Mount.	LES	Progno di Fumane	LESA	Porous aquifer of the Fumane basin	LESAP	10
Lessinian Mount.	LES	Progno di Fumane	LESA	Karst aquifer of the Fumane basin	LESAK	24
Lessinian Mount.	LES	Progno di Valpantena	LESB	Porous aquifer of the Valpantena basin	LESBP	24
Lessinian Mount.	LES	Progno di Valpantena	LESB	Karst aquifer of the Valpantena basin	LESBK	30
Lessinian Mount.	LES	Vaio di Squaranto	LESC	Porous aquifer of the Squaranto basin	LESCP	12
Lessinian Mount.	LES	Vaio di Squaranto	LESC	Karst aquifer of the Squaranto basin	LESCK	15
Lessinian Mount.	LES	Alpone-Tramigna	LESD	Porous aquifer of Alpone Tramigna basin	LESDP	16
Lessinian Mount.	LES	Alpone-Tramigna	LESD	Karst aquifer of Alpone Tramigna basin	LESDK	15
Krim Massif	KRI	Želimejščica	KRIA	Želimejščica (A) interstitial	KRIAP	4
Krim Massif	KRI	Želimejščica	KRIA	Želimejščica (A) karst: right bank of stream	KRIAK	6
Krim Massif	KRI	Iška	KRIB	Iška (B) interstitial	KRIBP	18
Krim Massif	KRI	Iška	KRIB	Iška (B) karst: epikarst above Vel. Pasjica	KRIBK	0,25
Krim Massif	KRI	Borovniščica	KRIC	Borovniščica (C) interstitial	KRICP	5
Krim Massif	KRI	Borovniščica	KRIC	karst (B-C): Jezero-Ponikve-Goričica	KRICK	15
Krim Massif	KRI	Podlipščica	KRID	Podlipščica (D) interstitial	KRIDP	4
Krim Massif	KRI	Podlipščica	KRID	Podlipščica (D) Pajsarjeva jama & springs	KRIDK	1
Roussillon	ROU	Agly	ROUA	Porous aquifer basin A	ROUAP	12
Roussillon	ROU	Agly	ROUA	Karst aquifer basin A-Agly	ROUAK	8
Roussillon	ROU	Tet	ROUB	Porous aquifer basin B	ROUBP	35
Roussillon	ROU	Tet	ROUB	Karst aquifer basin B-Tet	ROUBK	33
Roussillon	ROU	Aude	ROUC	Porous aquifer basin C	ROUCP	55
Roussillon	ROU	Aude	ROUC	karstic aquifer basin C-Aude	ROUCK	10
Roussillon	ROU	Tech	ROUD	Porous aquifer basin D-Tech	ROUDP	66
Roussillon	ROU	Tech	ROUD	karstic aquifer basin D-Tech	ROUDK	5

**Table 1:** Location, name, code, and area of aquifers retained for the partitioning of species richness.

Aquifers were selected so as to maximize the between-aquifer diversity in each basin (i.e. highest difference in species composition between aquifers). Although there were marked differences in size (i.e. area of the impluvium) between aquifers, the number of species did not increase significantly with increasing area of the aquifer ( $r=0.17$ ;  $n=48$ ).

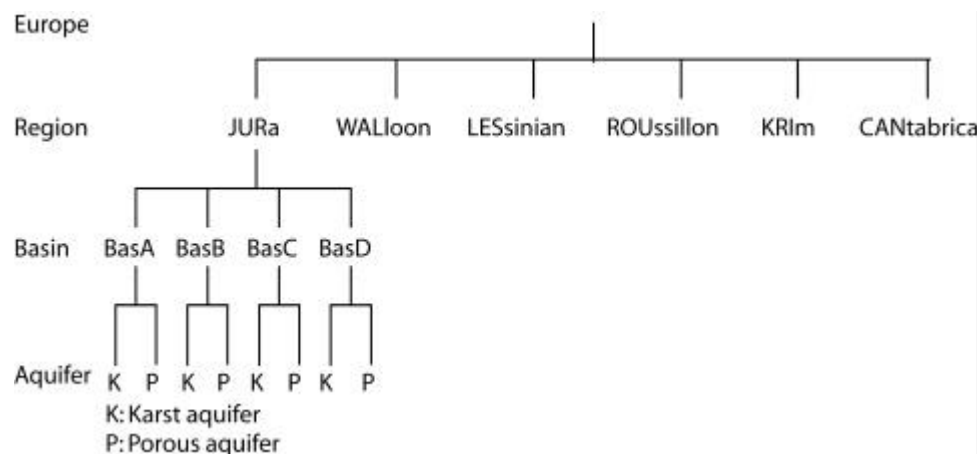
### 3.2.2 Data analysis :

#### *Variation in species richness of aquifers across spatial levels*

A two-level nested analysis of variance was used to test for variation in the mean richness of aquifers across basins and regions. Two-level nested analysis of variance followed by Tukey post hoc comparison tests were used to test for variation in the mean richness of aquifers across regions and aquifer type (i.e. karst and interstitial aquifers). Kruskal Wallis ANOVAs by ranks were used to test for differences in karst and interstitial aquifer richness among regions. Analyses were performed using Statistica software.

#### *Additive partitioning of total richness*

The hierarchical model we applied to determine how species diversity was distributed over spatial scales consisted of a mosaic of regions. Each region was fragmented into hydrogeographic basins which comprised distinct aquifers (Figure 1).



**Figure 1:** The four nested levels of diversity considered in the additive partitioning of species richness.

At each level of the hierarchy, total richness in the set of sampling units (i.e. regions, basins, and aquifers) was calculated as the sum of  $\alpha$  diversity and  $\beta$  diversity, where  $\alpha$  was the average (weighted sum) within-sampling unit diversity and  $\beta$  was the between-sampling unit diversity, or the average diversity not found in a single sampling unit (Lande 1996, Veech et al. 2002). Within the context of a hierarchy,  $\alpha$  diversity at any spatial level was simply the sum of the  $\alpha$  and  $\beta$  diversity at the next lowest level (see details in appendix 5.1). By substitution, total diversity ( $\gamma$ ), the total number of species collected in all regions, was equal

to the sum of within-aquifer diversity ( $\alpha_1$ ), between-aquifer diversity ( $\beta_1$ ), between-basin diversity ( $\beta_2$ ) and between-region diversity ( $\beta_3$ ). Because  $\alpha$  and  $\beta$  diversity corresponded to a number of species, total diversity was expressed as the proportional contributions of diversity due to each level in the hierarchy.

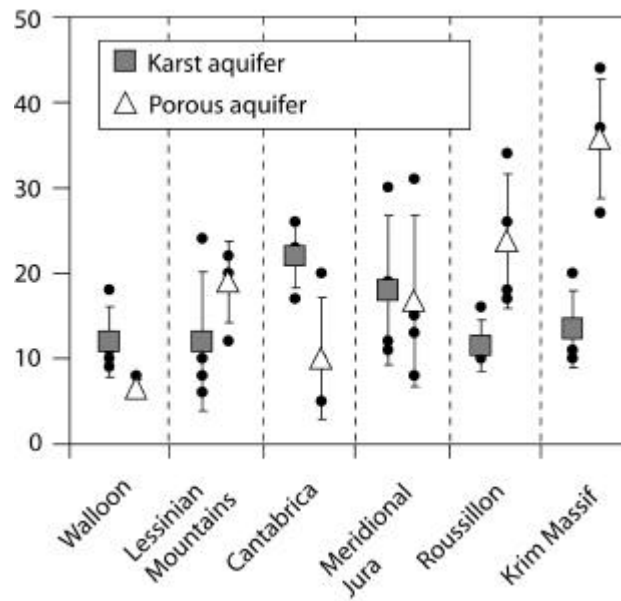
#### *Variation in species composition across spatial levels*

To examine the dissimilarity of stygobiotic communities among regions, basins and aquifers, we used hierarchical cluster analysis with the Sorensen index of dissimilarity (Sorensen 1948). 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. The reciprocal discrimination of correspondence analysis was used to obtain a simultaneous ordination of species and aquifers on the same graph (Thioulouse and Chessel 1992). Analysis was performed using ADE-4 software (Thioulouse et al. 1997).

### **3.3 RESULTS :**

#### **3.3.1 Variation in species richness of aquifers across spatial levels :**

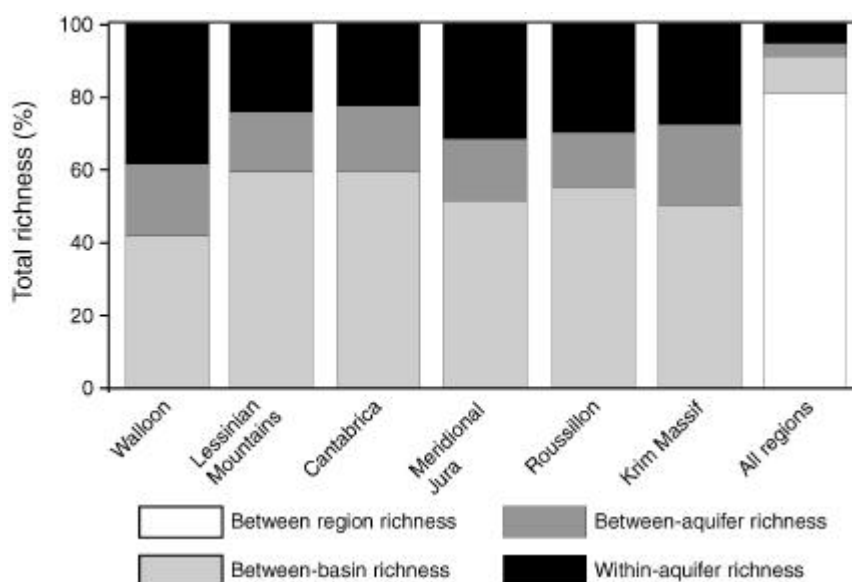
There were no significant differences in species richness of aquifers among regions and basins (Two-level nested ANOVA;  $p=0.09$  for region and  $p=0.81$  for basin) (Figure 2). Two-level nested ANOVA (i.e. regions and aquifer type) indicated significant differences among regions ( $p=0.002$ ) and aquifer type ( $p<0.0001$ ). However, post hoc comparison tests revealed that aquifer richness was only significantly higher in the Krim massif than in the Walloon karst. Kruskal Wallis ANOVA by ranks revealed that there were no differences in the species richness of karst aquifers among regions ( $p=0.08$ ) but significant differences in the species richness of interstitial aquifers among regions ( $p=0.005$ ).



**Figure 2:** Differences in the average species richness of karst (grey squares) and interstitial (white triangles) aquifers among regions. Black dots correspond to the species richness of aquifers.

### 3.3.2 Additive partitioning of total richness :

The between-region component accounted for 81% of the total richness whereas the between-basin, between-aquifer and within-aquifer components accounted for only 10, 4, and 5 % of the total richness, respectively (Figure 3).



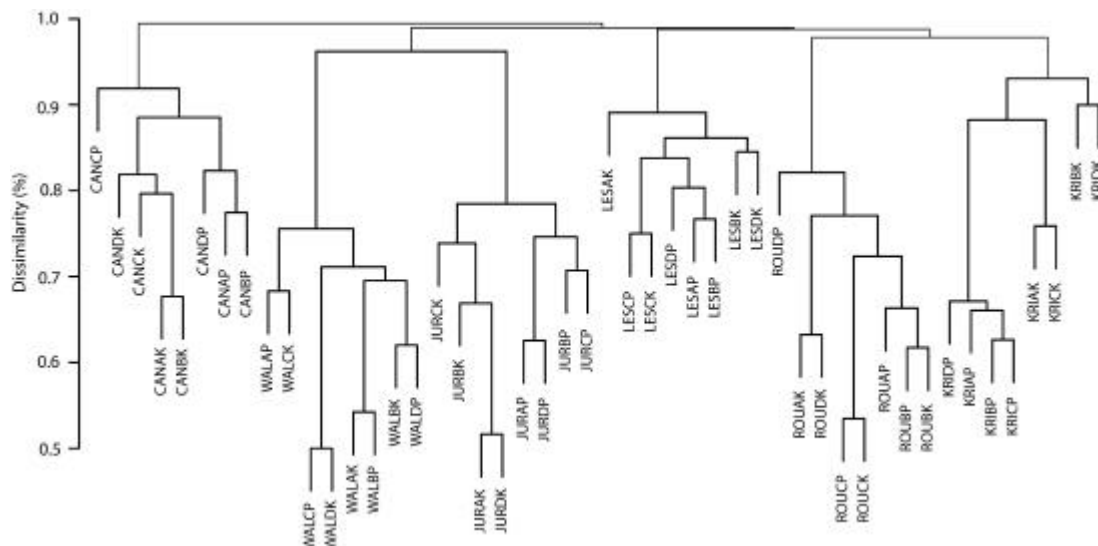
**Figure 3:** Additive partitioning of stygobiotic richness at the region extent (first six panels) and European region extent (last right panel). Bars show the proportion of total species richness explained by within and between components of richness at three spatial levels: aquifer, basin, and region.



The proportions of richness components varied little among regions. Between-basin richness was typically higher than between-aquifer and within-aquifer richness. The proportion of regional richness attributed to between-basin diversity was lower in the Walloon karst than in all other regions. Within-aquifer richness was higher than between aquifer richness in all regions.

### 3.3.3 Variation in species composition across spatial levels :

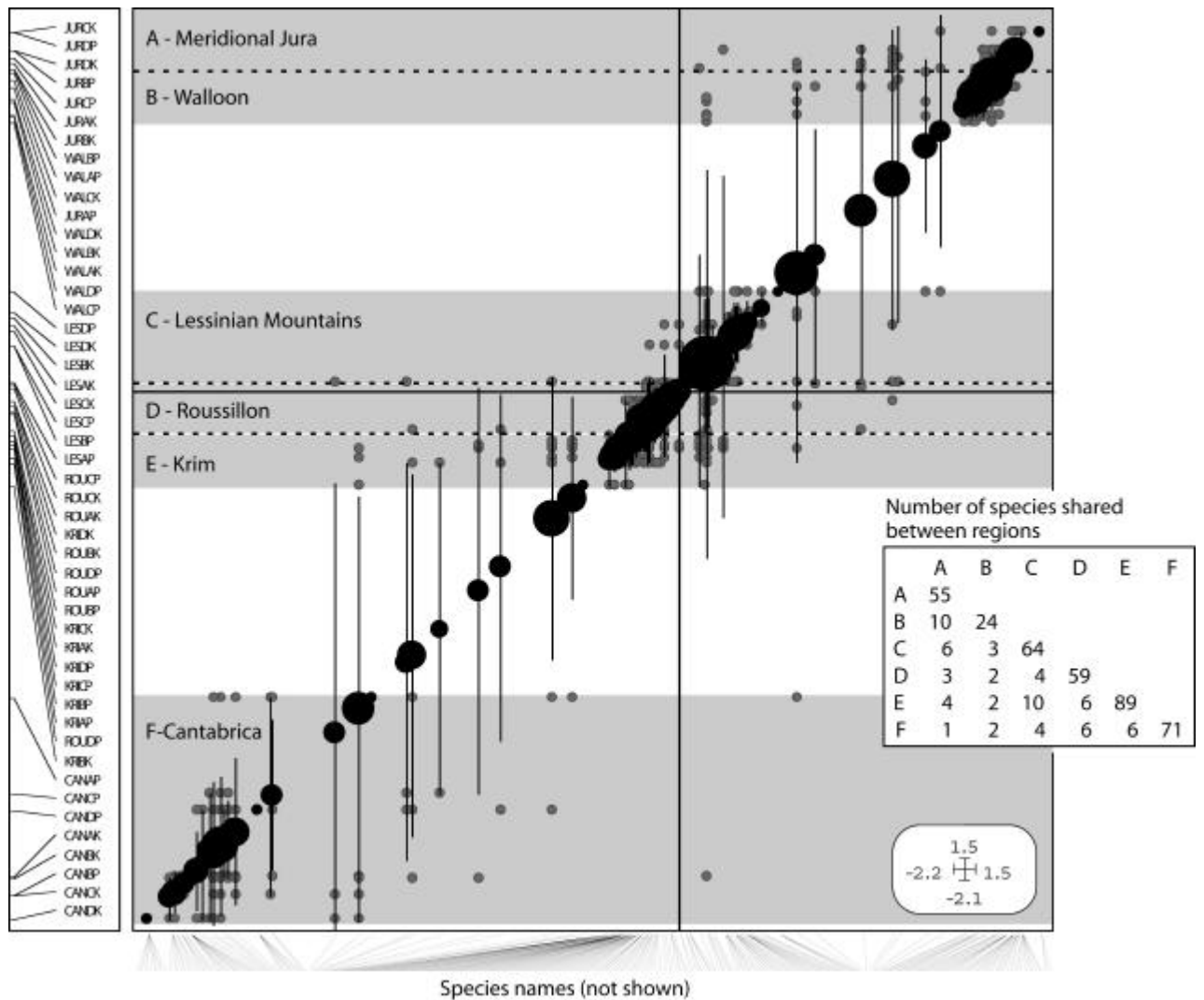
The cluster analysis grouped aquifers according to their regions and identified strong dissimilarities between regions (>90 %) (Figure 4). A region contained in average 60 species and the average number of species shared by any 2 regions was only 5 (Figure 5). The average dissimilarity between all pairs of aquifers was higher in the Cantabria (86 %), the Lessinian Mountains (85 %), and the Krim Massif (85 %) than in the Walloon karst (71 %), the meridional Jura (75 %) and the Roussillon region (74 %). In 3 of the 6 regions (Cantabria, meridional Jura and Krim massif), aquifers were grouped according to their type (i.e. karst and interstitial aquifers). Aquifers belonging to the same basin were almost never grouped together at the lowest level of the hierarchical tree.



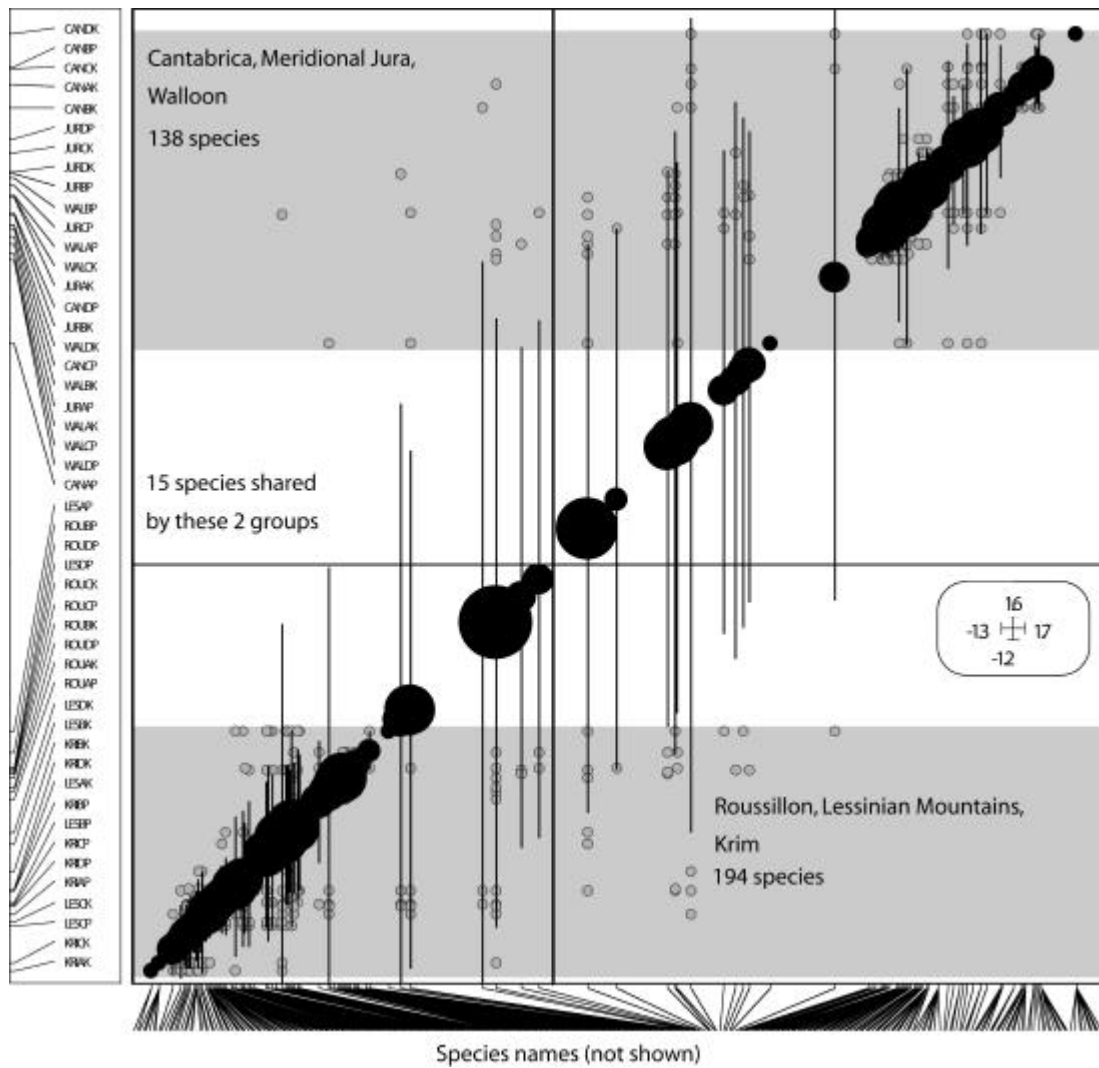
**Figure 4:** Degree of dissimilarity between stygobiotic communities in 48 aquifers, 24 basins, and 6 regions in southern Europe (see Table 1 for aquifer codes). The distance measure is percent dissimilarity based on the Sorensen coefficient.

The first axis of the correspondence analysis distinguished between 3 clusters of regions that had very dissimilar species composition:

1) the meridional Jura and Walloon karst; 2) the Lessinian Mountains, Krim Massif, and Roussillon; and 3) the Cantabria (Figure 5). Cluster 1 shared 10 and 2 species with clusters 2 and 3 respectively. Cluster 2 shared 11 species with cluster 3. The second axis of the analysis clearly separated between a “peri-Mediterranean” group of regions (Roussillon, Lessinian Mountains and Krim Massif) and a “non-Mediterranean” group (Walloon, Jura, and Cantabria) (Figure 6). These 2 groups shared only 15 species.



**Figure 5:** Conditional means of aquifers (left panel) and species (right panel) in the reciprocal discrimination model of correspondence analysis (axis 1 of the analysis). The sizes of black circles are proportional to the total frequencies of taxa. Grey circles represent sites in which a taxon occurs. Vertical lines correspond to standard deviations.



**Figure 6:** Conditional means of aquifers (left panel) and species (right panel) in the reciprocal discrimination model of correspondence analysis (axis 2 of the analysis). The sizes of black circles are proportional to the total frequencies of taxa. Grey circles represent sites in which a taxon occurs. Vertical lines correspond to standard deviations.

### **3.4 DISCUSSION :**

As expected, the between-region component made by far the highest contribution to the stygobiotic richness of southern Europe. This finding is in agreement with the results of recent studies demonstrating that the broad-scale effects of ecoregions had the strongest influence on invertebrate richness (Gering et al. 2003, Summerville et al. 2003, Atauri and de Lucio 2001). However, the contribution of between-region diversity to total richness of stygobiotic fauna (i.e. 81 %) was much higher than that observed in any other invertebrate groups. Stygobiotic community composition varied most importantly over broader spatial scales, even when differences in total richness between regions were relatively small. This was supported by the cluster analysis and discriminant model of correspondence analysis which identified marked differences in community composition among regions. As spatial scale increased (i.e. from aquifer to southern Europe), the contribution of between diversity became much higher than that of within diversity because the probability to encounter rare species increased. Because stygobiotic community contained many rare species the increase in the contribution of between-diversity with increasing spatial scale was particularly pronounced.

Additive partitioning is simply a mathematical approach for describing the pattern of within and between-component contribution to total richness over multiple spatial scales but it does not provide any explanation about the processes that determine this pattern. We suggest that the increase in the between-component contribution of diversity with increasing spatial scale is largely the product of multiple vicariant speciation events caused by the highly fragmented nature of groundwater systems.

This can happen also if there is no saturation : there is no correlation between aquifer area and species richness, suggesting that the environment may not be so complex, that the area per se is not important because large aquifers are most difficult to be colonized in all their parts; we have no evidence for competition; so, I suggest to let apart this sentences, if Florian agrees.

The patterns of species richness identified in the present study have strong implications for the assessment and conservation of stygobiotic diversity. The use of spatially extensive designs for assessing biodiversity in ground water is likely to produce unsaturated accumulation curves because of the high number or rare species (see section 1). However, spatially extensive designs would provide a better assessment of the heterogeneity of species diversity. This dilemma between obtaining a comprehensive list of species by sampling intensively over a limited spatial area and obtaining a better assessment of richness

heterogeneity by sampling extensively over large spatial areas was emphasized by Gering et al. (2003) for the assessment of arboreal beetle diversity. Our results clearly indicate that the most effective way to preserve stygobiotic diversity in southern Europe is to protect multiple aquifers within different regions, thereby maintaining regionally distinctive species-rich assemblages. Such a conservation strategy requires that ecoregions have to be more precisely defined in southern Europe. To this end, scientists should maintain their effort in identifying the stygobiotic communities of multiple aquifers in areas that have so far been poorly investigated.

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### 3.6 APPENDICES :

#### 3.6.1 The hierarchical model of species diversity, where the scale-specific components of within- and between-community richness are linked additively to form the richness at the next higher level

Levels	Within-community diversity	Between-community diversity
Europe	$\gamma = D_T$	
Region (level 3)	$\alpha_{3(\text{regions})} = \sum_r q_r D_{\text{within region } r}$	$\beta_{3(\text{regions})} = \sum_b q_b (D_T - D_{\text{within region } r})$
Basin (level 2)	$\alpha_{2(\text{basins})} = \sum_b q_b D_{\text{within basin } b}$	$\beta_{2(\text{basins})} = \sum_b q_b (D_R - D_{\text{within basin } b})$
Aquifer (level 1)	$\alpha_{1(\text{aquifers})} = \sum_a q_a D_{\text{within aquifer } a}$	$\beta_{1(\text{aquifers})} = \sum_a q_a (D_B - D_{\text{within aquifer } a})$
		$q_r = \frac{n_r}{\sum_r n_r}$
		$q_b = \frac{n_b}{\sum_b n_b}$
		$q_a = \frac{1}{n_a}$

$D_T$  : number of species in all the regions

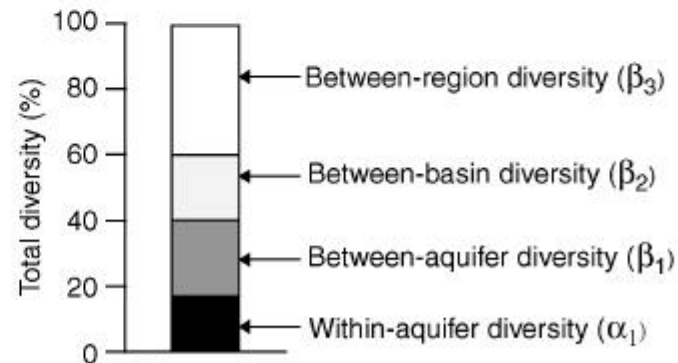
$D_R = D_{\text{within region } r}$  : number of species in region  $r$

$D_B = D_{\text{within basin } b}$  : number of species in basin  $b$

$n_r$  = number of aquifers in region  $r$

$n_b$  = number of aquifers in basin  $b$

$n_a$  = total number of aquifers in all the regions



Proportion of stygobite species richness explained by within-aquifer, between-aquifer, between-basin, and between-region diversity.

**3.6.2 List of species collected in the 48 aquifers retained for analysing the partitioning of species richness:**

Group	Species	Group	Species
Oligochaeta	<i>Abyssidrilus subterraneus</i>	Harpacticoida	<i>Elaphoidella cvetkae</i>
Oligochaeta	<i>Aktedrilus indet.</i>	Harpacticoida	<i>Elaphoidella elaphoides</i>
Oligochaeta	<i>Cernosvitoviella cf. parviseta</i>	Harpacticoida	<i>Elaphoidella jeanneli</i>
Oligochaeta	<i>Cernosvitoviella sp. I1</i>	Harpacticoida	<i>Elaphoidella indet. Aff. leruthi</i>
Oligochaeta	<i>Gianius cf. labouichensis</i>	Harpacticoida	<i>Elaphoidella phreatica</i>
Oligochaeta	<i>Gianius sp. I1</i>	Harpacticoida	<i>Elaphoidella sp. I1</i>
Oligochaeta	<i>Gianus cavealis</i>	Harpacticoida	<i>Elaphoidella sp. K1</i>
Oligochaeta	<i>Haber indet.</i>	Harpacticoida	<i>Elaphoidella sp. S3</i>
Oligochaeta	<i>Haber sp. K2</i>	Harpacticoida	<i>Lessinocamptus insoletus</i>
Oligochaeta	<i>Haber turquini</i>	Harpacticoida	<i>Lessinocamptus sp. I2</i>
Oligochaeta	<i>Krenedrilus indet.</i>	Harpacticoida	<i>Moraria (M.) sp. I1</i>
Oligochaeta	<i>Parvidrilidae indet.</i>	Harpacticoida	<i>Moraria (M.) sp. J1</i>
Oligochaeta	<i>Parvidrilus spelaeus</i>	Harpacticoida	<i>Moraria (M.) stankovitchi</i>
Oligochaeta	<i>Phalldrilineae gen. sp. I1</i>	Harpacticoida	<i>Morariopsis dumonti</i>
Oligochaeta	<i>Phalldrilineae gen. sp. K2</i>	Harpacticoida	<i>Nitocrella gr. hirta sp. J1</i>
Oligochaeta	<i>Phalldrilineae sp. K3</i>	Harpacticoida	<i>Nitocrella hirta</i>
Oligochaeta	<i>Phalldrilineae indet.</i>	Harpacticoida	<i>Nitocrella psammophila</i>
Oligochaeta	<i>Phalldrilineae indet.1</i>	Harpacticoida	<i>Paramorariopsis sp. I2</i>
Oligochaeta	<i>Phalldrilineae indet.2</i>	Harpacticoida	<i>Parapseudoleptomesochra italica</i>
Oligochaeta	<i>Phalldrilineae indet.3</i>	Harpacticoida	<i>Parastenocaris cf. cantabrica</i>
Oligochaeta	<i>Phalldrillus subterraneus</i>	Harpacticoida	<i>Parastenocaris cf. stammeri</i>
Oligochaeta	<i>Pristina sp. I1</i>	Harpacticoida	<i>Parastenocaris diana</i>
Oligochaeta	<i>Pristina sp. I3</i>	Harpacticoida	<i>Parastenocaris gertrudae</i>
Oligochaeta	<i>Rhyacodrilinae indet.</i>	Harpacticoida	<i>Parastenocaris glareola</i>
Oligochaeta	<i>Rhyacodriloides sp. K1</i>	Harpacticoida	<i>Parastenocaris italica</i>
Oligochaeta	<i>Rhyacodrilus balmensis</i>	Harpacticoida	<i>Parastenocaris nollii alpina</i>
Oligochaeta	<i>Rhyacodrilus cf. dolci</i>	Harpacticoida	<i>Parastenocaris phyllura</i>
Oligochaeta	<i>Rhyacodrilus gasparoi</i>	Harpacticoida	<i>Parastenocaris sp. J1</i>
Oligochaeta	<i>Rhyacodrilus indet.2</i>	Harpacticoida	<i>Parastenocaris sp. S1</i>
Oligochaeta	<i>Rhyacodrilus cf. lindbergi</i>	Harpacticoida	<i>Parastenocaris sp. I1</i>
Oligochaeta	<i>Rhyacodrilus sp. I2</i>	Harpacticoida	<i>Parastenocaris sp. I2</i>
Oligochaeta	<i>Rhyacodrilus subterraneus</i>	Harpacticoida	<i>Parastenocaris sp. I3</i>
Oligochaeta	<i>Spiridion sp. K1</i>	Harpacticoida	<i>Parastenocaris sp. J2</i>
Oligochaeta	<i>Trichodrilus capilliformis</i>	Harpacticoida	<i>Spelaeocamptus sp. S2</i>
Oligochaeta	<i>Trichodrilus cernosvitovi</i>	Isopoda	<i>Bragasellus aireyi</i>
Oligochaeta	<i>Trichodrilus longipenis</i>	Isopoda	<i>Caecosphaeroma virei</i>
Oligochaeta	<i>Trichodrilus pragensis</i>	Isopoda	<i>Faucheria indet.</i>
Oligochaeta	<i>Trichodrilus cf. leruthi</i>	Isopoda	<i>Microcharon sp. Rou1</i>
Oligochaeta	<i>Trichodrilus cf. pragensis</i>	Isopoda	<i>Microcharon sp. Rou2</i>
Oligochaeta	<i>Trichodrilus indet.</i>	Isopoda	<i>Microcharon sp. Rou3</i>
Oligochaeta	<i>Trichodrilus indet.2</i>	Isopoda	<i>Microcharon sp. Rou4</i>
Oligochaeta	<i>Trichodrilus indet.3</i>	Isopoda	<i>Microcharon angelieri</i>
Oligochaeta	<i>Trichodrilus indet.5</i>	Isopoda	<i>Microcharon indet.</i>
Oligochaeta	<i>Trichodrilus sp. K1</i>	Isopoda	<i>Microcharon reginae</i>
Oligochaeta	<i>Trichodrilus strandi</i>	Isopoda	<i>Monolistra (Typhlosphaeroma) berica</i>
Oligochaeta	<i>Tubificidae gen. sp. K2</i>	Isopoda	<i>Monolistra caeca absoloni</i>
Oligochaeta	<i>Tubificidae gen. sp. K3</i>	Isopoda	<i>Monolistra caeca intermedia</i>
Oligochaeta	<i>Tubificidae gen. sp. K4</i>	Isopoda	<i>Proasellus cantabricus</i>
Oligochaeta	<i>Tubificidae gen.sp. K 1</i>	Isopoda	<i>Proasellus cavaticus</i>



Gastropoda	<i>Acroloxus tetensi</i>	Isopoda	<i>Proasellus hermallensis</i>
Gastropoda	<i>Avenionia sp. pl.</i>	Isopoda	<i>Proasellus sp.</i>
Gastropoda	<i>Bythinella pupoides phreaticola</i>	Isopoda	<i>Proasellus valdensis</i>
Gastropoda	<i>Bythiospeum sp. pl.</i>	Isopoda	<i>Proasellus vulgaris</i>
Gastropoda	<i>Hadziella ephippiostoma</i>	Isopoda	<i>Proasellus walteri</i>
Gastropoda	<i>Hadziella krkae</i>	Isopoda	<i>Stenasellidae indet.</i>
Gastropoda	<i>Hauffenia cf michleri</i>	Isopoda	<i>Stenasellus buili</i>
Gastropoda	<i>Hauffenia indet.</i>	Isopoda	<i>Stenasellus virei angelieri</i>
Gastropoda	<i>Hauffenia indet. B (flattened)</i>	Isopoda	<i>Stenasellus virei buchneri</i>
Gastropoda	<i>Hauffenia indet. C (flat)</i>	Isopoda	<i>Stenasellus virei virei</i>
Gastropoda	<i>Iglica concii</i>	Isopoda	<i>Synasellus bragai</i>
Gastropoda	<i>Iglica gracilis</i>	Amphipoda	<i>Bogidiella albertimagni</i>
Gastropoda	<i>Iglica hauffeni</i>	Amphipoda	<i>Bogidiella nicolae</i>
Gastropoda	<i>Iglica indet. AB (wide)</i>	Amphipoda	<i>Crangonyx subterraneus</i>
Gastropoda	<i>Islamia minuta</i>	Amphipoda	<i>Echinogammarus indet.</i>
Gastropoda	<i>Islamia moquiniana</i>	Amphipoda	<i>Haploginglymus indet.</i>
Gastropoda	<i>Islamia sp. pl.</i>	Amphipoda	<i>Haploginglymus sp.S1</i>
Gastropoda	<i>Moitessiera simoniana</i>	Amphipoda	<i>Ingolfiella beatricis</i>
Gastropoda	<i>Moitessiera massoti</i>	Amphipoda	<i>Ingolfiella catalanensis</i>
Gastropoda	<i>Moitessiera indet.</i>	Amphipoda	<i>Niphargopsis casparyi</i>
Gastropoda	<i>Neohoratia subpiscinalis</i>	Amphipoda	<i>Niphargidae</i>
Gastropoda	<i>Paladilhopsiopsis(?) indet. KA</i>	Amphipoda	<i>Niphargus angelieri</i>
Gastropoda	<i>Paladilhopsiopsis(?) septentrionalis</i>	Amphipoda	<i>Niphargus aquilex</i>
Gastropoda	<i>Paladilhopsiopsis virei</i>	Amphipoda	<i>Niphargus delamarei</i>
Gastropoda	<i>Spiralix (Burgosia) burgensis</i>	Amphipoda	<i>Niphargus bajuvaricus grandii</i>
Gastropoda	<i>Spiralix vitrea</i>	Amphipoda	<i>Niphargus cf. forelii</i>
Gastropoda	<i>Zospeum frauenfeldi</i>	Amphipoda	<i>Niphargus cf. lessiniensis</i>
Ostracoda	<i>Candoninae gen. sp. (rectangular) I1</i>	Amphipoda	<i>Niphargus fontanus</i>
Ostracoda	<i>Candoninae gen. sp. (rectangular) I2</i>	Amphipoda	<i>Niphargus indet.</i>
Ostracoda	<i>Candoninae gen. sp. (trapez) I1</i>	Amphipoda	<i>Niphargus gr. giovanovici</i>
Ostracoda	<i>Candoninae gen. sp. (trapez) I2</i>	Amphipoda	<i>Niphargus giovanovici multipennatus</i>
Ostracoda	<i>Candoninae gen. sp. S4Triangular</i>	Amphipoda	<i>Niphargus kochianus</i>
Ostracoda	<i>Candoninae gen. sp. S5Triangular</i>	Amphipoda	<i>Niphargus kochianus dimorphopus</i>
Ostracoda	<i>Candoninae gen. sp. S2 Trapezoid</i>	Amphipoda	<i>Niphargus lessiniensis</i>
Ostracoda	<i>Candoninae gen. sp. S6 Triangular</i>	Amphipoda	<i>Niphargus "longicuspis"</i>
Ostracoda	<i>Cavernocypris subterranea</i>	Amphipoda	<i>Niphargus longidactylus</i>
Ostracoda	<i>Cryptocandona kieferi</i>	Amphipoda	<i>Niphargus "microstygius"</i>
Ostracoda	<i>Fabaeformiscandona aemona</i>	Amphipoda	<i>Niphargus pachypus</i>
Ostracoda	<i>Fabaeformiscandona breuili</i>	Amphipoda	<i>Niphargus rejici</i>
Ostracoda	<i>Fabaeformiscandona breuili sp. 2</i>	Amphipoda	<i>Niphargus rhenorhodanensis</i>
Ostracoda	<i>Fabaeformiscandona wegelini</i>	Amphipoda	<i>Niphargus schellenbergi</i>
Ostracoda	<i>Kovalevskiella cf. bretschkoi</i>	Amphipoda	<i>Niphargus similis</i>
Ostracoda	<i>Mixtacandona chappuisi</i>	Amphipoda	<i>Niphargus stygius+valvasori</i>
Ostracoda	<i>Mixtacandona lattingeriae</i>	Amphipoda	<i>Niphargus thienemanni</i>
Ostracoda	<i>Mixtacandona sp. K1</i>	Amphipoda	<i>Niphargus virei</i>
Ostracoda	<i>Mixtacandona sp. K2</i>	Amphipoda	<i>Pseudoniphargus burgensis</i>
Ostracoda	<i>Mixtacandona indet.</i>	Amphipoda	<i>Pseudoniphargus elongatus</i>
Ostracoda	<i>Mixtacandona stammeri</i>	Amphipoda	<i>Pseudoniphargus indet.</i>
Ostracoda	<i>Pseudocandona cavicola = P. pretneri</i>	Amphipoda	<i>Pseudoniphargus semielongatus</i>
Ostracoda	<i>Pseudocandona cf. eremita</i>	Amphipoda	<i>Pseudoniphargus sp.S1</i>
Ostracoda	<i>Pseudocandona zschokkei</i>	Amphipoda	<i>Salentinella sp.</i>
Ostracoda	<i>Schellencandona belgica</i>	Amphipoda	<i>Salentinella juberthieae</i>
Ostracoda	<i>Schellencandona spJ 3</i>	Amphipoda	<i>Salentinella delamarei</i>
Ostracoda	<i>Schellencandona spJ1 schellenbergi</i>	Amphipoda	<i>Salentinella sp. Roul</i>

Ostracoda	<i>Schellencandona cf. schellenbergi</i> sp. 2	Amphipoda	<i>Salentinella petiti</i>
Ostracoda	<i>Schellencandona</i> spJ2 <i>insueta</i>	Bathynellacea	<i>Bathynella</i> ?
Ostracoda	<i>Schellencandona</i> spJ4	Bathynellacea	<i>Bathynella natans</i>
Ostracoda	<i>Schellencandona triquetra</i>	Bathynellacea	<i>Bathynella slovenica</i> sp. K1
Ostracoda	<i>Schellencandona</i> <i>indet.</i> R1	Bathynellacea	<i>Bathynella</i> sp. K1
Ostracoda	<i>Dolekiella europaea</i>	Bathynellacea	<i>Bathynella</i> sp. K2
Cyclopoida	<i>Acanthocyclops</i> <i>cf. biarticulatus</i>	Bathynellacea	<i>Iberobathynella asturiensis</i>
Cyclopoida	<i>Acanthocyclops</i> <i>cf. venustus</i>	Bathynellacea	<i>Iberobathynella cantabriensis</i>
Cyclopoida	<i>Acanthocyclops hispanicus</i>	Bathynellacea	<i>Iberobathynella guarenensis</i>
Cyclopoida	<i>Acanthocyclops kiefferi</i>	Bathynellacea	<i>Iberobathynella imuniensis</i>
Cyclopoida	<i>Acanthocyclops sambugarea</i>	Bathynellacea	<i>Iberobathynella magna</i>
Cyclopoida	<i>Acanthocyclops sensitivus</i>	Bathynellacea	<i>Iberobathynella parasturiensis</i>
Cyclopoida	<i>Acanthocyclops</i> sp. S1	Bathynellacea	<i>Iberobathynella</i> sp.S1
Cyclopoida	<i>Acanthocyclops</i> sp. K1	Bathynellacea	<i>Iberobathynella</i> sp.S2
Cyclopoida	<i>Acanthocyclops venustus stammeri</i>	Bathynellacea	<i>Gallobathynella</i> sp. Rou1
Cyclopoida	<i>Diacyclops belgicus</i>	Bathynellacea	<i>Gallobathynella</i> sp. Rou2
Cyclopoida	<i>Diacyclops</i> <i>cf. belgicus</i>	Bathynellacea	<i>Gallobathynella</i> sp. Rou3
Cyclopoida	<i>Diacyclops clandestinus</i>	Bathynellacea	<i>Meridiobathynella</i> sp. II
Cyclopoida	<i>Diacyclops</i> <i>cf. maggii</i>	Bathynellacea	<i>Parabathynella</i> <i>cf. stygia</i>
Cyclopoida	<i>Diacyclops charon</i>	Bathynellacea	<i>Paradoxclamoussella fideli</i> sp. S1
Cyclopoida	<i>Diacyclops italianus</i>	Bathynellacea	<i>Paraiberobathynella fagei</i>
Cyclopoida	<i>Diacyclops languidoides</i>	Bathynellacea	<i>Syncarida</i> <i>gen.</i> S1 sp. S1
Cyclopoida	<i>Diacyclops paolae</i>	Bathynellacea	<i>Vejdovskybathynella edelweiss</i> sp.S1
Cyclopoida	<i>Diacyclops</i> <i>cf. paolae</i>	Bathynellacea	<i>Vejdovskybathynella</i> sp.S 2
Cyclopoida	<i>Diacyclops ruffoi</i>	Bathynellacea	<i>Vejdovskybathynella</i> sp.S3
Cyclopoida	<i>Diacyclops</i> sp. SB group <i>languidoides</i>	Thermosbaenacea	<i>Thermosbaenacea</i> <i>indet.</i>
Cyclopoida	<i>Diacyclops</i> sp. group <i>languidoides</i>	Coleoptera	<i>Siettitia avenionensis</i>
Cyclopoida	<i>Diacyclops</i> sp. II	Acari	<i>Acherontacarus</i> sp
Cyclopoida	<i>Diacyclops</i> sp. I2	Acari	<i>Albaxona</i> <i>indet.</i>
Cyclopoida	<i>Diacyclops</i> sp. I3	Acari	<i>Axonopsis (Paraxonopsis) vietsi</i>
Cyclopoida	<i>Diacyclops</i> sp.K1	Acari	<i>Balcanohydracarus alveolatus</i>
Cyclopoida	<i>Diacyclops</i> "clandestinus"-group	Acari	<i>Chappuisides thienemanni</i>
Cyclopoida	<i>Diacyclops zschokkei</i>	Acari	<i>Frontipodopsis reticulatifrons</i>
Cyclopoida	<i>Eucyclops graeteri</i>	Acari	<i>Frontipodopsis subterranea</i>
Cyclopoida	<i>Graeteriella (Graeteriella) unisetigera</i>	Acari	<i>Halacarellus phreaticus</i>
Cyclopoida	<i>Graeteriella Paragraeteriella Indet.</i>	Acari	<i>Hungarohydracarus subterraneus</i>
Cyclopoida	<i>Graeteriella</i> <i>indet.</i>	Acari	<i>Kongsbergia dentata</i>
Cyclopoida	<i>Graeteriella</i> <i>cf. boui</i>	Acari	<i>Kongsbergia</i> <i>indet.</i>
Cyclopoida	<i>Speocyclops cantabricus</i>	Acari	<i>Kongsbergia clypeata</i>
Cyclopoida	<i>Speocyclops infernus</i>	Acari	<i>Lethaxona cavifrons</i>
Cyclopoida	<i>Speocyclops</i> sp. II	Acari	<i>Ljania</i> <i>cf. macilenta</i>
Cyclopoida	<i>Speocyclops spelaeus</i>	Acari	<i>Lobohalacarus weberi</i>
Cyclopoida	<i>Speocyclops</i> spJ.3	Acari	<i>Lobohalacarus weberi quadriporus</i>
Harpacticoida	<i>Attheyella (A)</i> sp.J1	Acari	<i>Momonisia phreatica</i>
Harpacticoida	<i>Bryocamptus (R.) balcanicus</i>	Acari	<i>Soldanellonyx chappuisi</i>
Harpacticoida	<i>Bryocamptus (R.) pyrenaicus</i>	Acari	<i>Soldanellonyx visurgis</i>
Harpacticoida	<i>Bryocamptus</i> sp. J1	Acari	<i>Soldanellonyx monardi</i>
Harpacticoida	<i>Ceuthonectes serbicus</i>	Acari	<i>Stygomomonis latipes</i>
Harpacticoida	<i>Ceuthonectes gallicus</i>	Acari	<i>Hexaxonopsalbia lautieni</i>
Harpacticoida	<i>Ceuthonectes</i> sp. S1	Acari	<i>Wandesia stygophyla</i>
Harpacticoida	<i>Ceuthonectes</i> sp. S2	Acari	<i>Atractides similis</i>
Harpacticoida	<i>Ectinosomatidae</i> <i>gen. II</i> sp. II	Acari	<i>Hexaxonopsis inferorum</i>
Harpacticoida	<i>Elaphoidella cavatica</i>	Acari	<i>Lethaxoma pygmaea</i>
Harpacticoida	<i>Elaphoidella charon</i>		

#### **4 SECTION 4 : SELECTION OF BIODIVERSITY INDICATORS :**

##### **SUMMARY :**

Species richness is a simple measure of biodiversity and a widely used criterion for conservation planning. Unfortunately, estimates of species richness obtained from exhaustive field inventories over large spatial scales are expensive and time-consuming. For this reason, predictive models of species richness are developed herein. Environmental parameters, sets of indicator species as well as sets of higher taxa were used to develop successful regression models to predict groundwater habitats biodiversity. Species richness of stygobionts was a significant function of latitude, pH, nitrates and dissolved oxygen; the most efficient model selected using information criteria explained over 60% of the total variance of species richness. No area effect on biodiversity was detected.

Sets of indicator species and higher level taxa were selected using multiple regression models and statistically sound information criteria. In this initial case study, we found that a model based on the occurrence of five indicator species explained between 82-93% of the variance of species richness at a regional scale. Each indicator selected in a region belong to a different taxonomic group, in relation to the low species diversity of groundwater assemblages. At an European scale, a set of three indicator groups (Gastropoda, Harpacticoida, and Amphipoda) was detected; this model explained more than 80% of the variance of species richness. The inclusion of this set of indicators in large scale assessments of stygobiotic biodiversity in groundwaters is highly recommended.

#### **4.1 INTRODUCTION :**

Species richness is a simple measure of biodiversity and a widely used criterion for conservation planning. Natural resources managers need to determine which locations preferentially should be protected and how to maintain species diversity across the landscape. Data on species richness are obtained from exhaustive field inventories; unfortunately, inventories over large spatial scales are expensive and time-consuming. For this reason, ecologists need to develop predictive models of species richness as an alternative to conducting extensive field studies (Longino and Colwell, 1997; Mac Nally, 2000; Mac Nally et al., 2003). Successful biodiversity predictors can be identified in two ways.

The first method is based on environmental variables, sometimes named “environmental surrogates” of species richness (Araujo et al., 2001). Following this method, natural resources managers should be able to predict the species richness of particular areas on the basis of their environmental attributes (Mac Nally et al., 2003). The outcome of these predictive models can help to set priorities for locations for field inventories and monitoring efforts. If species richness can be modeled successfully as a function of easily quantified environmental variables, then the scientific foundation for making land-use decisions will be strengthened (Mac Nally et al., 2003).

The second method is based on the identification of a limited suite of species that reflects overall species richness of an entire biota (Pearson, 1994; Pimm et al. 2001). These species are named “biodiversity indicators”, and were recently defined by Mac Nally and Fleishman (2004) as “species with occurrence patterns that are correlated with the species richness of a larger group of organisms”. Mac Nally and Fleishman (2002, 2004) pointed out that it seems unlikely that indicator species from a single taxonomic group will provide information on the richness of an entire biota at scales meaningful for most land-use decisions (see also Mac Nally et al., 2002); however, following Fleishman et al. (2000), indicators still may be effective within limited taxonomic boundaries, and inter-group relationships can be predictive under some circumstances (Williams, 2001). Unfortunately, indicator relationships cannot always be assumed, because they can also be weak, absent or even negative, perhaps particularly when indicator and target organisms differ in their habitat associations because different ecological and historical factors govern their distributions (Su et al., 2004).

In recent times, the use of surrogate taxa (including “umbrella” species) and especially of environmental surrogates in conservation planning has become questionable because recent evidence suggests that their correlation with overall species richness is highly variable (Faith

& Walker, 1996; Su et al., 2004). Notwithstanding this fact, the search for indicators is still a much debated topic in conservation biology, because from a management-oriented perspective it may be much easier to measure the occurrence of indicators than to conduct comprehensive species inventories or habitat assessments. It is also easier to train field biologists and other personnel to identify a limited set of species or taxonomic groups and to design monitoring plans for a few indicators than to expect those personnel to recognize and track an entire fauna (Mac Nally & Fleishman, 2004). Finally, biodiversity indicators may be of paramount importance in selecting priority areas for conservation. Although measures of complementarity or other sophisticated statistical methods are used to build networks of protected sites based on selected taxonomic groups, the final currency of success is still total species richness (Su et al., 2004): a network of sites based on a surrogate taxon is considered successful if it also captures high species richness of non-target taxa, e.g. if and only if the surrogate taxon is a valid indicator.

Up to now, indicator species have been selected according to ad hoc criteria, such as their charisma or legal protection status (Andelman and Fagan, 2000). Mac Nally and Fleishman (2004) argued that statistically based selection of potential indicators is better justified and likely to be more effective: prediction of species richness should be regarded as a testable hypothesis in the form of a statistical model, e.g. a function of the occurrence of indicator species (Mac Nally et al., 2000).

The objective of the present section is to develop a statistical protocol to select potential indicators of stygobiotic species richness. The main steps followed in our study are listed herein:

- a) environmental predictors of biodiversity were identified following the suggestions of Mac Nally (2000, 2002) and Mac Nally et al. (2003)
- b) a statistical model based on the assumptions of Mac Nally and Fleishman (2002, 2004) was developed to identify associations of indicators at various taxonomic levels (“focal” groups sensu Kintsch & Urban, 2002) and spatial scales.

#### **4.2 MATERIALS AND METHODS :**

The selection of indicators was performed using a combinatorial model following the suggestions by Mac Nally and Fleishman (2002, 2004) and Mac Nally et al. (2003); the software was written using the Excel VBA (Visual Basic for Application) programming interface.

A set of 12 environmental variables (see table 1), including the standard deviations of 7 variables (Z, pH, Cond, DO, Ca, Mg, NO<sub>3</sub>) to account for heterogeneity, was used to predict species richness at the habitat level (Ku=unsaturated karst; Ks=saturated karst; Ph=hyporheic porous sediments; Ps=phreatic, e.g. saturated, porous sediments) for all PASCALIS countries. Each habitat unit within a hydrographic basin was considered as a sample.

<b>Log(Area)</b>	Area (km <sup>2</sup> , logarithmic value)
<b>Karst</b>	Karstic unit (1=Ku; 2=Ks)
<b>Por</b>	Porous unit (1=Ph; 2=Ps)
<b>Long</b>	Longitude (decimal degrees)
<b>Lat</b>	Latitude (decimal degrees)
<b>Z</b>	Altitude (m s.l.m.)
<b>pH</b>	pH value
<b>Cond</b>	Conductance (μS/cm)
<b>DO</b>	Dissolved oxygen
<b>Ca</b>	Calcium
<b>Mg</b>	Magnesium
<b>NO<sub>3</sub></b>	Nitrates

***Table 1:*** List of environmental variables included in the analyses at an European scale

With many independent variables (the number increases considering their squares and interactions), many of the ‘screening’ approaches to identify the most promising set of variables to retain (e.g. stepwise methods) are suspect for statistical reasons (Mac Nally, 2000). Mac Nally (2000) recommended using Schwarz’s information criterion (SIC) to identify the most efficient model, at least initially; an alternative is to use Akaike information criterion (AIC), the Hannan-Quin information criterion (HQC) or simply the adjusted  $r^2$  (McQuarrie and Tsai, 1998). Every possible model involving the independent variables and their standard deviations (19 variables), their squares (12 variables) and all their possible combinations ( $2^{31}$ ) in the hierarchy was tested and  $r$ , adjusted  $r^2$ , AIC, HQC, and SIC for each model were computed. Only non-interaction models were screened for tractability, because complete combinations of all the variables and their interactions are not tractable using the computing power of a PC. Hierarchical partitioning (Mac Nally, 2000) was used to confirm the choice of variables and exclude the importance of interaction terms.

The selection of indicator species and taxa was based on a slightly modified procedure; species richness, including and excluding the potential indicators (species, genera, families or higher taxa as defined in section 2), was modeled at each habitat as a function of a set of indicators drawn from the regional or European pool. Also the total number of possible

indicator sets was too high to be managed in a computer program in a reasonable computational time, considering that for  $n$  taxa  $2^n$  models must be tested. For this reason, the following scheme was adopted.

**a) *Species*.** Following the recommendations of Mac Nally and Fleishman (2004), species present within 25% and 75% of the sampled habitats were retained. In some cases most species were rare (below the limit of 75%). The number of combinations was retained, for practical reasons, below 5. The application of the results outside the study regions is impractical due to the high amount of beta diversity (see section 3). The analysis to genus and family species richness gave comparable results and presented the same problems of species due to the high turnover rates between regions and will not be discussed furthermore.

**b) *Higher taxa*:** Higher taxa as defined in section 2 are the focal topic of the analysis, being “functional” units for taxonomic studies (e.g. a unique taxonomist may identify each group); the model was tested for any region and for all the regions assembled together; in the last case karstic and porous habitats were modelled separately as well. The number of combinations was retained below 3 (e.g. only couples and trios of taxa were considered); higher rank combinations were discarded because 1) their correlation with residual biodiversity (e.g. total species richness minus indicator species richness) was low; 2) a higher number of indicators in sampling surveys become impractical. In an initial approach, the strength of the correlation between sets of indicators and species richness or residual species richness was tested using Spearman’s rank correlation. The inclusion of indicators (e.g. independent variables) in the dependent variable (species richness) as advocated by Mac Nally and Fleishman (2004), is statistically questionable, because the two data sets are not independent; for this reason a correlation with residual species richness was performed as well. Sets of indicators with highest Spearman scores were considered potential predictors of species richness. In a subsequent step, SIC, AIC, HQC and adjusted  $r^2$  were used to identify one predictive model from among the  $2^n$  combinations of potential indicator taxa (i.e., all possible pairs, trios, and so forth).

The model with a minimum value of SIC (Mac Nally, 2000) was considered the most statistically efficient, e.g. the model that optimizes fitting error against model complexity. Species richness was modelled against combinations of incidences of the predictor variables using ordinary multiple linear regression. From this preliminary screening, five indicator species were identified for each region, and three indicator taxa for each region and the whole PASCALIS area, whose incidences produced the minimum SIC among models of species richness.

### 4.3 RESULTS :

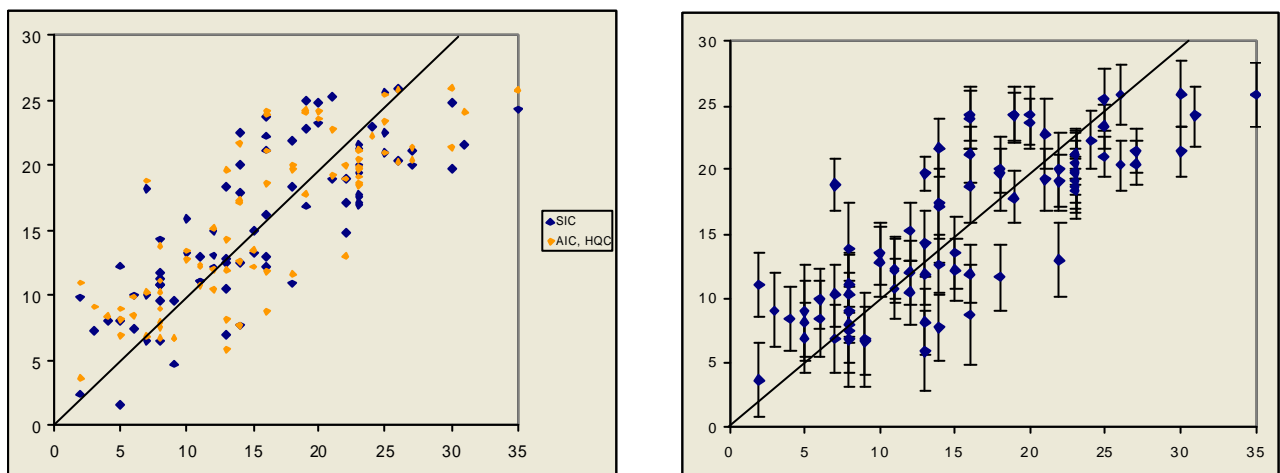
#### 4.3.1 Environmental predictors :

The output of the modelling process for selecting environmental parameters is reported below in Table 2.

Criterion	Parameters	R	R <sup>2</sup> adj	F	AIC	SIC	HQC
AIC, HQC, R <sup>2</sup> adj	Lat+pH+NO <sub>3</sub> +Lat <sup>2</sup> +DO <sup>2</sup>	0,811	0,634	28,365	3,144	3,323	3,216
SIC	pH+Ca+Lat <sup>2</sup>	0,788	0,605	41,378	3,197	3,316	3,244

**Table 2:** Selection of the “best” set of environmental predictors of biodiversity following different information criteria

Following three of the selected information criteria to identify the most statistically efficient model, latitude, pH, nitrates and dissolved oxygen explained more than 63% of the variance of species richness in the groundwater habitats studied in the PASCALIS countries. The SIC criterion, searching the best compromise between model complexity (e.g. number of selected parameters) and efficiency, indicated that latitude (in its quadratic form), pH and calcium content of water are good predictors of biodiversity. The explained variance was lower, but the predicting power of the model closely resembled that of the more complex model selected by AIC and HQC (Figure 1a).



**Figure 1a (left):** Relationship between actual and predicted species richness following the two models selected using different information criteria.

**Figure 1b (right):** The same for the 5parameters model selected by AIC; vertical bars are 95% confidence limits for predicted values.



The most informative model selects four variables; a hierarchical partitioning of variables indicates that their individual contribution in explaining biodiversity variance is high for latitude (31%, given as a non-linear relationship and assembling  $\text{Lat}^2$  – 25.01% - and  $\text{Lat}$  – 6.08%), lower for pH (6.37%) and nitrates (3.80%), and very low for  $\text{DO}^2$  (1.85%). The contribution of all the other variables (including area, habitat structure and mineralization) and of all their interaction terms was very low. The predictive multiple regression model is illustrated in Figure 1b; as it can be clearly seen, the whole model lets unexplained approximately 40% of biodiversity variance.

The results of the model agrees with those of OMI and redundancy analysis reported in section 2 as regards the predictive power of environmental variables. Their value as biodiversity predictors is enough to detect general trends of species richness across Europe, but too low if one wishes to predict biodiversity using environmental surrogates. The residual variance may be related to other, undetected factors, or to the fact that other parameters like habitat complexity deserve more attention. Similar explained values were found examining other ecological communities like butterflies (Mac Nally et al., 2003: 57% of the total deviance of species richness explained).

Some parameters like the area occupied by the habitat were unrelated with species richness; this fact was already observed at different spatial scales in section 3 (biodiversity partition). Perhaps this fact deserves a simple explanation; considering that the controversial relationship between area and species richness may depends mainly on the increase of habitat complexity with increasing area (Williamson, 1988), simply-structured groundwater habitats may not conform to this pattern. Moreover, larger subterranean areas does not allow an increased colonization facility, as it happens in epigeal landscapes; finally, groundwater communities may not be saturated, and hence not all spatial niches are already occupied, especially in post-glacial colonized areas.

#### **4.3.2 Selection of indicator species at the regional level :**

For each region the three most efficient sets of indicators are reported; they derive from the analysis of the species x habitat (presence/absence) matrix. Statistical parameters are coded as follows:  $R$  = multiple correlation coefficient;  $R^2_{\text{adj}}$  = adjusted square correlation coefficient;  $F$  = F test; AIC = Akaike's information criterion (logarithmic form); HQC = Hannan-Quin's information criterion (logarithmic form); SIC = Schwartz's information criterion (logarithmic

form). The set with the minimal SIC value is reported; in all the analyses performed, this coincides with the minimal AIC and HQC value, except for Walloon area. The same set of indicators are extracted using Spearman's rank correlation between the sum of incidences of species and total biodiversity; for this reasons these results are not reported below.

#### 4.3.2.1 Indicator species in the Lessinian mountains :

Indicators	R	R <sup>2</sup> adj	F	AIC	HQC	SIC
Diacyclops italianus+Ceuthonectes serbicus+Elaphoidella sp. I1+Paladilhliopsis virei+Graeteriella unisetigera	0,937	0,817	14,372	2,132	2,147	2,422
Diacyclops italianus+Speocyclops cf. infernus+Parvidrilus spelaeus+Graeteriella unisetigera	0,919	0,789	15,016	2,244	2,256	2,485
Diacyclops italianus+Speocyclops cf. infernus+Parvidrilus spelaeus+Paladilhliopsis virei+Graeteriella unisetigera	0,932	0,802	13,183	2,208	2,222	2,497

The most important indicators which accounted for 82% of biodiversity variance were combinations of a gastropod (*Paladilhliopsis virei*), two cyclopoids (*Diacyclops italianus*, *Graeteriella unisetigera*), and two harpacticoids (*Elaphoidella* sp., *Ceuthonectes serbicus*); alternative but less efficient models include two members of the unsaturated karstic assemblages, the cyclopoid *Speocyclops* cf. *infernus* and the oligochaete *Parvidrilus spelaeus*. Most of these species are widespread in northern Italian pre-alps, and hence this model is likely to be applied to other closely related areas as well.

#### 4.3.2.2 Indicator species in the meridional Jura :

Indicators	R	R <sup>2</sup> adj	F	AIC	HQC	SIC
Eucyclops graeteri+Attheyella sp.J1+Rhyacodrilus balmensis+Parabathynella cf. stygia+Elaphoidella phreatica	0,977	0,930	41,083	1,168	1,183	1,458
Eucyclops graeteri+Attheyella sp.J1+Rhyacodrilus balmensis+Parabathynella cf. stygia+Cryptocandona kieferi	0,972	0,9172	34,275	1,340	1,355	1,630
Eucyclops graeteri+Attheyella sp.J1+Rhyacodrilus balmensis+Parabathynella cf. stygia+Niphargus kochianus	0,972	0,917	34,105	1,345	1,359	1,634

The selected species account for 93% of total species richness variance, giving an optimal fit of the model to field data. The best model selected a cyclopoid (*Eucyclops graeteri*), widely distributed in European groundwaters, two harpacticoids (*Attheyella* sp., *Elaphoidella phreatica*), an oligochaete (*Rhyacodrilus balmensis*) and a syncarid (*Parabathynella* cf. *stygia*); alternative models include two widespread species, an ostracod (*Cryptocandona kieferi*) and an amphipod (*Niphargus kochianus*).

#### 4.3.2.3 Indicator species in the Krim massif :

Indicators	R	R <sup>2</sup> adj	F	AIC	HQC	SIC
Parastenocaris gertrudae +Mixtacandona chappuisi+Momonisia phreatica +Speocyclops infernus+Iglica hauffeni	0,972	0,918	34,658	1,407	1,422	1,697
Mixtacandona chappuisi+Proasellus vulgaris +Stygomomonia latipes+Momonisia phreatica +Acanthocyclops venustus stammeri	0,972	0,917	33,987	1,426	1,440	1,715
Mixtacandona chappuisi+Lethaxona cavifrons+Proasellus vulgaris +Momonisia phreatica +Acanthocyclops venustus stammeri	0,972	0,917	33,987	1,426	1,440	1,715

Also the most efficient model selected in Slovenian habitats accounted for more than 91% of species richness variance. The selected taxa included a harpacticoid (*Parastenocaris gertrudae*) and a cyclopoid (*Speocyclops infernus*), distributed both in karstic than in interstitial waters from Slovenia to northern Italy, together with an endemic gastropod (*Iglica hauffeni*), and acari.

#### 4.3.2.4 Indicator species in the Cantabrian region :

Indicators	R	R <sup>2</sup> adj	F	AIC	HQC	SIC
Iberobathynella imuniensis +Trichodrilus indet. 3+Paladilhiopsis(?) septentrionalis+Speocyclops cantabricus+Stygomomonia latipes	0,940	0,824	15,081	1,815	1,830	2,105
Iberobathynella imuniensis +Paladilhiopsis(?) septentrionalis+Speocyclops cantabricus+Stygomomonia latipes	0,920	0,791	15,176	1,960	1,973	2,202
Stenasellus virei buchneri+Iberobathynella imuniensis +Paladilhiopsis(?) septentrionalis+Speocyclops cantabricus+Stygomomonia latipes	0,926	0,787	12,075	2,009	2,023	2,298

Also Cantabrian habitat biodiversity can be efficiently predicted using a selection of indicators (more than 82% of variance explained) including oligochaeta (*Trichodrilus* sp.), gastropods (*Paladilhiopsis(?) septentrionalis*), bathynellaceans (*Iberobathynella imuniensis*), cyclopoids (*Speocyclops cantabricus*) and acari (*Stygomomonia latipes*); most of the species identified are local endemics.

#### 4.3.2.5 Indicator species in the Walloon region :

Indicators	R	R <sup>2</sup> adj	F	AIC	HQC	SIC
Pseudocandona zschokkei +Proasellus hermallensis+Niphargus aquilex +Diacyclops belgicus	0,948	0,863	24,609	1,010	1,022	1,251
Pseudocandona zschokkei +Proasellus hermallensis+Niphargus aquilex +Diacyclops belgicus+Acanthocyclops venustus s.l.	0,956	0,870	20,998	0,990	1,005	1,280
Pseudocandona zschokkei +Proasellus hermallensis+Niphargus aquilex +Diacyclops belgicus+Soldanellonyx visurgis	0,953	0,861	19,637	1,051	1,066	1,341

The predictive power of the optimal models (different following SIC or AIC and HQC) accounted for over 86-87% of the variance of species richness. Together with an ostracod (*Pseudocandona zschokkei*) and a cyclopoid (*Diacyclops belgicus*), the model included an isopod (*Proasellus hermallensis*) and an amphipod (*Niphargus aquilex*). All the species have a wide ecological tolerance and are widely distributed all over northern and central Europe.

#### 4.3.3 Selection of indicator taxa at the regional leve

The runs performed in each region using higher taxa and habitat units suffer for the limited number of available pairs of data (16); in any case they can give some useful informations. The results are summarized in the following table; Spearman's r value for 14 (16-2) degrees of freedom are 0.497 (p=0.05), 0.623 (p=0.01) and 0.742 (p=0.001).

Region	Indicator taxa	Rs(TOT)	Rs(RD)
CANTABRIA	GASTROPODA+HARPACTICOIDA+AMPHIPODA	0.950	0.834
JURA	GASTROPODA+CYCLOPOIDA+AMPHIPODA	0.974	0.892
WALLOON	CYCLOPOIDA+OSTRACODA+AMPHIPODA	0,943	0,186
LESSINIA	HARPACTICOIDA+AMPHIPODA+ACARI	0.910	0.788
KRIM	OLIGOCHAETA+GASTROPODA+ACARI	0,871	0,378

Region	Indicator taxa	R	R <sup>2</sup> adj	F	AIC	SIC
CANTABRIA	AMPHIPODA+BATHYNELLACEA+ACARI	0,929	0,828	25,149	1,724	1,917
JURA	GASTROPODA+CYCLOPOIDA+AMPHIPODA	0,978	0,947	90,018	0,831	1,024
WALLOON	CYCLOPOIDA+OSTRACODA+AMPHIPODA	0,963	0,910	51,542	0,552	0,745
LESSINIA	HARPACTICOIDA+AMPHIPODA+ACARI	0,954	0,889	41,383	1,556	1,749
KRIM	OLIGOCHAETA+GASTROPODA+HARPACTICOIDA	0,894	0,749	15,918	2,460	2,653

The taxa listed in the table are significantly correlated with overall species richness; the variance explained lies between 75% and 95%.

#### 4.3.4 Selection of indicator taxa at the European level :

The results of the combinatorial runs performed for each habitat type are summarized in the following table; the number of habitat units is 20; Spearman's r values for 18 degrees of freedom are 0.444 (p = 0.05), 0.561 (p=0.01), 0.679 (p=0.001).

Habitat	Indicator taxa	Rs(TOT)	Rs(RD)
Ku	GASTROPODA + HARPACTICOIDA + AMPHIPODA	0.880	0.390(n.s.)
Ks	CYCLOPOIDA + OSTRACODA + SYNCARIDA	0.858	0.778
Ph	GASTROPODA + HARPACTICOIDA + AMPHIPODA	0.966	0.902
Ps	HARPACTICOIDA + OSTRACODA + ISOPODA	0.940	0.893

Although group combinations differ between habitats, correlations with total biodiversity were very high as well.

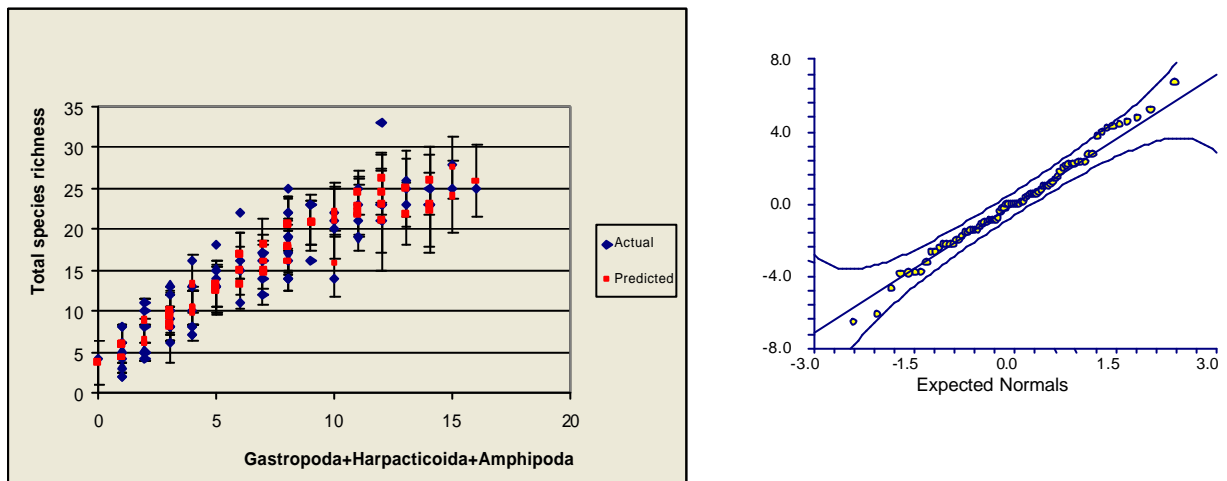
Finally, the combinatorial routines were run for all PASCALIS habitat units together; in this case the number of data is 80 and r values are significant approximately above 0.360 (p=0.001).

Indicator taxa	R(TOT)	R(RD)
GASTROPODA + HARPACTICOIDA+ AMPHIPODA	0.921	0.667
GASTROPODA + HARPACTICOIDA + OSTRACODA	0.917	0.678
ANNELIDA + GASTROPODA + CYCLOPOIDA	0.912	0.693

The results given by the multiple regression method are the same; explained variance is above 81% for the best model, but all alternative sets explain approximately the same percentage of variance.

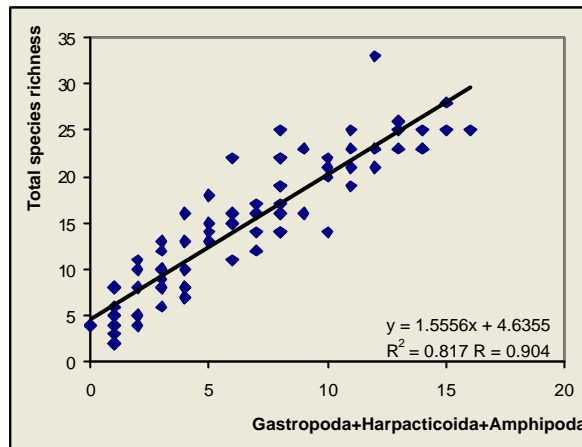
Indicator taxa	R	R <sup>2</sup> adj	F	AIC	SIC
GASTROPODA + HARPACTICOIDA + AMPHIPODA	0,908	0,818	119,093	2,322	2,441
GASTROPODA + HARPACTICOIDA + OSTRACODA	0,903	0,809	112,442	2,369	2,488
ANNELIDA+ GASTROPODA + CYCLOPOIDA	0,903	0,808	111,668	2,375	2,494

Taking in account the good performance of the combination of Gastropoda, Harpacticoida, and Amphipoda species richness as a predictor of total species richness, a categorical multiple linear regression was performed; this regression accounted for most of the variance (87.7%). The results of the model are summarized in Figure 2. A more simple, alternative linear model is reported in Figure 3; it accounted approximately for 81% of variance.



**Figure 2a (left):** Example of a categorical multiple linear regression between the predictors Gastropoda, Harpacticoida, and Amphipoda and total stygobiotic species richness of habitat units in PASCALIS countries (blue: original value; red: predicted values; bars: 95% confidence limits).

**Figure 2b (right):** normal probability plot of residuals, indicating the good performance of the model (curves represent 95% confidence limits).



**Figure 3:** Linear relationship between biodiversity predictors and total species richness.

#### 4.4 DISCUSSION :

Considering that each regional fauna differs from the others, indicators of biodiversity at the species level were confirmed to be different in each region. Usually a set of widespread species, including one or two representatives of each higher hierarchical taxonomic level, was selected by the model. This set should be interpreted merely in a statistical way; from an ecological point of view, it reflects the poor number of taxa in groundwater assemblages, which usually include a limited set of species belonging to different higher taxa. For this reason, the results illustrated above clearly demonstrate that in this case the choice of indicator species is likely to give results which can be applied exclusively at the regional level. The selection of higher taxa as indicators at the European level indicates that Gastropoda, Harpacticoida, and Amphipoda are reliable predictors of overall species richness. Although several authorities stated that true biodiversity indicators are uncommon or even absent in Nature (Faith & Walker, 1996; Su et al., 2004), the present research demonstrates that indicators and predictors can be identified within stygobiotic assemblages. The statistical methods applied in this section allowed to select species or higher level taxa which are highly correlated with overall species richness, accounting for a high percentage of biodiversity variance (usually higher than 80%). This fact may be due to the environmental constraints imposed by strong environmental gradients on the groundwater assemblages. The results of multivariate analyses (section 2) and the predictive models built using environmental variables (present section) identified some of the ecological and historical determinants of stygobiotic diversity in groundwaters. Biodiversity showed a negative correlation with latitude (which reflects the depauperation of faunas due to glaciations), anthropization (which affects mainly porous aquifers) and a positive correlation with dissolved oxygen and pH. This

correlation, even if accounting only for 60% of biodiversity variance, suggests that total stygobiotic species richness could be used in some way as an indicator of anthropogenic pressure, an important task which deserves further attention in forthcoming studies, considering that environmental requirements of stygobiotic species are poorly known (Notenboom et al., 1994). Groundwater quality, habitat structure as well as history (e.g. glaciations) clearly drive the biodiversity of most stygobiotic taxa in a similar way; this fact is reflected in a strong correlation between sets of indicators with total species richness. The analyses performed showed that it is possible to identify some sets of indicators, which allows the researcher to select the most useful taxa for inventory purposes.

From a methodological point of view, the models presented in this section suggest the following monitoring strategies to optimize the field surveys.

If the aim of the research is the detection of broad-scale biodiversity patterns, environmental parameters can be useful; however, the percentage of variance left unexplained by the model presented herein (40%) clearly indicates that further research is needed in this field and more precise environmental analyses are required. Moreover, species richness of different taxonomic groups may be driven by different environmental factors and may not be correlated at spatial and temporal scales relevant to natural resources managers (Mac Nally et al., 2003). For this reason the use of environmental surrogates of biodiversity should be treated with caution.

If the aim of the research is an assessment of stygobiotic species richness of groundwater habitats for conservation planning, as is the case of the PASCALIS field surveys, at least Gastropoda, Harpacticoida, and Amphipoda need to be included in the study. For each of these indicator groups, the collected specimens need to be identified at the species level, and their species richness can be used as a good surrogate of total stygobiotic species richness.

Finally, if more detailed analyses are to be performed in the selected regions, more accurate sets of indicator groups are indicated in the present sections; however, the choice of the correct model at local scale needs to be treated with caution, because the effect of sampling methods (see section 1) on biodiversity estimates is still poorly known.

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## **5 SECTION 5: PROPOSAL OF A METHOD FOR ASSESSING THE CONSERVATION VALUE OF SPECIES :**

### **SUMMARY :**

Indicators used to assess the conservation value of species are of paramount importance for management plans. Usually scores and indices, being simple surrogates of more complex mathematical models, may be easily used in GIS software and are a powerful tool to fill the gaps between hydrogeologists, conservation ecologists, cartographers and decision managers, allowing them to deal with the conservation value of species and communities. Conservationists usually assess species conservation value using two kinds of indices: indices of status (e.g. intrinsic properties of species, as endemism or phylogeny) and indices of risk (e.g. influenced by anthropogenic pressure, summarized in the IUCN categories). While widely accepted criteria exist for calculating the indices of risk, the indices of status are usually based on expert systems; they may be subjective and open to criticism. For this reason, the main objective of the present contribution was to develop a standard method to build conservation indices based on the information stored in the PASCALIS database and on the grid cells used to map the distribution of species over Europe.

Indices to assess degree of endemism, range-size rarity, habitat selection and taxonomic isolation (included phylogenetic relictuality) were developed using normalized values extracted from the PASCALIS database. The calculation of the degree of endemism was based on the log-transformed range of latitude and longitude reported in database. Range-size rarity was based on the log-transformed number of 0.2 x 0.2 decimal degrees cells in which the species was reported in the database. Habitat selection was based on the number of lower hierarchical units (e.g. unsaturated and saturated karst, hyporheos and saturated porous sediments) in which a species was reported; unfortunately, this information was lacking for more than one hundred species; for this reason this index is proposed herein but not used in further calculations. Finally, taxonomic isolation was calculated as the number of branches connecting a species with all the other species of the database; these values were weighted for relictuality, e. g. a higher weight was assigned to marine relicts as well as exclusively stygobiotic taxa above the species level.

Mean values of endemism, rarity, and taxonomic isolation (weighted for relictuality) were used to assign a cumulative conservation value to each of the 830 species included in the database. Limitations to this procedure are due to the high level of endemism of stygobiotic species (more than 83% of them can be defined as strict endemics) which prevents the selection of a threshold useful in discriminating priority species.

## **INTRODUCTION :**

This section deals with the selection of a method for assessing the conservation value of species and assemblages in the study regions using indices, allowing the selection of conservation indicators. To avoid confusion, a conservation indicator is here defined as a species having particular biogeographical, ecological, and phylogenetic value.

The assessment of the conservation value of species and species assemblages within habitat units is of paramount importance for selecting priority sites (Spellerberg, 1992; Villa, 1995; Williams, 2000). Scores and indices of conservation value, being simple surrogates of more complex mathematical models, may be easily used in GIS software (Williams, 2001), and are a powerful tool to exchange information between ecologists and policy-makers, filling the gap between hydrogeologists, conservation ecologists, cartographers and decision managers.

Different kinds of indices can be used, including indices of status and indices of risk. These indices, although interlinked, are based on different concepts; the indices of status are intrinsic properties of species (for example, endemism or phylogenetic relictuality), while indices of risk reflect the anthropogenic pressure on a species, on its habitat, or on its area of distribution (Froud, 1998), and are usually summarized following the I.U.C.N. classification. The present section deals with the assessment of indices of status for the stygobiotic species of the PASCALIS countries.

Up to now the assessment of scores was usually based on expert systems (Villa, 1995; Froud, 1998); in most of available studies, species scores were given by taxonomists and ecologists based on literature sources, direct experience in the field, I.U.C.N. status, and legislation as the inclusion in lists of protected species (Mariotti, 2001), thus intermixing status and risk. Although the role of expert systems may be useful in assessing the conservation value of species, scores and indices built in this way may be subjective and open to criticism. For this reason, the main objective of the present section is to develop a standard method to build conservation indices based on the information stored in the PASCALIS database. Moreover, a cumulative index for assessing the intrinsic conservation value of groundwater species and assemblages which summarizes the detailed conservation indices will be discussed.

## **5.1 MATERIAL AND METHODS :**

### **5.1.1 Data sets and data analysis :**

Data sets used for assessing the conservation value of species were stored in the PASCALIS database of regional species lists. The 4D database and the Mapinfo grid were converted to the MS Access format. After data check and correction, the database included 830 species; the calculations performed in the score-building process are illustrated herein.

a) ***Endemicity***: Defining endemism is usually a problem of scale (Peterson and Watson, 1998). Using minimum and maximum values of longitude and latitude, the extension of a species range was calculated as the area included between these limits; this area may be considered as a rough estimate of the area of occupancy, e.g. of the degree of endemism (Rapoport, 1982). Range-size data are lognormally distributed (see Rapoport, 1982, for a detailed discussion of this subject); a logarithmic (base 10) transformation was applied before using the data set in the scoring algorithm.

b) ***Rarity***: Rarity was defined as range-size rarity (Williams, 2001) and was calculated as the number of grid cells (0.2 x 0.2 decimal degrees) occupied by a species. Historical data were included in the calculation to avoid the possibility that rarity is due to anthropogenic effects, becoming an index of risk. Cell numbers are lognormally distributed as well; a logarithmic transformation was applied, as for endemism values.

c) ***Habitat selection***: Habitats were defined following the PASCALIS protocol; the database was corrected so that unique habitat types were assigned to each site where a species was collected. These data include PASCALIS sampling sites. The following classes were defined: 1) species restricted to a single habitat unit, coded as Kus (unsaturated karst), Ks (saturated karst), Ph (hyporheos), and Pp (phreatic unconsolidated sediments), received value 3; 2) species restricted to karstic (K), porous (P) or non-karstic (NK) areas, but present in more than one habitat unit, received value 2; 3) species present in all kinds of groundwaters (GW) received value 1. Unfortunately this information is still lacking in more than one hundred species; a conservative method suggests to use habitat selection values only when information is available, or alternatively to assign these species to the larger category (e.g. GW).

d) ***Taxonomic isolation***: It is well known that taxonomically isolated taxa, and especially phylogenetic relicts, deserve more attention in conservation plans, being irreplaceable natural resources. The taxonomic isolation of a species was measured following the algorithm used for taxonomic distinctness index, which is a good, powerful surrogate of the phylogenetic

value of a community or assemblage (Clarke and Warwick, 1998). Taxonomic isolation was defined as the cumulative taxonomic distance (measured as tree length) between the species and any other species of the pool. Information needed to calculate taxonomic isolation of each species were extracted from the Linnean classification assembled by the PASCALIS taxonomists, rebuilt following a standard scheme (including genus, family, order, class and phylum) and stored in the database. The taxonomic isolation for each species was calculated simply cumulating the number of branches connecting the species with all the other stygobiotic species of the pool, defined as the whole PASCALIS countries species pool. Each branch connecting a lower order taxon (for example a species) with the higher order taxon (in this case a genus) has a value of **1**; thus, tree length between pairs of congeneric species equals **2**, between species belonging to the same family but to different genera equals **4**, and so on. Finally a weighting procedure (Clarke and Warwick, 1998) was applied to the branches connecting a stygobiont taxon with a higher level taxon to account for relictuality. If the higher level taxon included both marine taxa as well as freshwater stygobionts (e.g. thalassoid stygobionts) the branch received weight **3**; if the higher level taxon included only freshwater stygobionts (e.g. limnicoid stygobionts) the branch received weight **2**; if the higher level taxon included freshwater stygobionts as well as freshwater surface taxa the branch received weight **1** (the lower level taxon being a limnicoid stygobiont of more recent origin).

### 5.1.2 Scoring indicator values :

Indicator values obtained from the database were normalized using the linear scaling transform:

$$x_n = (x - \min\{xI, xN\}) / (\max\{xI, xN\} - \min\{xI, xN\})$$

where  $\min\{xI, xN\}$  and  $\max\{xI, xN\}$  are the minimum and maximum values of the variable  $x$  within the  $N$  data. The normalization procedure needs to be repeated when adding data which are out of the old minimum – maximum range.

The linear data transform has some important statistical properties: a) introduces no distortion to the variable distribution; b) has a one-to-one relationship between the original and normalized values.

Normalization assumes the range 0-1; considering that some conservation indices may not accept null values, the range of the values was established between 1 and 5:

$$x_{(1,5)} = ((x - \min\{xI, xN\}) * (5-1) / (\max\{xI, xN\} - \min\{xI, xN\})) + 1$$

However, any kind of range can be chosen with 1 as the minimum value. Considering that in

some cases the minimum value should receive the highest score (as for range size), the linear transformation  $(5+1) - x_{(1,5)}$  was finally applied.

### 5.1.3 Calculating an index of conservation value :

Each species of the taxa included in PASCALIS (e.g. Annelida, Mollusca, Crustacea, Acari and a few stygobiotic coleoptera) and each species assemblage should receive the four status scores. There are several formulas to combine together these scores to assess the conservation value of each species; cumulating the conservation value of each species in an assemblage, a conservation value can be attributed to a spatial unit (e.g. a habitat, a region, a grid cell, and so on).

**Mean values or weighted averages:** The simplest way to assess the conservation value of a species or of an assemblage consists in calculating the mean of the four scores (for a species) or of the scores of every species (for assemblages). Weights can be assigned giving a different importance to the indices in different conservation plans; for example, weights were assigned to taxonomic isolation to emphasize the relictuality of a species giving weight 2 to any limnicoid stygobiont belonging to a stygobiotic genus, and 3 to any thalassoid stygobiont. Mean values are useful when we are comparing assemblages which differ in species richness; moreover, partition of the index into its components is very simple.

**Sums and percentages:** This method consists simply in calculating the sum of conservation scores of species or assemblages; percentages on the maximum conservation value can be used (roots of arcsin transformed percentages should be used in statistical analyses). If species richness should be included in the conservation value of an assemblage, a simple sum of the indices of each species may be a good choice.

**Storie index:** Mean values and sums can penalize species which have only one or two high scores, while the others are low; if a conservation plan should be effective in protecting species or assemblages which have at least one high value (for example strict endemic species), the Storie index modified by Villa (1995; Mariotti, 2001) can be applied. The formulation of this index is as follows:

$$I = \Omega(k; A_1, A_2, \dots, A_n) = k - \left[ \prod_{i=1}^n (k - A_i + 1) \right] \frac{1}{k^{(n-1)}}$$

where: k is the maximum value of a score (here 5), n is the number of indices (here 4) and  $A_i$  are the individual scores.

## 5.2 RESULTS :

The stygobiotic species included in the database retained for the analysis are 830. Endemic species are very common among stygobiotic taxa; over 83% have an endemism score above 4; as regards rarity, 69.6% of species have a score above 4. Percentages in each score and index are represented in the table below:

Scores	Endemicity	Rarity	Habitat Selection	Taxonomic isolation (unweighted)	Storie Index
4-5	83.37	69.64	41.92	7.84	91.57
3-4	6.51	18.07	17.59	9.77	5.66
2-3	6.75	9.52	-	13.51	1.45
1-2	3.25	2.65	40.48 (*)	68.87	1.33

(\*) overestimated, including species with no habitat details available in the data set

The results clearly demonstrate the the high degree of endemism and rarity of the stygobiotic species for conservation plans. Storie index is clearly inefficient in discriminating species values, considering that over 90% of the species fall within the highest score category; mean values are a better way to compare species conservation values. Even using averages, more than 44% of the species receives a very high score; this is not an artifact, but reflects a true, high degree of rarity and endemism, e.g. a high conservation value, of stygobiotic species.

Conservation values of species are reported in the appendix; the whole data set of scores was included in the database in MS Access format.

## 5.3 DISCUSSION :

Thanks to the PASCALIS database that reflects the available knowledge on species for six European countries, the scoring method proposed herein appears to be the best suitable method that can be applied taking in account ecology and phylogeny with however some limitations and criticisms.

**ENDEMICITY** – Over 83% of western European stygobiotic fauna includes strict endemic species, i.e. 689 endemic species out of a total of 830. This percentage is probably likely to

increase in a near future with more intensive sampling surveys; local surveys planned in poorly known areas will lead to the discovery of species new to science, most of them endemics. This was clearly demonstrated during the field studies performed in the PASCALIS regions.

Endemics are of paramount interest for conservation plans: usually, patterns of endemic species richness are more useful for conservation than biodiversity patterns (Araujo and Williams, 2000; Williams, 2000; Williams et al., 2000). The scoring method proposed herein may be resumed in this simple scheme given for reference:

Strict endemic species	4-5
Regional endemic or sub-endemic species	3-4
Widely distributed species	2-3
Species with a large distributional area in western Europe	1-2

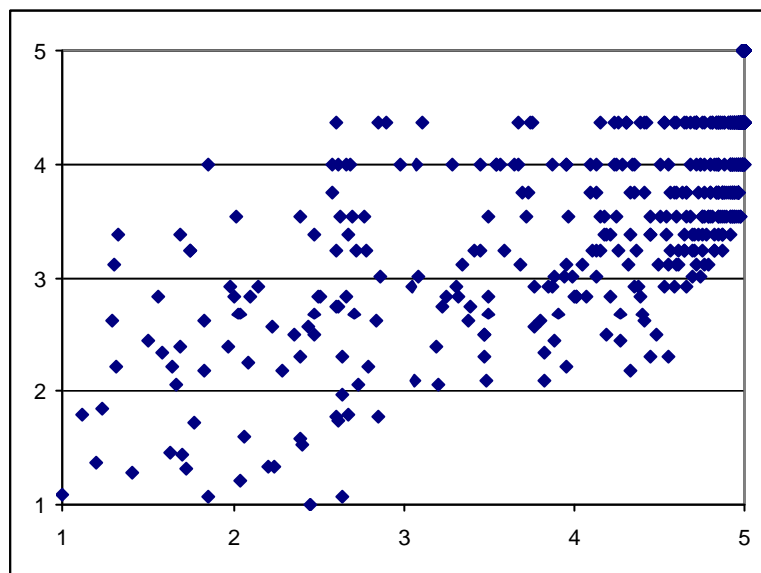
Apart from endemics, a country or an administrative region has a particular responsibility in preserving species which are present at the limit of their distributional area; it is well known from macro-ecological theory that peripheral populations are more prone to extinction, e.g., vulnerable (Brown, 1995). Marginality should be taken in account by each regional or national conservation agency; at the European level considered by the PASCALIS project, marginality is not included in this index; moreover, the area of occupancy of a species is calculated only on the data included in western-European countries and may be misleading..

In this preliminary essay, the calculation of the distributional range was roughly estimated from longitude-latitude limits stored in the database. More sophisticated methods exist to obtain species ranges from a grid (Rapoport, 1982), first of all intersection with GIS layers including landmasses borders. This time-consuming task is likely to give unsatisfactory results because it does not consider paleogeographic borders: landmasses and seas had a different extension during Miocene, Pliocene and even during the last glaciation, and considering actual borders may be misleading. Finally, the calculated values should be treated with caution as applied at an European scale; data sets up to now available refers only to PASCALIS countries and Portugal, so that the ranges obtained need to be updated in the future when data from other European countries will be stored into the database.

**RARITY** – More than 69% of western European stygobiotic species are rare. Rarity may be defined in several ways (Gaston, 1994). Considering that in stygobiotic communities we



cannot deal with abundances in a satisfactory way, and stygohabitats are too dissimilar to be compared on a quantitative basis, range-size rarity (Williams, 2000) was adopted herein. In some softwares as Worldmap (Williams, 2001) range-size rarity is considered synonym of endemism; even if a statistically significant correlation exists between the two indices (Pearson's correlation coefficient 0.764 is significant at a level of  $p < 0.001$ , indicating that endemism explains approximately 58% of rarity variance), they represent completely different historical and ecological phenomena. The correlation exists because narrow endemics are obviously rare; however rarity may be high even in widely distributed species (large gaps between cells), and, on the other hand, several regional endemics may be widespread in the whole region under study.



**Figure 1:** Relationship between endemism scores (horizontal axis) and rarity scores (vertical axis) in the set of 830 stygobiotic species included in the database; the statistically significant correlation is mainly due to the higher rarity scores of strict endemics (plotted in the right part of the graph)

For this reason the two parameters should be considered distinct, even if they emphasize the conservation status of narrow endemics; this is not a negative aspect in nature protection plans. To overcome this problem, range-size rarity may be normalized using the area of occupancy, or residuals of the regression can be used; unfortunately, these procedures may lower the conservation value of strict endemics, which are rare by definition. For this reason, as a first approach range-size rarity calculated on the whole area (in our case PASCALIS countries) was retained.

A further word of caution should be spent as regards rarity. A species can be rare simply because of our lack of knowledge; up to now we are obliged to take into account only what is

known in the literature; extensive field surveys in the future will allow to store more distributional records in the database and rarity scores will have to be recalculated.

The meaning of the scores resulting from the database can be summarized in the following table:

Rare species	4-5
Frequent species	3-4
Common species	2-3
Very common species	1-2

**HABITAT SELECTION** - Some species (more than 41%) are closely linked only to one of the hierarchical levels established in the PASCALIS protocol; these species were individuated using multivariate analyses (section 2) and are usually highly specialized to their habitat. Specialists are more vulnerable and good indicators for monitoring studies (Pearson, 1994). Unfortunately, for a large set of species (more than one hundred species) no detailed information on habitat requirements are available, and also the informations available in the database cannot be checked being based on literature data sources dealing mainly with faunistics and taxonomy, not with ecology. For this reason, the value of this index remains highly speculative, may be applied only in well known regions and was not included in the recalculation of the cumulative conservation index. Its use for assessing conservation value at an European level is not recommended until new ecological data will be available for the stygobiotic species.

**TAXONOMIC ISOLATION** – Approximately 7.8% of western European species are highly isolated from a taxonomic point of view (unweighted scores). Old phylogenetic lineages, which colonized groundwaters in ancient, usually pre-Quaternary ages, are of great cultural and scientific value (Danielopol, 1999), and have to be the main target of groundwater fauna conservation plans. The taxonomic isolation index is a surrogate of the phylogenetic value which can be assessed only using molecular techniques or traditional cladistic analysis; unfortunately these data are available only for a very limited subset of European stygobionts. The related measures of average taxonomic distinctness was applied in assessing the conservation value of stygohabitats (Danielopol et al., 2002), as well as in environmental impact assessments (Warwick and Clarke, 1998), being correlated with habitat degradation. Weighting was used to account for relictuality as well. Other weighting procedures proportional to taxon rank (Clarke and Warwick, 1999) are possible and their utility in better

tuning this index should be tested. The weighting procedure proposed in this section is strictly related to the interest and history of stygobiotic communities. One can argue that the taxonomic value of a species is higher if the whole genus is stygobiotic as well, and much higher if the whole family, or even the whole order, is stygobiotic too. Moreover, a marine relictuality (thalassoid stygobionts) may be more important in assessing conservation values than a freshwater relictuality (limnicoid stygobionts); this is a debatable assertion which deserves more attention in the future. Taxon weights were applied following a very simple procedure; more complex weighting methods can be tested in the future.

Considering that our taxonomic knowledge on stygobionts is evolving in a dramatic way, also the taxonomic isolation index needs to be updated when new taxa will be added to the database in the future, or when the tree will change following major taxonomic revisions.

*CUMULATIVE INDICES* - A cumulative index may be based on various choices. After performing a test on the available dataset, the Storie index, widely used for plant and bird species (Mariotti, 2001) as well as for soil arthropods (Storie, 1976), is not recommended for stygobionts due to its poor discriminant ability. Following this index, more than 90% of stygobionts have a very high conservation value. This reflects the high level of rarity and endemism in stygobiotic species and hence their importance in conservation plans, but the selection of such a high number of important species is impractical for conservation plans.

The method suggested in this preliminary essay is the calculation of the mean values of endemism, rarity, and taxonomic isolation (weighted for relictuality) for all the species stored in the database; habitat selection was not used due to the large gaps in our data set. Even with this method, it is difficult to recommend a discriminant threshold for the species to be strictly protected; the value of this procedure relies more on its usage in selecting priority sites for conservation than in recommending species to be included in legislation, like the annexes of the Habitat Directive. The structure of the index is likely to be amended in the future with the advance of our knowledge on groundwater fauna.

In summary, it can be reminded that the underground environment is peculiar compared to the surface environments because it includes a large number of endemics and rare species. The PASCALIS project allowed to establish that over 83 % of the stygobiotic species are to be classified as strict endemics and over 69% are to be considered very rare. Thus, following the criteria of the Habitat Directive, most of the European stygobiotic fauna is in urgent need of strict protection.

Considering that statistically sound criteria for selecting priority species and sites are needed, the application of a mean conservation value is proposed herein and it will be tested together

with species richness as a tool to produce meaningful maps to be used for planning groundwater conservation. This testing procedure and map production is the objective of WP8 workpackage.

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**5.5 APPENDIX – SPECIES SCORES (IN DECREASING ORDER OF CONSERVATION VALUE) :**

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Annelida	Marifugia cavatica	5	5	4,860728745	4,227370994	4,742456998
Crustacea	Troglocaris hercegovinensis	5	5	1,91659919	3,543848106	4,514616035
Crustacea	Microcerberus ruffoi	5	5	1,534412955	3,455117962	4,485039321
Crustacea	Microcerberus remanei	5	5	1,534412955	3,455117962	4,485039321
Crustacea	Metacirolana ponsi	5	5	1,524696356	3,452862111	4,48428737
Crustacea	Monolistra (Typhlosphaeroma) lavalensis	5	5	1,463157895	3,438575054	4,479525018
Crustacea	Monolistra (Typhlosphaeroma) pavani	5	5	1,463157895	3,438575054	4,479525018
Crustacea	Monolistra (Microlistra) calopyge	5	5	1,463157895	3,438575054	4,479525018
Crustacea	Monolistra (Microlistra) spinosissima	4,996400784	5	1,463157895	3,438575054	4,478325279
Crustacea	Monolistra (Microlistra) schottlaenderi	4,999655221	4,374248846	1,463157895	3,438575054	4,270826374
Crustacea	Monolistra (Microlistra) bolei	4,991831406	4,374248846	1,463157895	3,438575054	4,268218435
Crustacea	Monolistra (Monolistrella) velkovrhi	4,988763339	4,374248846	1,463157895	3,438575054	4,267195746
Crustacea	Typhlocirolana margalefi	4,973576105	4,374248846	1,52145749	3,452110161	4,266645037
Crustacea	Monolistra (Microlistra) spinosa	4,980588291	4,374248846	1,463157895	3,438575054	4,26447073
Crustacea	Sphaeromides virei	4,913530586	4,374248846	1,52145749	3,452110161	4,246629864
Crustacea	Limnosbaena finki	5	5	1,929554656	2,540495661	4,18016522
Crustacea	Tethysbaena siracusae	4,992520394	5	1,923076923	2,53949306	4,177337818
Crustacea	Teruelbathynella ramosae	5	5	1,654251012	2,49788514	4,165961713
Crustacea	Guadalpebathynella puchi	5	5	1,654251012	2,49788514	4,165961713
Crustacea	Paraiberobathynella (O.) notenboomii	5	5	1,651012146	2,497383839	4,165794613
Crustacea	Hexaiberobathynella hortezuelensis	5	5	1,651012146	2,497383839	4,165794613
Crustacea	Pseudobathynella magniezi	5	5	1,644534413	2,496381239	4,165460413
Crustacea	Hexabathynella valdecasasi	5	5	1,644534413	2,496381239	4,165460413
Crustacea	Sardobathynella cottarellii	5	5	1,644534413	2,496381239	4,165460413
Crustacea	Parameridiobathynella gardenis	5	5	1,644534413	2,496381239	4,165460413
Crustacea	Delamareibathynella motasi	5	5	1,641295547	2,495879939	4,165293313
Crustacea	Delamareibathynella debouttevillei	5	5	1,641295547	2,495879939	4,165293313
Crustacea	Paradoxicalamousella pinhaoensis	5	5	1,63805668	2,495378638	4,165126213
Crustacea	Meridiobathynella bedoyai	5	5	1,63805668	2,495378638	4,165126213
Crustacea	Meridiobathynella bragae	5	5	1,63805668	2,495378638	4,165126213
Crustacea	Meridiobathynella rouchi	5	5	1,63805668	2,495378638	4,165126213
Crustacea	Paradoxicalamousella fideli	4,999850113	5	1,63805668	2,495378638	4,16507625
Crustacea	Clamousella lusitanica	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Clamousella paralusitanica	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Vejdovskybathynella espattyensis	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Clamousella valencianensis	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Gallobathynella (Gallobathynella) boui	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Vejdovskybathynella leclerci	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Vejdovskybathynella balazuci	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Gallobathynella (Gallobathynella) juberthiae	5	5	1,634817814	2,494877338	4,164959113
Crustacea	Vejdovskybathynella edelweiss	4,999261055	5	1,634817814	2,494877338	4,164712798
Crustacea	Bathynella pyrenaica	5	5	1,628340081	2,493874738	4,164624913
Crustacea	Bathynella lombardica	5	5	1,628340081	2,493874738	4,164624913
Crustacea	Bathynella ruffoi	5	5	1,628340081	2,493874738	4,164624913
Crustacea	Bathynella hispanica	5	5	1,628340081	2,493874738	4,164624913

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	Paradoxicalamousella huescalensis	4,997519384		5 1,63805668	2,495378638	4,164299341
Crustacea	Iberobathynella pedroi	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Iberobathynella) gracilipes	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Iberobathynella) barcelensis	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Asturibathynella) celiana	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Asturibathynella) ortizi	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Espanobathynella) espaniensis	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Asturibathynella) serbani	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Asturibathynella) guarenensis	5		5 1,599190283	2,489363035	4,163121012
Crustacea	Iberobathynella (Asturibathynella)	4,997459652		5 1,599190283	2,489363035	4,162274229
Crustacea	Iberobathynella (Espanobathynella)	4,995717622		5 1,599190283	2,489363035	4,161693552
Crustacea	Metaingolfiella mirabilis	5		5 1,466396761	2,468809725	4,156269908
Crustacea	Metacrangonyx ilvanus	5		5 1,459919028	2,467807125	4,155935708
Crustacea	Pseudoniphargus eborarius	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus daviui	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus affinis	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus granadensis	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus grandis	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus vomeratus	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus inconditus	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus latipes	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus longispinum	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus gorbeanus	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus margalefi	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus stocki	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus triasi	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus mateusorum	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus spiniferus	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus sorbasiensis	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus sodalis	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus unisexualis	5		5 1,252631579	2,435723909	4,145241303
Crustacea	Pseudoniphargus burgensis	4,999261055		5 1,252631579	2,435723909	4,144994988
Chelicerata	Halacarellus phreaticus	4,999972839		5 4,873684211	2,379076981	4,12634994
Annelida	Abyssidrilus subterraneus	5		5 4,507692308	2,336591785	4,112197262
Annelida	Aktedrilus argatxae	5		5 4,507692308	2,336591785	4,112197262
Annelida	Aktedrilus ruffoi	5		5 4,507692308	2,336591785	4,112197262
Annelida	Gianius crypticus	5		5 4,504453441	2,33621581	4,112071937
Annelida	Gianius labouichensis	5		5 4,504453441	2,33621581	4,112071937
Crustacea	Troglocaris inermis	4,889812177	3,748497694	1,91659919	3,543848106	4,060719326
Crustacea	Monolistra (Typhlosphaeroma) boldorii	4,884649121	3,748497694	1,463157895	3,438575054	4,02390729
Crustacea	Schizopera (Schizopera) lindae	5		5 1,761133603	2,017764828	4,005921609
Crustacea	Schizopera (Schizopera) lagrecai	5		5 1,761133603	2,017764828	4,005921609
Crustacea	Pseudectinosoma kunzi	5		5 1,75465587	2,017012877	4,005670959
Crustacea	Pseudectinosoma reductum	5		5 1,75465587	2,017012877	4,005670959
Crustacea	Nitokra reducta	5		5 1,676923077	2,007989473	4,002663158
Crustacea	Nitokra divaricata	5		5 1,676923077	2,007989473	4,002663158
Crustacea	Nitokra intermedia	5		5 1,676923077	2,007989473	4,002663158
Crustacea	Microcharon sisyphus	5		5 1,456680162	1,98242316	3,994141053
Crustacea	Microcharon doueti	5		5 1,456680162	1,98242316	3,994141053
Crustacea	Microcharon nuragicus	5		5 1,456680162	1,98242316	3,994141053
Crustacea	Microcharon arganoi	5		5 1,456680162	1,98242316	3,994141053

Phylum	Species	Endemicy ty	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	Microcharon silverii	5	5	1,456680162	1,98242316	3,994141053
Crustacea	Metahadzia tavaresi	5	5	1,456680162	1,98242316	3,994141053
Crustacea	Microcharon comasi	5	5	1,456680162	1,98242316	3,994141053
Crustacea	Microcharon angelicae	5	5	1,456680162	1,98242316	3,994141053
Crustacea	Microcharon coineauae	5	5	1,456680162	1,98242316	3,994141053
Crustacea	Ingolfiella cottarellii	5	5	1,453441296	1,982047185	3,994015728
Crustacea	Ingolfiella catalanensis	5	5	1,453441296	1,982047185	3,994015728
Crustacea	Longigammarus planasiae	5	5	1,450202429	1,98167121	3,993890403
Crustacea	Hadzia fragilis	5	5	1,450202429	1,98167121	3,993890403
Crustacea	Bogidiella aprutina	5	5	1,4048583	1,976407557	3,992135852
Crustacea	Bogidiella helenae	5	5	1,4048583	1,976407557	3,992135852
Crustacea	Bogidiella ichnusae	5	5	1,4048583	1,976407557	3,992135852
Crustacea	Bogidiella vandeli	5	5	1,4048583	1,976407557	3,992135852
Crustacea	Bogidiella silverii	5	5	1,4048583	1,976407557	3,992135852
Crustacea	Bogidiella semidenticulata	4,999884465	5	1,4048583	1,976407557	3,992097341
Crustacea	Hexabathynella nicoleiana	4,997936167	4,374248846	1,644534413	2,496381239	3,956188751
Crustacea	Monolistra (Typhlosphaeroma) berica	4,881010943	3,547050817	1,463157895	3,438575054	3,955545605
Crustacea	Iberobathynella (Espanobathynella) magna	4,990065218	4,374248846	1,599190283	2,489363035	3,9512257
Crustacea	Iberobathynella (Iberobathynella) lusitanica	4,979596388	4,374248846	1,599190283	2,489363035	3,94773609
Crustacea	Vandelibathynella vandeli	4,96656718	4,374248846	1,644534413	2,496381239	3,945732422
Crustacea	Clamourella delayi	4,967979594	4,374248846	1,634817814	2,494877338	3,945701926
Crustacea	Pseudoniphargus gracilis	4,991355577	4,374248846	1,252631579	2,435723909	3,933776111
Crustacea	Pseudoniphargus jereanus	4,99095963	4,374248846	1,252631579	2,435723909	3,933644128
Crustacea	Pseudoniphargus gibraltarius	4,967205101	4,374248846	1,252631579	2,435723909	3,925725952
Crustacea	Iberobathynella (Iberobathynella) paragracilipes	4,912579581	4,374248846	1,599190283	2,489363035	3,925397154
Chelicerata	Acherontacarus bicornis	5	5	4,880161943	1,762477676	3,920825892
Chelicerata	Balcanohydracarus alveolatus	5	5	4,876923077	1,762227026	3,920742342
Chelicerata	Hungarohydracarus subterraneus	4,999902119	5	4,876923077	1,762227026	3,920709715
Chelicerata	Stygomamersopsis viedmai	5	5	4,873684211	1,761976376	3,920658792
Chelicerata	Stygomamersopsis anisitsipalpis	5	5	4,873684211	1,761976376	3,920658792
Crustacea	Pseudoniphargus guernicae	4,947282568	4,374248846	1,252631579	2,435723909	3,919085108
Annelida	Delaya bureschi	5	5	4,565991903	1,738164614	3,912721538
Annelida	Delaya cantabronensis	5	5	4,565991903	1,738164614	3,912721538
Annelida	Delaya corbarensis	5	5	4,565991903	1,738164614	3,912721538
Annelida	Cookidrilus speluncaeus	5	5	4,553036437	1,737162014	3,912387338
Annelida	Krenedrilus sergei	5	5	4,510931174	1,733903562	3,911301187
Annelida	Stochidrilus glandulosus	5	5	4,510931174	1,733903562	3,911301187
Crustacea	Sphaeromides raymondi	4,681958848	3,547050817	1,52145749	3,452110161	3,893706609
Crustacea	Typhlocaris salentina	4,33044763	3,748497694	1,929554656	3,546855908	3,875267077
Mollusca	Henrigirardia wienini	5	5	3,027530364	1,619105806	3,873035269
Mollusca	Lanzaiopsis savinica	4,999904174	5	3,027530364	1,619105806	3,873003327
Mollusca	Palacanthiopsis margritae	5	5	3,024291498	1,618855156	3,872951719
Mollusca	Istriana falkneri	5	5	3,024291498	1,618855156	3,872951719
Mollusca	Palaospeum nanum	5	5	3,024291498	1,618855156	3,872951719
Mollusca	Sardohoratia sulcata	5	5	3,024291498	1,618855156	3,872951719
Mollusca	Horatia gatao	5	5	3,024291498	1,618855156	3,872951719
Mollusca	Sardohoratia islamioides	5	5	3,024291498	1,618855156	3,872951719
Mollusca	Neohoratia coronadoi	5	5	3,017813765	1,618353855	3,872784618
Mollusca	Neohoratia gasulli	5	5	3,017813765	1,618353855	3,872784618
Mollusca	Neohoratia herreroi	5	5	3,017813765	1,618353855	3,872784618



Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Mollusca	Horatia supracarinata	4,999020891		5 3,024291498	1,618855156	3,872625349
Mollusca	Paladilhopsis sublesta		5	5 3,011336032	1,617852555	3,872617518
Mollusca	Paladilhopsis kostanjeviceae		5	5 3,011336032	1,617852555	3,872617518
Mollusca	Kerkia kusceri	4,998422007		5 3,027530364	1,619105806	3,872509271
Mollusca	Hadziella thermalis	4,999299069		5 3,014574899	1,618103205	3,872467425
Mollusca	Spiralix burgundina		5	5 3,0048583	1,617351255	3,872450418
Mollusca	Hauffenia (Hauffenia) solitaria		5	5 3,0048583	1,617351255	3,872450418
Mollusca	Islamia consolationis		5	5 3,0048583	1,617351255	3,872450418
Mollusca	Islamia germaini		5	5 3,0048583	1,617351255	3,872450418
Mollusca	Hauffenia (Hauffenia) minuta		5	5 3,0048583	1,617351255	3,872450418
Mollusca	Spiralix corsica		5	5 3,0048583	1,617351255	3,872450418
Mollusca	Spiralix hofmanni	4,999346883		5 3,0048583	1,617351255	3,872232713
Mollusca	Alzoniella feneriensis		5	5 2,9951417	1,616599304	3,872199768
Mollusca	Alzoniella haicabia		5	5 2,9951417	1,616599304	3,872199768
Mollusca	Alzoniella navarrensis		5	5 2,9951417	1,616599304	3,872199768
Mollusca	Alzoniella pyrenaica		5	5 2,9951417	1,616599304	3,872199768
Mollusca	Alzoniella junqua		5	5 2,9951417	1,616599304	3,872199768
Mollusca	Iglica aedlaueri		5	5 2,9951417	1,616599304	3,872199768
Mollusca	Hauffenia (Hauffenia) subcarinata	4,999194863		5 3,0048583	1,617351255	3,872182039
Mollusca	Iglica pezzolii	4,999824832		5 2,9951417	1,616599304	3,872141379
Mollusca	Moitessieria nezi		5	5 2,988663968	1,616098004	3,872032668
Mollusca	Moitessieria cocheti		5	5 2,988663968	1,616098004	3,872032668
Mollusca	Moitessieria bourguignati		5	5 2,988663968	1,616098004	3,872032668
Mollusca	Alzoniella sigestra	4,998484422		5 2,9951417	1,616599304	3,871694575
Mollusca	Hauffenia (Vrania) wagneri	4,997595791		5 3,0048583	1,617351255	3,871649015
Mollusca	Bythiospeum racovitzae		5	5 2,972469636	1,614844754	3,871614918
Mollusca	Bythiospeum drouetianum		5	5 2,972469636	1,614844754	3,871614918
Mollusca	Bythiospeum anglesianum		5	5 2,972469636	1,614844754	3,871614918
Mollusca	Bythiospeum terveri		5	5 2,972469636	1,614844754	3,871614918
Mollusca	Erythromatiana verdica	4,994333328		5 3,024291498	1,618855156	3,871062828
Mollusca	Alzoniella manganellii	4,984564305		5 2,9951417	1,616599304	3,867054536
Crustacea	Gelyella droguei		5	5 2,295546559	1,562458878	3,854152959
Crustacea	Graeteriella (Paragraeteriella) laisi		5	5 1,848582996	1,527869161	3,842623054
Crustacea	Graeteriella (Paragraeteriella) vandeli		5	5 1,848582996	1,527869161	3,842623054
Crustacea	Graeteriella (Paragraeteriella) gelyensis		5	5 1,848582996	1,527869161	3,842623054
Crustacea	Graeteriella (Graeteriella) brehmi	4,999141106		5 1,848582996	1,527869161	3,842336756
Crustacea	Pseudolimnocythere hypogea	4,998038879	4,374248846	2,930364372	2,15349187	3,841926532
Crustacea	Speocyclops hellenicus		5	5 1,816194332	1,525362659	3,841787553
Crustacea	Speocyclops orcinus		5	5 1,816194332	1,525362659	3,841787553
Crustacea	Speocyclops sardus		5	5 1,816194332	1,525362659	3,841787553
Crustacea	Hexabathynella knoepffleri	4,653235545	4,374248846	1,644534413	2,496381239	3,841288543
Crustacea	Speocyclops sisyphus	4,993438867		5 1,816194332	1,525362659	3,839600509
Crustacea	Speocyclops proserpinae	4,990224249		5 1,816194332	1,525362659	3,838528969
Crustacea	Stygonitocrella guadalensis		5	5 1,680161943	1,514835354	3,838278451
Crustacea	Nitocrellopsis rouchi		5	5 1,680161943	1,514835354	3,838278451
Crustacea	Stygonitocrella dubia		5	5 1,680161943	1,514835354	3,838278451
Crustacea	Parapseudoleptomesochra baeticola		5	5 1,670445344	1,514083404	3,838027801
Crustacea	Parapseudoleptomesochra guadalhorcensis		5	5 1,670445344	1,514083404	3,838027801
Crustacea	Nitocrella dussarti		5	5 1,631578947	1,511075602	3,837025201
Crustacea	Nitocrella stochi		5	5 1,631578947	1,511075602	3,837025201
Crustacea	Nitocrella pescei		5	5 1,631578947	1,511075602	3,837025201

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	Nitocrella kunzi	5	5	1,631578947	1,511075602	3,837025201
Crustacea	Nitocrella vasconica	5	5	1,631578947	1,511075602	3,837025201
Crustacea	Nitocrella omega	5	5	1,631578947	1,511075602	3,837025201
Crustacea	Nitocrella juturna	5	5	1,631578947	1,511075602	3,837025201
Crustacea	Nitocrella fedelitae	4,999916646	5	1,631578947	1,511075602	3,836997416
Crustacea	Nitocrella delayi	4,998114186	5	1,631578947	1,511075602	3,836396596
Crustacea	Paramorariopsis anae	5	5	1,55708502	1,505310649	3,83510355
Crustacea	Morariopsis dumonti	4,999720767	5	1,553846154	1,505059999	3,834926922
Crustacea	Antrocampthus stygius	5	5	1,544129555	1,504308049	3,83476935
Crustacea	Antrocampthus longifurcatus	5	5	1,544129555	1,504308049	3,83476935
Crustacea	Ceuthonectes vievilleae	5	5	1,534412955	1,503556099	3,8345187
Crustacea	Ceuthonectes chappuisi	5	5	1,534412955	1,503556099	3,8345187
Crustacea	Ceuthonectes boui	5	5	1,534412955	1,503556099	3,8345187
Crustacea	Ceuthonectes bulbiseta	5	5	1,534412955	1,503556099	3,8345187
Crustacea	Antrocampthus catherinae	4,998114186	5	1,544129555	1,504308049	3,834140745
Crustacea	Stenasellus magniezi	5	5	1,492307692	1,500297647	3,833432549
Crustacea	Antrocampthus chappuisi	4,995931758	5	1,544129555	1,504308049	3,833413269
Crustacea	Psammogammarus burri	5	5	1,450202429	1,497039195	3,832346398
Crustacea	Tyrrhenogammarus sardous	5	5	1,446963563	1,496788545	3,832262848
Crustacea	Pseudoniphargus illustris	4,68624335	4,374248846	1,252631579	2,435723909	3,832072035
Crustacea	Parastenocaris nicolasi	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris acherusia	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris veneris	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris lusitanica	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris micheli	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris calliroe	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris stellae	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris sardoa	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris ruffoi	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris rivi	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris ranae	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris oligoalina	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris triphyda	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris amatheia	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris cantabrica	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris conimbrigensis	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris crenobia	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris cruzi	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris dentulatus	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris andalusica	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris lorenzae	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris amyclaea	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris kalypso	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris glacialis	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris hera	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris hippuris	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris ima	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris aedes	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris kabyloides	5	5	1,424291498	1,495033994	3,831677998
Crustacea	Parastenocaris etrusca	4,999744265	5	1,424291498	1,495033994	3,831592753
Crustacea	Parastenocaris silvana	4,999674866	5	1,424291498	1,495033994	3,83156962

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue	
Crustacea	Parastenocaris federici	4,999631976		5	1,424291498	1,495033994	3,831555323
Crustacea	Parastenocaris phyllura	4,998679108		5	1,424291498	1,495033994	3,831237701
Crustacea	Parastenocaris mangini	4,998114186		5	1,424291498	1,495033994	3,831049393
Crustacea	Parastenocaris nertensis	4,998046319		5	1,424291498	1,495033994	3,831026771
Crustacea	Parastenocaris vandeli	4,995931758		5	1,424291498	1,495033994	3,830321917
Crustacea	Chthonasellus bodoni		5	5	1,288259109	1,484506689	3,828168896
Crustacea	Bragasellus seabrai		5	5	1,265587045	1,482752138	3,827584046
Crustacea	Bragasellus pauloae		5	5	1,265587045	1,482752138	3,827584046
Crustacea	Bragasellus frontellum		5	5	1,265587045	1,482752138	3,827584046
Crustacea	Bragasellus peltatus		5	5	1,265587045	1,482752138	3,827584046
Crustacea	Bragasellus escolai		5	5	1,265587045	1,482752138	3,827584046
Crustacea	Synasellus albicastrensis		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus pireslimai		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus nobrei		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus minutus		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus longicauda		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus mateusi		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus exiguus		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus barcelensis		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus transmontanus		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus brigantinus		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus fragilis		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus meirelesi		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus lafonensis		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Synasellus serranus		5	5	1,236437247	1,480496287	3,826832096
Crustacea	Niphargobates orophobata		5	5	1,220242915	1,479243037	3,826414346
Crustacea	Niphargopsis legeri		5	5	1,217004049	1,478992387	3,826330796
Crustacea	Haploginglymus lobatus		5	5	1,213765182	1,478741736	3,826247245
Crustacea	Haploginglymus mateusi		5	5	1,213765182	1,478741736	3,826247245
Crustacea	Niphargus tauri		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus balazuci		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus burgundus		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus vandeli		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus carniolicus		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus parenzani		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus timavi		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus subtypicus		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus strouhali		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus hebereri		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus jalzici		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus kieferi		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus microcerberus		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus pachytelson		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus dolienianensis		5	5	1	1,462198828	3,820732943
Crustacea	Niphargus boulangei	4,999955664		5	1	1,462198828	3,820718164
Crustacea	Niphargus armatus	4,999913207		5	1	1,462198828	3,820704012
Crustacea	Niphargus labacensis	4,997815851		5	1	1,462198828	3,820004893
Crustacea	Spelaemysis bottazzii	4,788759107	3,12274654	1,936032389	3,548359808	3,819955152	
Crustacea	Pseudoniphargus incantatus	4,970778502	4,008207887	1,252631579	2,435723909	3,804903433	
Crustacea	Bogidiella nicolae	4,999452123	4,374248846	1,4048583	1,976407557	3,783369509	
Crustacea	Microcharon boui	4,982795157	4,374248846	1,456680162	1,98242316	3,779822388	

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Annelida	Gianius cavealis	4,990799647	4,008207887	4,504453441	2,33621581	3,778407781
Crustacea	Hadzia minuta	4,962357742	4,374248846	1,450202429	1,98167121	3,772759266
Crustacea	Microcharon juberthiei	4,942404177	4,374248846	1,456680162	1,98242316	3,766358728
Crustacea	Iberobathynella (Iberobathynella) valbonensis	4,412497131	4,374248846	1,599190283	2,489363035	3,758703004
Crustacea	Sphaeromicola cebennica	4,724465513	4,374248846	2,910931174	2,151236018	3,749983459
Crustacea	Galloobathynella (Galloobathynella) coiffaiti	4,74103679	4,008207887	1,634817814	2,494877338	3,748040672
Crustacea	Stygiomysis hydruntina	4,875739118	3,748497694	1,936032389	2,541498261	3,721911691
Chelicerata	Axonopsis (Paraxonopsis) vietsi	5	5	4,870445344	1,144750446	3,714916815
Chelicerata	Kongsbergia dentata	4,999986382	5	4,870445344	1,144750446	3,714912276
Crustacea	Pseudoniphargus nevadensis	4,960109306	3,748497694	1,252631579	2,435723909	3,71477697
Tracheata	Siettita balsetensis	4,99527516	4,374248846	5	1,771751731	3,713758579
Annelida	Pachydrilus fossor	5	5	4,588663968	1,133847166	3,711282389
Annelida	Trichodrilus tenuis	5	5	4,520647773	1,13121534	3,710405113
Annelida	Trichodrilus rouchi	5	5	4,520647773	1,13121534	3,710405113
Annelida	Psammoryctides hadzii	5	5	4,510931174	1,130839365	3,710279788
Annelida	Trichodrilus sketi	4,999366478	5	4,520647773	1,13121534	3,710193939
Annelida	Rhyacodrilus caudosetosus	5	5	4,481781377	1,129711439	3,709903813
Annelida	Rhyacodrilus omodei	5	5	4,481781377	1,129711439	3,709903813
Annelida	Rhyacodrilus dolcei	5	5	4,481781377	1,129711439	3,709903813
Annelida	Rhyacodrilus maculatus	5	5	4,481781377	1,129711439	3,709903813
Annelida	Rhyacodrilus okamikae	4,99979976	5	4,481781377	1,129711439	3,709837066
Chelicerata	Momonisia phreatica	4,992785148	4,374248846	4,876923077	1,762227026	3,709753673
Annelida	Rhyacodrilus sketi	4,999530734	5	4,481781377	1,129711439	3,709747391
Annelida	Tubificoides galarzai	4,396288658	4,374248846	4,510931174	2,33696776	3,702501755
Annelida	Haber turquini	4,989065152	4,374248846	4,507692308	1,733652912	3,69898897
Chelicerata	Neoacarus hibernicus	4,941293832	4,374248846	4,880161943	1,762477676	3,692673451
Mollusca	Marstoniopsis croatica	5	5	3,027530364	1,073440486	3,691146829
Mollusca	Graziana cezairensis	5	5	3,024291498	1,073315161	3,691105054
Crustacea	Alona sketi	5	5	3,0048583	1,072563211	3,690854404
Crustacea	Alona stochi	5	5	3,0048583	1,072563211	3,690854404
Crustacea	Alona bessei	5	5	3,0048583	1,072563211	3,690854404
Mollusca	Bythinella viridis	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Belgrandiella dunalina	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella galerae	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Belgrandiella andalucensis	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella reyniesi	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella padiraci	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Belgrandiella cantabrica	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella abbreviata	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella bouloti	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella geisserti	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Belgrandiella pageti	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella pupoides	5	5	2,991902834	1,072061911	3,690687304
Mollusca	Bythinella gonzalezi	4,99979224	5	2,991902834	1,072061911	3,69061805
Mollusca	Belgrandiella globulosa	4,998974058	5	2,991902834	1,072061911	3,690345323
Mollusca	Belgrandiella robusta	4,995207777	5	2,991902834	1,072061911	3,689089896
Crustacea	Typhlocypris schmeili	5	5	2,784615385	1,064041107	3,688013702
Crustacea	Cryptocandona leruthi	5	5	2,781376518	1,063915782	3,687971927
Crustacea	Fabaeformiscandona latens	5	5	2,774898785	1,063665131	3,687888377
Crustacea	Mixtacandona juberthieae	5	5	2,771659919	1,063539806	3,687846602
Crustacea	Mixtacandona lattingerae	5	5	2,771659919	1,063539806	3,687846602

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	Pseudocandona pretneri	5	5	2,758704453	1,063038506	3,687679502
Crustacea	Pseudocandona simililampadis	5	5	2,758704453	1,063038506	3,687679502
Crustacea	Pseudocandona cavicola	5	5	2,758704453	1,063038506	3,687679502
Crustacea	Pseudocandona rouchi	4,995931758	5	2,758704453	1,063038506	3,686323421
Crustacea	Troglocaris anophthalmus	4,592589264	2,921299663	1,91659919	3,543848106	3,685912344
Crustacea	Parasalentinella rouchi	4,842973435	3,748497694	1,446963563	2,465801924	3,685757684
Crustacea	Gallobathynella (Gallobathynella) tarissei	4,803889189	3,748497694	1,634817814	2,494877338	3,682421407
Crustacea	Eucyclops ibleicus	5	5	1,858299595	1,028198139	3,676066046
Crustacea	Eucyclops puteincola	5	5	1,858299595	1,028198139	3,676066046
Crustacea	Eucyclops longispinosus	5	5	1,858299595	1,028198139	3,676066046
Crustacea	Metacyclops postojnae	5	5	1,855060729	1,028072814	3,676024271
Crustacea	Metacyclops trisetosus	5	5	1,855060729	1,028072814	3,676024271
Crustacea	Hexabathynella minuta	4,157045796	4,374248846	1,644534413	2,496381239	3,67589196
Crustacea	Acanthocyclops agamus	5	5	1,83562753	1,027320863	3,675773621
Crustacea	Acanthocyclops hypogeus	5	5	1,83562753	1,027320863	3,675773621
Crustacea	Diacyclops nuragicus	5	5	1,806477733	1,026192938	3,675397646
Crustacea	Diacyclops antrincola	5	5	1,806477733	1,026192938	3,675397646
Crustacea	Diacyclops balearicus	5	5	1,806477733	1,026192938	3,675397646
Crustacea	Diacyclops nagysalloensis	5	5	1,806477733	1,026192938	3,675397646
Crustacea	Diacyclops ichnusae	5	5	1,806477733	1,026192938	3,675397646
Crustacea	Paracampius gasparoi	5	5	1,55708502	1,016542908	3,672180969
Crustacea	Moraria (Moraria) denticulata	5	5	1,547368421	1,016166933	3,672055644
Crustacea	Moraria (Moraria) michelettoae	5	5	1,547368421	1,016166933	3,672055644
Crustacea	Bryocampius (Rheocampius) alosensis	5	5	1,544129555	1,016041608	3,672013869
Crustacea	Elaphoidella madiracensis	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella oglasae	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella boui	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella federicae	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella dubia	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella franci	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella garbetensis	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella calypsonis	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella reducta	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella pyrenaica	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella longifurcata	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella brehieri	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella aprutina	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella subplutonis	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella tiberina	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella vandeli	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella apostolovi	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella cottarellii	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella ruffoi	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella janas	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella italica	5	5	1,434008097	1,011780556	3,670593519
Crustacea	Elaphoidella coiffaiti	4,998114186	5	1,434008097	1,011780556	3,669964914
Crustacea	Asellus (Asellus) aquaticus ssp.	4,999317295	5	1,285020243	1,006015603	3,668444299
Crustacea	Moraria (Moraria) catalana	4,984057576	5	1,547368421	1,016166933	3,666741503
Crustacea	Proasellus parvulus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus bellesi	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus exiguus	5	5	1,129554656	1	3,666666667

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	Proasellus vignai	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus spinipes	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus faesulanus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus espanoli	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus rouchi	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus comasi	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus meridianus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus margalefi	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus lusitanicus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus ligusticus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus lagari	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus jaloniacus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus solanasi	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus amiterminus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus chappuisi	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus bouianus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus goubaultae	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus burgundus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus arthrodilus	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus claudei	5	5	1,129554656	1	3,666666667
Crustacea	Proasellus spelaeus	4,994639007	5	1,129554656	1	3,664879669
Crustacea	Pseudectinosoma vandeli	4,601771862	4,374248846	1,75465587	2,017012877	3,664344528
Mollusca	Istriana mirnae	4,999831822	4,374248846	3,024291498	1,618855156	3,664311941
Mollusca	Pezzolia radapalladis	4,997475641	4,374248846	3,027530364	1,619105806	3,663610098
Mollusca	Alzoniella finalina	4,998267577	4,374248846	2,9951417	1,616599304	3,663038576
Mollusca	Iglica giustii	4,998263446	4,374248846	2,9951417	1,616599304	3,663037199
Mollusca	Plagigeyeria deformata	4,99537391	4,374248846	3,024291498	1,618855156	3,662825971
Mollusca	Paladilhioopsis grobbeni	4,995504263	4,374248846	3,011336032	1,617852555	3,662535221
Mollusca	Moitessieria fontsainteii	4,99695384	4,374248846	2,988663968	1,616098004	3,662433563
Mollusca	Islamia gaiteri	4,995110714	4,374248846	3,0048583	1,617351255	3,662236938
Mollusca	Iglica robiciana	4,989983249	4,374248846	2,9951417	1,616599304	3,660277133
Mollusca	Iglica luxurians	4,986168735	4,374248846	2,9951417	1,616599304	3,659005628
Mollusca	Plagigeyeria stochi	4,982537594	4,374248846	3,024291498	1,618855156	3,658547199
Mollusca	Sardopaladilhia plagigeyeric	4,979970176	4,374248846	3,027530364	1,619105806	3,657774943
Mollusca	Paladilhia pontmartiniana	4,975358675	4,374248846	3,014574899	1,618103205	3,655903575
Mollusca	Paladilhia gloeri	4,973847391	4,374248846	3,014574899	1,618103205	3,655399814
Mollusca	Palaospeum bessoni	4,968909151	4,374248846	3,024291498	1,618855156	3,654004384
Crustacea	Hadzia adriatica	4,595146801	4,374248846	1,450202429	1,98167121	3,650355619
Mollusca	Moitessieria juvenisanguis	4,958283367	4,374248846	2,988663968	1,616098004	3,649543406
Mollusca	Paladilhia umbilicata	4,951929759	4,374248846	3,014574899	1,618103205	3,648093937
Crustacea	Spelaodiaptomus rouchi	4,985328916	4,374248846	2,289068826	1,561957577	3,64051178
Crustacea	Bogidiella balearica	4,92355563	4,008207887	1,4048583	1,976407557	3,636057025
Crustacea	Graeteriella (Paragraeteriella) bertrandi	4,998627672	4,374248846	1,848582996	1,527869161	3,633581893
Crustacea	Iberobathynella (Asturibathynella) asturiensis	4,661623494	3,748497694	1,599190283	2,489363035	3,633161408
Crustacea	Speocyclops franciscoi	4,999689036	4,374248846	1,816194332	1,525362659	3,63310018
Crustacea	Monodella stygicola	4,350578603	4,008207887	1,919838057	2,53899176	3,63259275
Mollusca	Alzoniella fabrianensis	4,906582393	4,374248846	2,9951417	1,616599304	3,632476848
Crustacea	Speocyclops arregladensis	4,996978563	4,374248846	1,816194332	1,525362659	3,632196689
Crustacea	Speocyclops anomalus	4,983744895	4,374248846	1,816194332	1,525362659	3,627785467
Crustacea	Graeteriella (Graeteriella) rouchi	4,974886725	4,374248846	1,848582996	1,527869161	3,625668244
Crustacea	Lessinocamptus caoduroi	4,997024888	4,374248846	1,550607287	1,504809349	3,625361028

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	Chappuisides thienemanni	4,978995304	4,374248846	1,774089069	1,522104208	3,625116119
Crustacea	Nitocrella hirta	4,982068918	4,374248846	1,631578947	1,511075602	3,622464455
Crustacea	Antrocamptus coiffaiti	4,988079659	4,374248846	1,544129555	1,504308049	3,622212185
Crustacea	Parastenocaris psammica	4,996501006	4,374248846	1,424291498	1,495033994	3,621927949
Mollusca	Spiralix burgensis	4,873333457	4,374248846	3,0048583	1,617351255	3,621644519
Crustacea	Stenasellus breuili	4,987697013	4,374248846	1,492307692	1,500297647	3,620747835
Crustacea	Nitocrellopsis elegans	4,967614841	4,374248846	1,680161943	1,514835354	3,61889968
Crustacea	Bragasellus aireyi	4,997391205	4,374248846	1,265587045	1,482752138	3,61813073
Crustacea	Salentinella gracillima	4,842578171	3,547050817	1,430769231	2,463295423	3,61764147
Crustacea	Tyrrhenogammarus catacumbae	4,977825764	4,374248846	1,446963563	1,496788545	3,616287718
Crustacea	Ceuthonectes pescei	4,968539795	4,374248846	1,534412955	1,503556099	3,615448247
Crustacea	Microniphargus leruthi	4,989014714	4,374248846	1,220242915	1,479243037	3,614168866
Crustacea	Sphaeromicola stammeri	4,682410015	4,008207887	2,910931174	2,151236018	3,613951307
Mollusca	Paladilhia conica	4,846042123	4,374248846	3,014574899	1,618103205	3,612798058
Mollusca	Islamia bourguignati	4,844730268	4,374248846	3,0048583	1,617351255	3,612110123
Crustacea	Niphargus stenopus	4,997839347	4,374248846	1	1,462198828	3,611429007
Crustacea	Pseudoniphargus longicarpus	4,851341974	3,547050817	1,252631579	2,435723909	3,611372233
Crustacea	Niphargus rejici	4,99719028	4,374248846	1	1,462198828	3,611212651
Crustacea	Niphargus tridentinus	4,991413362	4,374248846	1	1,462198828	3,609287012
Crustacea	Nitocrella slovenica	4,936893081	4,374248846	1,631578947	1,511075602	3,607405843
Crustacea	Niphargus costozzae	4,969264605	4,374248846	1	1,462198828	3,601904093
Crustacea	Carinurella paradoxa	4,941308981	4,374248846	1,220242915	1,479243037	3,598266955
Mollusca	Bythiospeum garnieri	4,801984255	4,374248846	2,972469636	1,614844754	3,597025952
Crustacea	Iberobathynella (Asturibathynella) rouchi	4,287464565	4,008207887	1,599190283	2,489363035	3,595011829
Crustacea	Nitocrella morettii	4,882656269	4,374248846	1,631578947	1,511075602	3,589326906
Crustacea	Niphargus pectinicauda	4,92997805	4,374248846	1	1,462198828	3,588808575
Crustacea	Niphargus corsicanus	4,929440796	4,374248846	1	1,462198828	3,58862949
Crustacea	Synasellus mariae	4,908731195	4,374248846	1,236437247	1,480496287	3,587825443
Crustacea	Niphargus pupetta	4,92462832	4,374248846	1	1,462198828	3,587025331
Crustacea	Niphargus arbiter	4,915549829	4,374248846	1	1,462198828	3,583999168
Crustacea	Gallasellus heilyi	4,885468803	4,374248846	1,288259109	1,484506689	3,581408113
Crustacea	Parastenocaris tyrrhenidis	4,860285104	4,374248846	1,424291498	1,495033994	3,576522648
Crustacea	Parastenocaris pasquini	4,85091821	4,374248846	1,424291498	1,495033994	3,57340035
Crustacea	Caecosphaeroma (Caecosphaeroma) virei	4,351147216	2,921299663	1,495546559	3,446094558	3,572847146
Crustacea	Ceuthonectes rouchi	4,835474541	4,374248846	1,534412955	1,503556099	3,571093162
Annelida	Delaya navarrensis	4,957640685	4,008207887	4,565991903	1,738164614	3,568004395
Crustacea	Pseudectinosoma janineae	4,93702054	3,748497694	1,75465587	2,017012877	3,56751037
Crustacea	Pseudoniphargus vasconiensis	4,877051079	3,382456735	1,252631579	2,435723909	3,565077241
Crustacea	Microcharon reginae	4,961319439	3,748497694	1,456680162	1,98242316	3,564080098
Crustacea	Balkanostenasellus skopljensis	4,814473125	4,374248846	1,514979757	1,502052198	3,56359139
Crustacea	Microcharon angelieri	4,947176984	3,748497694	1,456680162	1,98242316	3,559365946
Crustacea	Niphargus steueri	4,817503585	4,374248846	1	1,462198828	3,551317086
Mollusca	Moitessieria ollerii	4,660257886	4,374248846	2,988663968	1,616098004	3,550201579
Crustacea	Parastenocaris stammeri	4,768924713	4,374248846	1,424291498	1,495033994	3,546069184
Crustacea	Schizopera (Schizopera) subterranea	4,24163396	4,374248846	1,761133603	2,017764828	3,544549211
Mollusca	Alzoniella cornucopia	4,99776351	4,008207887	2,9951417	1,616599304	3,5408569
Mollusca	Bythiospeum vallei	4,992269524	4,008207887	2,972469636	1,614844754	3,538440722
Crustacea	Speocyclops castereti	4,709562323	4,374248846	1,816194332	1,525362659	3,536391276
Crustacea	Salentinella gineti	4,598744932	3,547050817	1,430769231	2,463295423	3,536363724
Crustacea	Faucheria faucheri	4,130685066	3,016415775	1,524696356	3,452862111	3,533320984
Mollusca	Moitessieria heideae	4,974824761	4,008207887	2,988663968	1,616098004	3,533043551

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Mollusca	Paladilhiosis septentrionalis	4,972215944	4,008207887	3,011336032	1,617852555	3,532758795
Mollusca	Islamia spirata	4,966383997	4,008207887	3,0048583	1,617351255	3,530647713
Mollusca	Iglica gracilis	4,959534152	4,008207887	2,9951417	1,616599304	3,528113781
Mollusca	Bythiospeum klemmi	4,952107031	4,008207887	2,972469636	1,614844754	3,525053224
Crustacea	Niphargus aberrans	4,720459288	4,374248846	1	1,462198828	3,518968987
Mollusca	Hauffenia (Hauffenia) subpiscinalis	4,928568707	4,008207887	3,0048583	1,617351255	3,518042616
Mollusca	Phreatica bolei	4,923063796	4,008207887	3,027530364	1,619105806	3,516792496
Mollusca	Erythropomatiana erythropomatia	4,919523672	4,008207887	3,024291498	1,618855156	3,515528905
Mollusca	Moitessieria rhodani	4,915875707	4,008207887	2,988663968	1,616098004	3,513393654
Annelida	Aeolosoma gineti	4,996350815	4,374248846	4,860728745	1,144374471	3,504991377
Mollusca	Hadziella anti	4,888085016	4,008207887	3,014574899	1,618103205	3,504798703
Crustacea	Stenasellus assorgiai	4,999729969	4,008207887	1,492307692	1,500297647	3,502745168
Annelida	Trichodrilus ptujensis	4,988458525	4,374248846	4,520647773	1,13121534	3,497974237
Crustacea	Kieferiella delamarei	4,946597452	4,008207887	1,868016194	1,529373061	3,494726133
Crustacea	Synasellus bragai	4,990238088	4,008207887	1,236437247	1,480496287	3,492980754
Crustacea	Niphargus montellianus	4,995908185	4,008207887	1	1,462198828	3,488771633
Mollusca	Bythiospeum michaudi	4,836800872	4,008207887	2,972469636	1,614844754	3,486617838
Annelida	Parvidrilus spelaeus	4,262871586	3,24329442	4,588663968	2,952063164	3,48607639
Mollusca	Graziana provincialis	4,999563069	4,374248846	3,024291498	1,073315161	3,482375692
Annelida	Haber monfalconensis	4,963775244	3,748497694	4,507692308	1,733652912	3,481975283
Mollusca	Bythinella saxatilis	4,997219539	4,374248846	2,991902834	1,072061911	3,481176765
Mollusca	Sadleriana schmidti	4,993827578	4,374248846	3,027530364	1,073440486	3,480505637
Mollusca	Belgrandiella kusceri	4,991672248	4,374248846	2,991902834	1,072061911	3,479327668
Crustacea	Candonopsis boui	4,999576272	4,374248846	2,784615385	1,064041107	3,479288742
Crustacea	Niphargus longiflagellum	4,965636935	4,008207887	1	1,462198828	3,478681217
Mollusca	Bythinella eutrepha	4,986579747	4,374248846	2,991902834	1,072061911	3,477630168
Crustacea	Mixtacandona laisi	4,99291321	4,374248846	2,771659919	1,063539806	3,476900621
Crustacea	Niphargus lessiniensis	4,9591789	4,008207887	1	1,462198828	3,476528538
Mollusca	Bythiospeum bourguignati	4,805504296	4,008207887	2,972469636	1,614844754	3,476185646
Mollusca	Belgrandiella schleschi	4,977242344	4,374248846	2,991902834	1,072061911	3,4745177
Crustacea	Pseudocandona aemonae	4,986055691	4,374248846	2,758704453	1,063038506	3,474447681
Crustacea	Mixtacandona chappuisi	4,982588014	4,374248846	2,771659919	1,063539806	3,473458889
Mollusca	Belgrandiella superior	4,973512717	4,374248846	2,991902834	1,072061911	3,473274491
Crustacea	Bragasellus comasi	4,926049327	4,008207887	1,265587045	1,482752138	3,472336451
Crustacea	Monolistra (Typhlosphaeroma) racovitzae	4,480470567	2,496995386	1,463157895	3,438575054	3,472013669
Crustacea	Ilvanella inexpectata	4,532956958	4,374248846	1,450202429	1,497039195	3,468081666
Crustacea	Niphargus spoeckeri	4,926077997	4,008207887	1	1,462198828	3,465494904
Crustacea	Elaphoidella mauro	4,999793537	4,374248846	1,434008097	1,011780556	3,46194098
Crustacea	Elaphoidella infernalis	4,991840482	4,374248846	1,434008097	1,011780556	3,459289961
Crustacea	Proasellus lescherae	4,999175518	4,374248846	1,129554656	1	3,457808121
Crustacea	Proasellus nolli	4,998650032	4,374248846	1,129554656	1	3,457632959
Crustacea	Synurella ambulans	4,982407174	4,374248846	1,463157895	1,012908481	3,4565215
Crustacea	Proasellus chauvini	4,983607747	4,374248846	1,129554656	1	3,452618864
Crustacea	Cypria cavernae	4,915103005	4,374248846	2,852631579	1,066672933	3,452008261
Crustacea	Elaphoidella vasconica	4,967614841	4,374248846	1,434008097	1,011780556	3,451214748
Crustacea	Proasellus vandeli	4,975051611	4,374248846	1,129554656	1	3,449766819
Annelida	Trichodrilus intermedius	4,839316578	4,374248846	4,520647773	1,13121534	3,448260255
Crustacea	Elaphoidella mabelae	4,9563317	4,374248846	1,434008097	1,011780556	3,447453701
Crustacea	Proasellus patrizii	4,96206075	4,374248846	1,129554656	1	3,445436532
Mollusca	Iglica tellinii	4,970322144	3,748497694	2,9951417	1,616599304	3,445139714
Crustacea	Sensonator valentiensis	4,823518532	4,008207887	1,450202429	1,497039195	3,442921871



Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	<i>Pseudoniphargus montanus</i>	4,762743315	3,12274654	1,252631579	2,435723909	3,440404588
Crustacea	<i>Sphaeromicola hamigera</i>	4,420825097	3,748497694	2,910931174	2,151236018	3,44018627
Mollusca	<i>Hadziella krkae</i>	4,920554743	3,748497694	3,014574899	1,618103205	3,429051881
Crustacea	<i>Bragasellus lagari</i>	4,423053266	4,374248846	1,265587045	1,482752138	3,42668475
Mollusca	<i>Avenionia parvula</i>	4,912650277	3,748497694	3,014574899	1,618103205	3,426417059
Crustacea	<i>Metacrangonyx longipes</i>	4,566723412	3,24329442	1,459919028	2,467807125	3,425941652
Crustacea	<i>Speocyclops sebastianus</i>	4,720911038	4,008207887	1,816194332	1,525362659	3,418160528
Mollusca	<i>Spiralix collieri</i>	4,883771363	3,748497694	3,0048583	1,617351255	3,416540104
Crustacea	<i>Elaphoidella paraelaphoides</i>	4,859367191	4,374248846	1,434008097	1,011780556	3,415132198
Crustacea	<i>Nitocrella gracilis</i>	4,963391548	3,748497694	1,631578947	1,511075602	3,407654948
Mollusca	<i>Alzoniella elliptica</i>	4,845710832	3,748497694	2,9951417	1,616599304	3,40360261
Mollusca	<i>Bythiospeum francomontanum</i>	4,841401896	3,748497694	2,972469636	1,614844754	3,401581448
Crustacea	<i>Parastenocaris nolli</i>	4,960323322	3,748497694	1,424291498	1,495033994	3,401285003
Crustacea	<i>Morariopsis scotenophila</i>	4,926460478	3,748497694	1,553846154	1,505059999	3,39333939
Crustacea	<i>Parastenocaris xyrophora</i>	4,305488629	4,374248846	1,424291498	1,495033994	3,39159049
Crustacea	<i>Parastenocaris mateusi</i>	4,305488629	4,374248846	1,424291498	1,495033994	3,39159049
Crustacea	<i>Parastenocaris amalasuntae</i>	4,929242521	3,748497694	1,424291498	1,495033994	3,390924736
Mollusca	<i>Bythiospeum moussonianum</i>	4,801633115	3,748497694	2,972469636	1,614844754	3,388325188
Crustacea	<i>Pseudocandona trigonella</i>	4,723417564	4,374248846	2,758704453	1,063038506	3,386901639
Crustacea	<i>Speocyclops cantabricus</i>	4,870978406	3,748497694	1,816194332	1,525362659	3,38161292
Crustacea	<i>Monolistra (Monolistra) caeca</i>	4,194068629	2,496995386	1,463157895	3,438575054	3,376546356
Mollusca	<i>Bythiospeum dorvani</i>	4,761096854	3,748497694	2,972469636	1,614844754	3,374813101
Mollusca	<i>Islamia bomangiana</i>	4,959578356	3,547050817	3,0048583	1,617351255	3,374660143
Crustacea	<i>Bogidiella calicali</i>	4,135968589	4,008207887	1,4048583	1,976407557	3,373528011
Crustacea	<i>Proasellus acutianus</i>	4,720871138	4,374248846	1,129554656	1	3,365039995
Mollusca	<i>Hadziella ephippiostoma</i>	4,927280836	3,547050817	3,014574899	1,618103205	3,364144953
Mollusca	<i>Spiralix vitrea</i>	4,919957991	3,547050817	3,0048583	1,617351255	3,361453354
Annelida	<i>Troglochaetus beranecki</i>	2,606963193	3,24329442	4,860728745	4,227370994	3,359209536
Crustacea	<i>Iberobathynella (Asturibathynella) cavadoensis</i>	3,571802579	4,008207887	1,599190283	2,489363035	3,356457834
Mollusca	<i>Belgrandiella substricta</i>	4,985765394	4,008207887	2,991902834	1,072061911	3,355345064
Crustacea	<i>Fabaeformiscandona aemonae</i>	4,994006266	4,008207887	2,774898785	1,063665131	3,355293095
Annelida	<i>Spiridion phreaticola</i>	4,179539705	3,547050817	4,510931174	2,33696776	3,354519427
Crustacea	<i>Niphargus stefanellii</i>	4,848196511	3,748497694	1	1,462198828	3,352964344
Crustacea	<i>Pseudocandona eremita</i>	4,985237776	4,008207887	2,758704453	1,063038506	3,35216139
Crustacea	<i>Niphargus minor</i>	4,839581625	3,748497694	1	1,462198828	3,350092716
Annelida	<i>Rhyacodrilus lindbergi</i>	4,53534074	4,374248846	4,481781377	1,129711439	3,346433675
Crustacea	<i>Lessinocamptus pivai</i>	4,9770667	3,547050817	1,550607287	1,504809349	3,342975622
Crustacea	<i>Lessinocamptus insoletus</i>	4,937992061	3,547050817	1,550607287	1,504809349	3,329950742
Crustacea	<i>Metacyclops gasparoi</i>	4,949816259	4,008207887	1,855060729	1,028072814	3,328698987
Crustacea	<i>Niphargus bajuvaricus</i>	4,513078515	4,008207887	1	1,462198828	3,32782841
Crustacea	<i>Acanthocyclops gordani</i>	4,945543888	4,008207887	1,83562753	1,027320863	3,327024213
Crustacea	<i>Niphargus similis</i>	4,97080523	3,547050817	1	1,462198828	3,326684958
Mollusca	<i>Palacanthilhiopsis vervierii</i>	4,807920109	3,547050817	3,024291498	1,618855156	3,324608694
Crustacea	<i>Synasellus meijersae</i>	4,942302855	3,547050817	1,236437247	1,480496287	3,32328332
Mollusca	<i>Moitessieria lescherae</i>	4,803197626	3,547050817	2,988663968	1,616098004	3,322115482
Crustacea	<i>Elaphoidella charon</i>	4,926001134	4,008207887	1,434008097	1,011780556	3,315329859
Crustacea	<i>Niphargus julius</i>	4,913023007	3,547050817	1	1,462198828	3,307424217
Crustacea	<i>Stenasellus nuragicus</i>	4,665628731	3,748497694	1,492307692	1,500297647	3,304808024
Mollusca	<i>Hauffenia (Hauffenia) media</i>	4,74960563	3,547050817	3,0048583	1,617351255	3,304669234
Crustacea	<i>Diacyclops slovenicus</i>	4,84816454	4,008207887	1,806477733	1,026192938	3,294188455
Chelicerata	<i>Lethaxona cavifrons</i>	4,960323322	3,748497694	4,870445344	1,144750446	3,284523821

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	<i>Cypria stygia</i>	4,770551628	4,008207887	2,852631579	1,066672933	3,281810816
Crustacea	<i>Pseudoniphargus semielongatus</i>	4,15984379	3,24329442	1,252631579	2,435723909	3,279620706
Crustacea	<i>Speocyclops spelaeus</i>	4,562042281	3,748497694	1,816194332	1,525362659	3,278634211
Mollusca	<i>Paladilhia pleurotoma</i>	4,66409884	3,547050817	3,014574899	1,618103205	3,276417621
Annelida	<i>Haplotaxis leruthi</i>	4,946443318	3,748497694	4,575708502	1,133345866	3,276095626
Crustacea	<i>Proasellus dianae</i>	4,799642165	4,008207887	1,129554656	1	3,269283351
Mollusca	<i>Acroloxus tetensi</i>	4,929526964	3,748497694	4,018623482	1,111789955	3,263271538
Annelida	<i>Trichodrilus cernovsytovi</i>	4,909369936	3,748497694	4,520647773	1,13121534	3,263027657
Mollusca	<i>Pseudavenionia pedemontana</i>	4,777270811	3,382456735	3,027530364	1,619105806	3,259611117
Mollusca	<i>Bythinella cylindracea</i>	4,951347433	3,748497694	2,991902834	1,072061911	3,257302346
Crustacea	<i>Niphargus renei</i>	4,922567701	3,382456735	1	1,462198828	3,255741088
Crustacea	<i>Hispanobathynella catalanensis</i>	2,89654567	4,374248846	1,644534413	2,496381239	3,255725252
Mollusca	<i>Bythiospeum bressanum</i>	4,75837296	3,382456735	2,972469636	1,614844754	3,251891483
Crustacea	<i>Acanthocyclops sambugarae</i>	4,962844949	3,748497694	1,83562753	1,027320863	3,246221169
Mollusca	<i>Hauffenia (Hauffenia) michleri</i>	4,874350948	3,24329442	3,0048583	1,617351255	3,244998874
Mollusca	<i>Bythiospeum diaphanoides</i>	4,728059511	3,382456735	2,972469636	1,614844754	3,241787
Mollusca	<i>Hadziella deminuta</i>	4,54811717	3,547050817	3,014574899	1,618103205	3,237757064
Mollusca	<i>Iglica hauffeni</i>	4,708958321	3,382456735	2,9951417	1,616599304	3,236004787
Crustacea	<i>Niphargus transitivus</i>	4,233960482	4,008207887	1	1,462198828	3,234789066
Mollusca	<i>Avenionia berenguieri</i>	4,699783794	3,382456735	3,014574899	1,618103205	3,233447911
Mollusca	<i>Neohortia subpiscinalis</i>	4,824139095	3,24329442	3,017813765	1,618353855	3,22859579
Mollusca	<i>Iglica vobarnensis</i>	4,510883008	3,547050817	2,9951417	1,616599304	3,224844376
Crustacea	<i>Medigidella chappuisi</i>	2,690135988	3,547050817	1,434008097	3,431807501	3,222998102
Crustacea	<i>Microcharon rouchi</i>	4,550736744	3,12274654	1,456680162	1,98242316	3,218635481
Crustacea	<i>Speocyclops gallicus</i>	4,354679097	3,748497694	1,816194332	1,525362659	3,20951315
Crustacea	<i>Stenasellus galhanoae</i>	3,751890222	4,374248846	1,492307692	1,500297647	3,208812238
Crustacea	<i>Asellus (Asellus) cavernicolus</i>	4,871171339	3,748497694	1,285020243	1,006015603	3,208561545
Crustacea	<i>Proasellus franciscocoli</i>	4,876807849	3,748497694	1,129554656	1	3,208435181
Crustacea	<i>Parapseudoleptomesochra subterranea</i>	4,820934743	3,24329442	1,670445344	1,514083404	3,192770856
Crustacea	<i>Elaphoidella nuragica</i>	4,552534277	4,008207887	1,434008097	1,011780556	3,190840907
Crustacea	<i>Parapseudoleptomesochra minoricae</i>	3,678887981	4,374248846	1,670445344	1,514083404	3,18907341
Mollusca	<i>Spiralix puteana</i>	4,546440217	3,382456735	3,0048583	1,617351255	3,182082736
Crustacea	<i>Pseudoniphargus branchiatus</i>	3,086732992	4,008207887	1,252631579	2,435723909	3,176888263
Annelida	<i>Rhyacodrilus gasparoi</i>	4,640709439	3,748497694	4,481781377	1,129711439	3,172972857
Crustacea	<i>Monodella argentarii</i>	2,600660364	4,374248846	1,919838057	2,53899176	3,171300323
Crustacea	<i>Proasellus beroni</i>	4,954511745	3,547050817	1,129554656	1	3,167187521
Crustacea	<i>Elaphoidella stammeri</i>	4,935100513	3,547050817	1,434008097	1,011780556	3,164643962
Crustacea	<i>Stenasellus buili</i>	4,444993801	3,547050817	1,492307692	1,500297647	3,164114088
Mollusca	<i>Belgrandiella crucis</i>	4,860643537	3,547050817	2,991902834	1,072061911	3,159918755
Crustacea	<i>Metacyclops stammeri</i>	4,874967075	3,547050817	1,855060729	1,028072814	3,150030235
Mollusca	<i>Bythiospeum rhenanum</i>	4,44554641	3,382456735	2,972469636	1,614844754	3,147615966
Crustacea	<i>Acanthocyclops troglophilus</i>	4,791166018	3,547050817	1,83562753	1,027320863	3,121845899
Crustacea	<i>Proasellus boui</i>	4,604033626	3,748497694	1,129554656	1	3,11751044
Crustacea	<i>Niphargus tamaninii</i>	4,646880369	3,24329442	1	1,462198828	3,117457872
Mollusca	<i>Iglica forumjuliana</i>	4,612021856	3,12274654	2,9951417	1,616599304	3,117122567
Tracheata	<i>Siettita avenionensis</i>	4,196310531	3,382456735	5	1,771751731	3,116839666
Crustacea	<i>Parastenocaris admete</i>	4,608804644	3,24329442	1,424291498	1,495033994	3,115711019
Crustacea	<i>Proasellus slovenicus</i>	4,59217659	3,748497694	1,129554656	1	3,113558095
Mollusca	<i>Paladilhopsis robiciana</i>	4,695693468	3,016415775	3,011336032	1,617852555	3,109987266
Crustacea	<i>Diacyclops tantalus</i>	4,752876432	3,547050817	1,806477733	1,026192938	3,108706729
Crustacea	<i>Pseudocandona delamarei</i>	4,250761569	4,008207887	2,758704453	1,063038506	3,107335987

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Mollusca	Bythiospeum diaphanum	4,157982035	3,547050817	2,972469636	1,614844754	3,106625869
Crustacea	Troglodiptomus sketi	4,607364138	3,12274654	2,289068826	1,561957577	3,097356085
Crustacea	Niphargus serbicus	4,445156013	3,382456735	1	1,462198828	3,096603859
Crustacea	Pseudoniphargus elongatus	4,014559827	2,835256675	1,252631579	2,435723909	3,095180137
Annelida	Rhyacodrilus amphigenus	3,764731599	4,374248846	4,481781377	1,129711439	3,089563961
Crustacea	Niphargus galvagnii	4,251796833	3,547050817	1	1,462198828	3,087015493
Crustacea	Iberobathynella (Asturibathynella) imuniensis	3,849525658	2,921299663	1,599190283	2,489363035	3,086729452
Crustacea	Ingolfiella thibaudi	3,729425741	3,547050817	1,453441296	1,982047185	3,086174581
Crustacea	Proasellus cantabricus	4,874203657	3,382456735	1,129554656	1	3,085553464
Crustacea	Proasellus adriaticus	4,84998553	3,382456735	1,129554656	1	3,077480755
Mollusca	Avenionia ligustica	4,491317387	3,12274654	3,014574899	1,618103205	3,077389044
Crustacea	Proasellus aquaecalidae	4,828938483	3,382456735	1,129554656	1	3,070465073
Crustacea	Bathynella gallica	2,688044959	4,008207887	1,628340081	2,493874738	3,063375861
Crustacea	Parastenocaris orcina	3,659268545	4,008207887	1,424291498	1,495033994	3,054170142
Crustacea	Diacyclops crassicaudis	4,095897319	4,008207887	1,806477733	1,026192938	3,043432715
Annelida	Trichodrilus longipenis	4,749432878	3,24329442	4,520647773	1,13121534	3,041314213
Annelida	Trichodrilus leruthi	3,964530014	4,008207887	4,520647773	1,13121534	3,03465108
Mollusca	Avenionia bourguignati	3,736050682	3,748497694	3,014574899	1,618103205	3,034217194
Crustacea	Speocyclops kieferi	4,179528144	3,382456735	1,816194332	1,525362659	3,029115846
Crustacea	Niphargus wolffi	4,366282745	3,24329442	1	1,462198828	3,023925331
Annelida	Rhyacodrilus balmensis	4,693755341	3,24329442	4,481781377	1,129711439	3,022253733
Crustacea	Parastenocaris trinacriae	3,54430219	4,008207887	1,424291498	1,495033994	3,015848024
Crustacea	Proasellus vulgaris	4,645442361	3,382456735	1,129554656	1	3,009299699
Crustacea	Megacyclops brachypus	3,962812077	4,008207887	1,868016194	1,028574114	2,999864693
Crustacea	Parastenocaris meridionalis	3,121148427	4,374248846	1,424291498	1,495033994	2,996810422
Annelida	Abyssidrilus cuspis	2,618085131	4,008207887	4,507692308	2,336591785	2,987628268
Crustacea	Elaphoidella jeanneli	4,704339502	3,24329442	1,434008097	1,011780556	2,986471493
Crustacea	Nitocrella achaiae	3,698842399	3,748497694	1,631578947	1,511075602	2,986138565
Crustacea	Nitocrella beatricis	4,318890553	3,12274654	1,631578947	1,511075602	2,984237565
Mollusca	Heraultia exilis	4,376882079	2,921299663	3,027530364	1,619105806	2,972429183
Crustacea	Acanthocyclops orientalis	3,878182564	4,008207887	1,83562753	1,027320863	2,971237105
Crustacea	Proasellus intermedius	4,647527921	3,24329442	1,129554656	1	2,963607447
Crustacea	Graeteriella (Graeteriella) boui	4,110276557	3,24329442	1,848582996	1,527869161	2,960480046
Crustacea	Proasellus pavani	4,132696135	3,748497694	1,129554656	1	2,960397943
Crustacea	Parastenocaris dianae	4,131912301	3,24329442	1,424291498	1,495033994	2,956746905
Crustacea	Elaphoidella cavatica	4,104177852	3,748497694	1,434008097	1,011780556	2,954818701
Mollusca	Arganiella pescei	4,391078265	2,835256675	3,027530364	1,619105806	2,948480249
Crustacea	Proasellus hermaliensis	4,714552163	3,12274654	1,129554656	1	2,945766234
Crustacea	Parabathynella stygia	2,584086796	3,748497694	1,654251012	2,49788514	2,943489877
Crustacea	Typhlocirolana moraguesi	2,600646567	2,75670558	1,52145749	3,452110161	2,936487436
Crustacea	Salentinella juberthieae	3,768514726	2,55258704	1,430769231	2,463295423	2,929022951
Mollusca	Bythiospeum articense	4,047350948	3,12274654	2,972469636	1,614844754	2,928314081
Crustacea	Bryocamptus (Rheocamptus) balcanicus	4,737561198	3,016415775	1,544129555	1,016041608	2,923339527
Crustacea	Proasellus deminutus	4,335723853	3,382456735	1,129554656	1	2,906060196
Crustacea	Diacyclops lindae	3,675989306	4,008207887	1,806477733	1,026192938	2,903463377
Mollusca	Bythiospeum charpyi	4,408752016	2,684445579	2,972469636	1,614844754	2,902680783
Crustacea	Salentinella delamarei	3,312339936	2,921299663	1,430769231	2,463295423	2,898978341
Annelida	Tubifex pescei	3,554710716	4,008207887	4,510931174	1,130839365	2,897919323
Mollusca	Fissuria boui	4,214187126	2,835256675	3,027530364	1,619105806	2,889516536
Crustacea	Microcharon marinus	2,663342988	4,008207887	1,456680162	1,98242316	2,884658012
Mollusca	Iglica concii	4,412483837	2,617543265	2,9951417	1,616599304	2,882208802

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Mollusca	Avenionia brevis	3,997335264	3,016415775	3,014574899	1,618103205	2,877284748
Crustacea	Diacyclops ruffoi	4,216252113	3,382456735	1,806477733	1,026192938	2,874967262
Crustacea	Diacyclops charon	4,655710287	2,921299663	1,806477733	1,026192938	2,867734296
Crustacea	Proasellus slavus	3,972717545	3,547050817	1,129554656	1	2,839922787
Mollusca	Moitessieria locardi	4,014698927	2,835256675	2,988663968	1,616098004	2,822017869
Crustacea	Elaphoidella cvetkae	4,529707771	2,921299663	1,434008097	1,011780556	2,82092933
Crustacea	Bathynella natans	2,724027863	3,24329442	1,628340081	2,493874738	2,820399007
Crustacea	Haploginglymus bragai	2,971577579	4,008207887	1,213765182	1,478741736	2,819509067
Crustacea	Salentinella petiti	3,483812269	2,496995386	1,430769231	2,463295423	2,814701026
Crustacea	Niphargus delamarei	4,270215737	2,684445579	1	1,462198828	2,805620048
Crustacea	Parastenocaris gertrudae	4,073433818	2,835256675	1,424291498	1,495033994	2,801241496
Crustacea	Niphargus speziae	3,883832719	3,016415775	1	1,462198828	2,787482441
Annelida	Trichodrilus moravicus	2,851791762	4,374248846	4,520647773	1,13121534	2,785751983
Mollusca	Hauffenia (Hauffenia) tellinii	4,269665244	2,442265413	3,0048583	1,617351255	2,776427304
Crustacea	Diacyclops maggii	3,294580608	4,008207887	1,806477733	1,026192938	2,776327144
Chelicerata	Soldanellonyx visurgis	2,395143035	3,547050817	4,873684211	2,379076981	2,773756944
Crustacea	Niphargus angelieri	4,557357698	2,295548509	1	1,462198828	2,771701678
Crustacea	Niphargus longidactylus	3,882699062	2,921299663	1	1,462198828	2,755399184
Chelicerata	Lobohalacarus weberi	2,469705968	3,382456735	4,873684211	2,379076981	2,743746561
Mollusca	Paladilhopsis virei	3,910659792	2,684445579	3,011336032	1,617852555	2,737652642
Crustacea	Parastenocaris glareola	3,415903595	3,24329442	1,424291498	1,495033994	2,718077336
Crustacea	Bryocamptus (Limocamptus) dacicus	3,957504307	3,12274654	1,544129555	1,016041608	2,698764152
Crustacea	Elaphoidella bouillonii	3,506494004	3,547050817	1,434008097	1,011780556	2,688441792
Crustacea	Proasellus albigenis	3,952093982	3,016415775	1,129554656	1	2,656169919
Crustacea	Parastenocaris hispanica	3,346643841	3,12274654	1,424291498	1,495033994	2,654808125
Crustacea	Niphargus stygius	4,332680353	2,169375889	1	1,462198828	2,65475169
Mollusca	Spiralix rayi	3,886953072	2,442265413	3,0048583	1,617351255	2,64885658
Crustacea	Schellencandona belgica	3,689632928	3,12274654	2,781376518	1,063915782	2,62543175
Crustacea	Diacyclops hypnicolus	3,596854318	3,24329442	1,806477733	1,026192938	2,622113892
Crustacea	Moraria (Moraria) stankovitchi	4,005508871	2,835256675	1,547368421	1,016166933	2,618977493
Annelida	Trichodrilus capilliformis	3,452952001	3,24329442	4,520647773	1,13121534	2,60915392
Crustacea	Niphargus laisi	2,770276503	3,547050817	1	1,462198828	2,593175383
Crustacea	Proasellus synaselloides	3,766306722	2,921299663	1,129554656	1	2,562535462
Mollusca	Moitessieria massoti	2,674749341	3,382456735	2,988663968	1,616098004	2,557768027
Crustacea	Stenasellus racovitzai	3,323336918	2,835256675	1,492307692	1,500297647	2,552963747
Crustacea	Mixtacandona stammeri	2,58415132	4,008207887	2,771659919	1,063539806	2,551966338
Crustacea	Speocyclops italicus	3,098833282	3,016415775	1,816194332	1,525362659	2,546870572
Crustacea	Niphargus puteanus	2,624797551	3,547050817	1	1,462198828	2,544682399
Mollusca	Islamia minuta	3,478594483	2,496995386	3,0048583	1,617351255	2,530980375
Crustacea	Antrobathynella stammeri	1,688475811	3,382456735	1,644534413	2,496381239	2,522437928
Crustacea	Sphaeromicola topsenti	2,705982321	2,684445579	2,910931174	2,151236018	2,513887973
Crustacea	Schellencandona triquetra	3,805626213	2,617543265	2,781376518	1,063915782	2,495695087
Crustacea	Parastenocaris proserpina	3,042295293	2,921299663	1,424291498	1,495033994	2,48620965
Crustacea	Speocyclops infernus	3,831267377	2,094101632	1,816194332	1,525362659	2,483577223
Crustacea	Caecosphaeroma (Vireia) burgundum	2,394960353	1,583754369	1,495546559	3,446094558	2,474936427
Crustacea	Elaphoidella pseudophreatica	3,500824623	2,835256675	1,434008097	1,011780556	2,449287285
Crustacea	Hexaiberobathynella mateusi	2,227149663	2,555258704	1,651012146	2,497383839	2,426597402
Crustacea	Ceuthonectes gallicus	3,479212643	2,295548509	1,534412955	1,503556099	2,42610575
Mollusca	Belgrandiella saxatilis	3,395390287	2,75670558	2,991902834	1,072061911	2,408052593
Crustacea	Diacyclops italianus	3,831169723	2,341854459	1,806477733	1,026192938	2,39973904
Crustacea	Proasellus valdensis	3,963331901	2,209505521	1,129554656	1	2,390945807

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	<i>Diacyclops paralanguidoides</i>	3,258465803	2,835256675	1,806477733	1,026192938	2,373305139
Crustacea	<i>Speocyclops racovitzai</i>	3,488292141	2,094101632	1,816194332	1,525362659	2,369252144
Crustacea	<i>Cryptocandona kieferi</i>	3,390757855	2,617543265	2,781376518	1,063915782	2,357405634
Crustacea	<i>Elaphoidella plutonis</i>	2,779362815	3,24329442	1,434008097	1,011780556	2,344812597
Crustacea	<i>Acanthocyclops kieferi</i>	3,238045889	2,75670558	1,83562753	1,027320863	2,340690777
Crustacea	<i>Niphargus setiferus</i>	2,661628834	2,835256675	1	1,462198828	2,319694779
Crustacea	<i>Niphargus ciliatus</i>	2,835188248	2,617543265	1	1,462198828	2,30497678
Crustacea	<i>Metacyclops subdolos</i>	2,857059768	3,016415775	1,855060729	1,028072814	2,300516119
Crustacea	<i>Bryocamptus (Rheocamptus) dentatus</i>	1,854620028	4,008207887	1,544129555	1,016041608	2,292956508
Crustacea	<i>Parastenocaris fontinalis</i>	2,503023301	2,835256675	1,424291498	1,495033994	2,277771323
Crustacea	<i>Pseudoniphargus africanus</i>	1,558705216	2,835256675	1,252631579	2,435723909	2,276561933
Crustacea	<i>Niphargus thienemanni</i>	2,610414435	2,75670558	1	1,462198828	2,276439614
Crustacea	<i>Bogidiella albertimagni</i>	2,355753569	2,496995386	1,4048583	1,976407557	2,276385504
Mollusca	<i>Moitessieria rolandiana</i>	3,074367164	2,094101632	2,988663968	1,616098004	2,261522267
Crustacea	<i>Ceuthonectes serbicus</i>	3,206520528	2,058694425	1,534412955	1,503556099	2,256257017
Chelicerata	<i>Frontipodopsis reticulatifrons</i>	2,015065928	3,547050817	4,870445344	1,144750446	2,235622397
Crustacea	<i>Niphargus orcinus</i>	2,474020463	2,684445579	1	1,462198828	2,20688829
Crustacea	<i>Proasellus strouhali</i>	3,198621787	2,390664621	1,129554656	1	2,196428803
Crustacea	<i>Nitocrella psammophila</i>	2,788290379	2,209505521	1,631578947	1,511075602	2,169623834
Chelicerata	<i>Stygomonomia latipes</i>	1,327915739	3,382456735	4,876923077	1,762227026	2,157533167
Crustacea	<i>Niphargus gallicus</i>	2,433519325	2,555258704	1	1,462198828	2,150325619
Crustacea	<i>Niphargus jovanovici</i>	2,47780917	2,496995386	1	1,462198828	2,145667795
Crustacea	<i>Parastenocaris italica</i>	2,642629568	2,295548509	1,424291498	1,495033994	2,144404024
Crustacea	<i>Acanthocyclops rhenanus</i>	2,494274125	2,835256675	1,83562753	1,027320863	2,118950554
Crustacea	<i>Pseudoniphargus adriaticus</i>	1,287434415	2,617543265	1,252631579	2,435723909	2,113567196
Mollusca	<i>Moitessieria simoniana</i>	2,849979056	1,764913467	2,988663968	1,616098004	2,076996842
Crustacea	<i>Salentinella angelieri</i>	1,667446899	2,058694425	1,430769231	2,463295423	2,063145582
Crustacea	<i>Diacyclops zschokkei</i>	2,143666874	2,921299663	1,806477733	1,026192938	2,030386492
Crustacea	<i>Halicyclops troglodytes</i>	1,750429069	3,24329442	1,868016194	1,028574114	2,007432534
Crustacea	<i>Diacyclops belgicus</i>	1,750387331	3,24329442	1,806477733	1,026192938	2,006624896
Crustacea	<i>Alona phreatica</i>	2,09532813	2,835256675	3,0048583	1,072563211	2,001049339
Crustacea	<i>Paraiberobathynella (Paraiberobathynella) fagei</i>	1,76688096	1,716103305	1,651012146	2,497383839	1,993456035
Crustacea	<i>Parapseudoleptomesochra italica</i>	2,287799247	2,169375889	1,670445344	1,514083404	1,990419513
Crustacea	<i>Acanthocyclops hispanicus</i>	1,985216689	2,921299663	1,83562753	1,027320863	1,977945738
Crustacea	<i>Niphargus ladmiraulti</i>	2,670660943	1,790345237	1	1,462198828	1,974401669
Crustacea	<i>Elaphoidella phreatica</i>	2,004616468	2,835256675	1,434008097	1,011780556	1,950551233
Crustacea	<i>Pseudocandona zschokkei</i>	2,728762154	2,058694425	2,758704453	1,063038506	1,950165028
Crustacea	<i>Niphargopsis casparyi</i>	2,617804953	1,740178548	1,217004049	1,478992387	1,945658629
Crustacea	<i>Niphargus gineti</i>	2,607240479	1,764913467	1	1,462198828	1,944784258
Crustacea	<i>Acanthocyclops sensitivus</i>	2,0394677	2,684445579	1,83562753	1,027320863	1,917078047
Crustacea	<i>Fabaeformiscandona wegelini</i>	2,390159153	2,295548509	2,774898785	1,063665131	1,916457598
Crustacea	<i>Elaphoidella leruthi</i>	2,034857387	2,684445579	1,434008097	1,011780556	1,910361174
Crustacea	<i>Proasellus walteri</i>	2,642694345	1,960112789	1,129554656	1	1,867602378
Annelida	<i>Trichodrilus pragensis</i>	1,305828308	3,12274654	4,520647773	1,13121534	1,853263396
Crustacea	<i>Diacyclops paolae</i>	1,832539477	2,617543265	1,806477733	1,026192938	1,825425227
Crustacea	<i>Niphargus robustus</i>	2,402780633	1,524209619	1	1,462198828	1,79639636
Crustacea	<i>Bryocamptus (Rheocamptus) pyrenaicus</i>	1,967292269	2,390664621	1,544129555	1,016041608	1,791332833
Crustacea	<i>Eucyclops graeteri</i>	2,088109353	2,251502306	1,858299595	1,028198139	1,789269933
Crustacea	<i>Niphargus rhenorhodanensis</i>	2,636414672	1,078551094	1	1,462198828	1,725721531
Mollusca	<i>Islamia moquiniana</i>	2,199482425	1,334361635	3,0048583	1,617351255	1,717065105
Crustacea	<i>Fabaeformiscandona breuili</i>	1,694078569	2,390664621	2,774898785	1,063665131	1,716136107

Phylum	Species	Endemicity	Rarity	Phylogeny	WPhylogeny	ConsValue
Crustacea	<i>Niphargus fontanus</i>	2,070160507	1,60450857	1	1,462198828	1,712289302
Crustacea	<i>Stenasellus virei</i>	2,242722737	1,334361635	1,492307692	1,500297647	1,692460673
Crustacea	<i>Cavernocypris subterranea</i>	1,826799818	2,169375889	2,859109312	1,066923583	1,687699763
Crustacea	<i>Niphargus longicaudatus</i>	1,310528057	2,209505521	1	1,462198828	1,660744135
Crustacea	<i>Acanthocyclops venustus</i> ssp.	1,506394325	2,442265413	1,83562753	1,027320863	1,6586602
Crustacea	<i>Crangonyx subterraneus</i>	1,57913369	2,341854459	1,463157895	1,012908481	1,64463221
Crustacea	<i>Niphargus virei</i>	2,453537426	1	1	1,462198828	1,638578751
Crustacea	<i>Elaphoidella elaphoides</i>	1,640411446	2,209505521	1,434008097	1,011780556	1,620565841
Crustacea	<i>Niphargus plateaui</i>	2,036148904	1,204137692	1	1,462198828	1,567495141
Crustacea	<i>Niphargus foreli</i>	1,701800199	1,432943271	1	1,462198828	1,532314099
Crustacea	<i>Nitocrella stammeri</i>	1,231490592	1,843464561	1,631578947	1,511075602	1,528676918
Crustacea	<i>Niphargus pachypus</i>	1,720692119	1,318929326	1	1,462198828	1,500606758
Crustacea	<i>Niphargus schellenbergi</i>	1,85584738	1,066902311	1	1,462198828	1,461649506
Crustacea	<i>Niphargus kochianus</i>	1,408898402	1,288834271	1	1,462198828	1,386643834
Crustacea	<i>Proasellus cavaticus</i>	1,626357173	1,4504733	1,129554656	1	1,358943491
Crustacea	<i>Niphargus aquilex</i>	1,194493064	1,366040959	1	1,462198828	1,34091095
Crustacea	<i>Diacyclops clandestinus</i>	1,121084603	1,790345237	1,806477733	1,026192938	1,312540926
Crustacea	<i>Diacyclops languidoides</i>	1	1,090352153	1,806477733	1,026192938	1,038848364

## **6 RECOMANDATION AND CONCLUSION :**

### **6.1 RECOMMENDATIONS FOR OPTIMIZING THE SAMPLING STRATEGY :**

After a careful statistical analysis of the large data sets including both environmental variables (Brancelj, 2004) and species occurrences, some main proposals for optimizing the field sampling strategy and improving the PASCALIS protocol (Malard et al., 2002) can be suggested.

***Hierarchical sampling strategy:*** The general hierarchical scheme gave satisfactory results only in a subset of the study regions (section 1). For this reason, the structure of the hierarchy requires to be improved in new areas taking into account other sources of environmental heterogeneity, such as elevation, distance from Würmian glacier borders, habitat complexity and anthropogenic disturbance; all these factors are known to affect species distribution in groundwaters (Gibert et al., 1994; Stoch, 1995), and are clearly related with the structure of stygobiotic assemblages and the patterns of species richness in the PASCALIS regions (section 2). The stratification considering karstic areas and porous sediments as different sampling units should be used in every sampling design, being statistically significant in discriminating different species assemblages (section 1 and 2).

***Number of sampling stations:*** The high sampling effort performed during PASCALIS surveys (192 sites per region established in the protocol: Malard et al., 2002) was not satisfactory in some areas, as clearly demonstrated through the use of SACs (Species Accumulation Curves: Colwell, 1997) (section 1), due to the high amount of rare species and strict endemics. Even if the use of spatially extensive designs for assessing biodiversity in groundwaters is likely to produce unsaturated accumulation curves, the use of spatially extensive designs for assessing biodiversity in groundwaters is confirmed to produce more exhaustive results than intensive sampling in smaller areas, and it is highly recommended for future assessment of biodiversity trends (high between-region variation: section 3).

***Selection of biodiversity indicators:*** Considering that large scale sampling surveys are needed, and that such inventories are expensive and time-consuming, the use of biodiversity indicators (sensu Mac Nally and Fleishman, 2002, 2004) is highly recommended. Unfortunately, indicator species varies between regions as a function of species diversity heterogeneity (sections 3 and 4); at larger spatial scales, indicators should include higher taxa like gastropods, harpacticoids, and amphipods, which appear to be significantly correlated with total species richness (section 4).

*Selection of environmental surrogates:* As a preliminary screening, environmental parameters can be used as “surrogates” of species richness (sensu Mac Nally et al., 2003); detailed statistical analyses demonstrated (section 2) that they can be useful predictors for a first assessment of species richness within the PASCALIS countries.

The statistical analyses performed during WP7 indicate that impediments to the application of a standard sampling protocol over large spatial scales are due to the fact that species distribution within hierarchical units differs from one region to another, and that species similarity between regions is low or very low due to the high level of endemism. Consequently, it is recommended to search for a sampling strategy adapted to each region; regional history and structural complexity need to be examined in advance before preliminary field surveys.

## **6.2 BIODIVERSITY-ENVIRONMENT RELATIONSHIPS: IMPLICATIONS FOR BIODIVERSITY ASSESSMENT AND CONSERVATION :**

In five regions out of six, geology was the main variable explaining the structure of stygobiotic assemblages at the species level (section 2). The effect of elevation, distance to the Würmian glaciers, and land cover (related to anthropogenic pressure) was statistically significant in some regions as well. Except for the northernmost regions (e.g. Walloon), depauperated during the Quaternary glaciations and recolonized by generalist species, a significant influence of environmental and palaeogeographic variables on species distribution was detected according to the known literature (Camacho, 1992 not found in the list; Gibert et al., 1994; Stoch, 1995).

As regards species richness, trends of biodiversity were evidenced by the multivariate statistical analyses, indicating statistically significant effects of geography as well as history, habitat structure, water chemistry and pollution (section 2). The taxa which account for most of overall variance were the harpacticoid copepods, followed by gastropods, ostracods and amphipods. These groups may be considered as markers of biodiversity patterns at an European scale.

As regards biodiversity partitioning, the between-region component made by far the highest contribution to the stygobiotic species richness of southern Europe, e.g. community composition varied most importantly over broader spatial scales, even when differences in total richness between regions were relatively small. The increase of the between-component contribution of diversity with increasing spatial scale may be largely the product of multiple



vicariant speciation events caused by the highly fragmented nature of groundwater systems (Rouch and Danielopol, 1987).

The patterns of species richness identified in the present study have strong implications for the assessment and conservation of stygobiotic diversity. Furthermore, the results clearly indicate that the most effective way to preserve stygobiotic diversity in southern Europe is to protect multiple aquifers within different regions and with different environmental features, thereby maintaining regionally distinctive species-rich assemblages.

### **6.3 RECOMMENDATIONS FOR SELECTING BIODIVERSITY INDICATORS :**

The exhaustive surveys suggested by the heterogeneous distribution of stygobiotic species in the fragmented groundwater systems of southern Europe, and the high sampling effort required to collect rare and endemic species strongly support the use of biodiversity indicators (see Favreau et al., 2004, for an exhaustive review of this subject). For this reason, predictive models of species richness were developed using environmental parameters, sets of indicator species as well as sets of higher taxa as indicators of groundwater biodiversity at the habitat level (section 4). Species richness was a significant function of latitude, pH, nitrates and dissolved oxygen; the most efficient model (sensu Mac Nally et al., 2003) selected using information criteria explained over 60% of the total variance of species richness. Furthermore, sets of indicator species and higher level taxa were selected using multiple regression models and statistically sound information criteria (Mac Nally and Fleishman, 2002, 2004). A model based on the occurrence of five indicator species explained between 82-93% of the variance of species richness at a regional scale. At the scale of southern Europe, a set of three indicator groups (gastropods, harpacticoids, and amphipods) was detected; this model explained more than 80% of the variance of species richness. The inclusion of this set of indicators in large scale assessments of stygobiotic diversity in groundwaters is highly recommended.

Considering that each regional fauna differs from the others (section 3), indicators of biodiversity at the species level were confirmed to be different in each region (section 4). However, environmental parameters and higher taxa species richness can be used as successful indicators and predictors of species richness at large scales. This important fact may be explained considering the environmental constraints imposed by strong environmental gradients on the groundwater assemblages, evidenced by the results of the multivariate analyses (section 2). Paleogeography, habitat structure and groundwater quality drive the

biodiversity of most stygobiotic taxa in a similar way; this fact is reflected in a strong correlation between sets of indicators with total species richness.

The models developed suggests the following optimization of the indicator selection process:

- a) if the aim of the research is the detection of broad-scale biodiversity patterns, environmental parameters can be selected as useful predictors;
- b) if the aim of the research is an assessment of stygobiotic species richness of groundwater habitats for conservation planning, higher taxa species richness of indicator groups (such as gastropods, harpacticoids, and amphipods for southern Europe) is recommended as a satisfactory surrogate; for this purpose, the specimens of each indicator group need to be identified at the species level to calculate indicator species richness;
- c) finally, if more detailed analyses are to be performed in the selected regions, more accurate sets of indicators are required, e.g. a limited suite of species that reflects overall species richness of the entire biota (Pimm et al. 2001).

The selection of indicators should be performed in any new region following sound and comparable statistical techniques. In fact, up to now indicator species have been selected according to ad hoc criteria, such as their charisma or legal protection status. Mac Nally and Fleishman (2004) argued that statistically based selection of potential indicators is better justified and likely to be more effective: prediction of species richness should be regarded as a testable hypothesis in the form of a statistical model, e.g. a function of the occurrence of indicators. Therefore, the models developed in section 4 are highly recommended.

#### **6.4 RECOMMENDATIONS FOR ASSESSING THE CONSERVATION VALUE OF SPECIES AND ASSEMBLAGES ;**

Species richness alone is not the only criterion to be taken in account in management plans (Kerr, 1997). Indicators used to assess the conservation value of individual species are needed, and may be of paramount importance for selecting priority sites (Spellerberg, 1992; Williams, 2000). Scores and indices of conservation value, being simple surrogates of more complex mathematical models, may be easily used in GIS software (Williams, 2001), and are a powerful tool to exchange information between ecologists and policy-makers, filling the gap between hydrogeologists, conservation ecologists, cartographers and decision managers.

Unfortunately, up to now conservationists usually assessed species conservation value using indices of status based on expert systems, and hence open to criticism. A standard method to

build conservation indices based on the information stored in the PASCALIS database was proposed in chapter 5. Indices to assess degree of endemism, range-size rarity, habitat selection, and taxonomic isolation (including phylogenetic relictuality, whose importance was stressed by Botosaneanu and Holsinger, 1991 and Coineau and Boutin, 1992) were developed using normalized values extracted from the database. Considering that statistically sound criteria for selecting priority species and sites are needed (Williams, 2001), the application of a mean conservation value was proposed and will be tested during WP8 together with species richness as a tool to produce meaningful maps to be used for planning groundwater conservation. Mean values of endemism, rarity, and taxonomic isolation were used to assign a cumulative conservation value to each of the 830 stygobiotic species included in the database. A mean index is recommended also for assessing the conservation value of stygobiotic species assemblages; such an index does not take in account species richness and is likely to give important complementary informations.

## **6.5 DISCUSSION ON THE EFFECTIVENESS OF THE PROPOSED MODELS**

The effectiveness of the recommendations and models suggested following the main results of the WP7 workpackage rely on the peculiar structure of groundwater ecosystems (Gibert et al., 1994). The number of sampling sites required for an exhaustive field survey across southern European groundwater systems appears to be very high and quite variable depending on geographical location, habitat structure and anthropogenic impact on the study area. The complexity, geographical variability and high fragmentation of the subterranean environment prevents the application of a standard protocol (Malard et al., 2002) over broad spatial scales. The sampling strategy needs to be refined as a function of geographical location, paleogeographic informations, habitat structure and environmental determinants of biodiversity. This complex, time expensive surveys require the use of biodiversity indicators (*sensu* Mac Nally and Fleishmann, 2004).

Unfortunately, species richness of different taxonomic groups may be driven by different environmental factors in the different regions, and this may not be correlated at spatial and temporal scales relevant to the sampling design (Mac Nally et al., 2003). For this reason, the environmental surrogates of biodiversity should be treated with caution. The choice of indicators and the effectiveness of sampling procedures are heavily influenced by the high rate of endemism (more than 80% of southern European stygobionts are likely to be strict

endemics) and by the rarity of groundwater species. Moreover, habitat requirements of a large percentage of stygobiotic species are still poorly known, several new species were discovered during PASCALIS field surveys and several others are likely to be discovered extending such surveys to other regions, especially in the southernmost part of Europe (Stoch, 2000). Finally, limitations to the effectiveness of the models due to the high level of endemism of stygobiotic species prevents the establishment of a threshold in conservation indices useful in discriminating priority species for environmental conservation plans.

Notwithstanding the fact that the techniques and models proposed herein should be treated with caution when applied outside the regions sampled during the PASCALIS project, the simple structure of groundwater assemblages, the presence of strong environmental gradients driving biodiversity patterns and the link between species distribution and environmental parameters allowed the building of effective models for predicting species richness. For this reason, the methodology used herein in the model building process is likely to be useful in further sampling programs, and the high conservation values of stygobiotic assemblages (including mainly rare and endemic species), following the criteria established by the Habitat Directive, clearly suggests their importance in developing conservation strategies for the landscape extended to the subsurface environment.

Thanks to the PASCALIS database that reflects the available knowledge on species for six European countries, the methods proposed in this report appear to be the best suitable ones that can be applied. However new data are needed to update the taxonomic status, distribution patterns and biological and ecological aspects with the purpose to make knowledge on groundwater fauna advance.

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