



**Protocols for the ASsessment and CConservation of Aquatic Life
In the Subsurface**

CNRS - Université Claude Bernard Lyon1

Symposium on World Subterranean Biodiversity

Villeurbanne 8, 9 and 10 December 2004

Programme and abstracts

Organizer

**UMR-CNRS 5023- Laboratory of Ecology of Fluvial Hydrosystems
Subterranean Hydrobiology and Ecology research team**

**University Claude Bernard Lyon 1
43, Bd du 11 Novembre 1918
69422 VILLEURBANNE CEDEX - France**

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Acknowledgements

The organizing Committee is deeply grateful to the following Institutions for their generous support :

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Agence de l'Eau Rhône-Corse-Méditerranée, directeur Alain Pialat, 2-4, allée de Lodz, 69363 Lyon cedex 07.

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Scientific programme

Tuesday 7th December

14.00-18.30	Registration session
18.30-19.00	Bus departure to Villeurbanne town hall
19.00-20.00	Welcoming reception by the mayor of Villeurbanne

Wednesday 8th December

9.00-9.30	J. Gibert (organizer), P. Lantéri (University Lyon 1) and B. Andral (CNRS)	<i>Opening session in presence of institutional representatives</i>
General introduction to biodiversity		
9.30-10.00	S. Morand CNRS-IRD – France	"Global change and biodiversity"
10.00-10.30	P. Joly University Claude Bernard Lyon 1 - France	"Values of biodiversity and motivation for its conservation"
Break		
11.00-11.30	D. C. Culver American University – Washington DC – USA	"The struggle to measure subterranean biodiversity"
11.30-12.00	D. L. Danielopol Austrian Academy of Sciences - Austria	"Why and how to take care of subterranean aquatic microcrustaceans"
General presentation of PASCALIS European research programme <i>Chairman : J. Gibert</i>		
12.00-12.30	J. Gibert Project coordinator	"How to assess and conserve groundwater biodiversity. The PASCALIS European project in the international context : main results"
Lunch		
14.00-14.30	L. Deharveng Natural History Museum – Paris - France	"The PASCALIS data base and the diversity of stygobitic fauna in Europe."
14.30-15.00	P. Trontelj, C. Douady University of Ljubljana – Slovenia	"Cryptic diversity of selected groundwater taxa."
15.00-15.30	F. Stoch University of L'Aquila – Italy	"Biodiversity indicators"
Break		
16.00-16.30	F. Malard CNRS-University Claude Bernard Lyon 1 – France	"Additive partitioning of stygobiont species richness in Southern Europe"
16.30-17.00	F. Stoch University of L'Aquila – Italy	"Assessing the conservation value of groundwater species"
17.00-17.30	G. Michel, F. Malard Walloon Commission for Study and Protection of Subterranean Sites – Belgium	"Design of a network of priority sites for conserving groundwater biodiversity in Europe"
17.30-18.30	Poster session	<i>20 posters</i>

Thursday 9th December

Session 1	Australian Stygofauna and Western Australian project (Pilbara region)	<i>Chairman : Dan Danielopol Reporter : David Culver</i>
9.00-9.20	W.F. Humphreys, C.H.S. Watts, J.H. Bradbury Western Australian Museum – South Australia	"Emerging knowledge of diversity, distribution and origins of some Australian stygofauna"
9.20-9.50	S. Eberhard, S. Halse Department of Conservation and Land Management - Australia	"Assessment and conservation of aquatic life in the subsurface of the Pilbara region in Western Australia."

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9.50-10.10	J. Reeves Australian National University - Australia	"The relationship between Ostracod species diversity and water chemistry in the subsurface of the Pilbara region, Western Australia"
10.10-10.30	T. Finston, M.S. Johnson, S. Eberhard, S. Halse The University of Western Australia – Crawley - Australia	"Allozyme and haplotype diversity in Pilbarus Millsi, a widespread groundwater species of amphipod from the Pilbara, Western Australia"
Break		
11.00-11.20	C. J. Francis, M.S. Johnson, T. Finston The University of Western Australia – Perth - Australia	"Population structure of stygobitic isopods in the Pilbara, Western Australia."
Session 2 Phylogeny, Phylogeography and Taxonomy		
<i>Chairman : Ana Camacho</i> <i>Reporter : Louis Deharveng</i>		
11.20-11.40	A. Campanaro, V. Ketmaier, R. Argano University of Rome "La Sapienza" - Italy	"COI data indicate a complex evolutionary history for Italian subterranean and surface populations of Proasellus (Crustacea, Isopoda)"
11.40-12.00	T. Lefébure, C.J. Douady, M. Gouy, P. Trontelj, J. Briolay, J. Gibert University Claude Bernard Lyon 1 – France	"Phylogeography and taxonomic status of Niphargus virei (subterranean amphipod)"
12.00-12.20	C. J. Douady, T. Lefébure University Claude Bernard Lyon 1 – France	"Are Bayesian inferences a useful tool for phylogeographers ?"
Lunch		
14.00-14.20	P.J. Wood, G. S. Proudlove Loughborough University - UK	"Subterranean biodiversity in Great-Britain and Ireland : a predominantly post-glacial colonising fauna with possible trans-glacial elements"
14.20- 14.40	R. Huys, D. Galassi, P. De Laurentiis The Natural History Museum – London - UK	"Ectinosomatidae (Copepoda, Harpacticoida) in groundwater : radiation or multiple invasion ?"
14.40-15.00	A. Brancelj, N. Mori National Institute of Biology – Ljubljana - Slovenia	" Cladocera and Calanoida – two interesting groups of stygobionts"
15.00-15.20	M. Artheau University Paul Sabatier – Toulouse - France	"Distribution of the speciose genus Vestalenula, Rosseti & Martens, 1998 (Darwinulidae, Ostracoda), with the description of a new species from the Roussillon Region (South Eastern France)"
Break		
Session 3 Biodiversity patterns		
<i>Chairman : Diana Galassi</i> <i>Reporter : Anton Brancelj</i>		
16.00-16.20	D.C. Culver, M.C. Christman, M. Madden, D. White American University – Washington DC – USA	"Patterns of endemism of the Eastern North American cave fauna"
16.20-16.40	D. Ferreira, F. Malard, M.-J. Dole-Olivier, J. Gibert University Claude Bernard Lyon 1 – France	"Hierarchical pattern of obligate groundwater biodiversity in France"
16.40-17.00	H.J. Hahn, A. Fuchs University of Koblenz-Landau - Germany	" Mapping the stygofauna of the state Baden-Württemberg, Southwest Germany."
17.00-17.20	K. Schneider, D.C. Culver, H.H. Hobbs, D. Fong University of Maryland – College Park - USA	"The blind misleading the blind : false negatives and estimations of subterranean biodiversity."
20.00	Bus departure from the University campus to the restaurant	
20.15-23.00	Banquet dinner and return by bus	

Friday 10th December

Session 3 Biodiversity patterns : continuation		
9.30-9.50	P. Martin, C. De Broyer, F. Fiers, G. Michel, R. Sablon, K. Wouters Royal Belgian Institute of Natural Sciences – Brussels	"Biodiversity of Belgian groundwaters : the Meuse Basin"
9.50-10.10	T. Pipan, D.C. Culver Karst Research Institute – Postojna – Slovenia	"Epikarst fauna from North American caves"

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10.10-10.30	F. Malard, M. Lafont, Cemagref – Lyon - France	"Riverscape dynamics and the distribution of the hyporheos in a glacial river-floodplain system"
Break		
11.00-11.20	F. Paran, F. Malard, J. Mathieu, M. Lafont, D. Galassi, P. Marmonier Ecole National Supérieure des Mines – Saint-Etienne – France	"Distribution of groundwater invertebrates along an environmental gradient in a shallow water-table aquifer"
11.20-11.40	W. K. Jones Karst Waters Institute – Charles Town – USA	"Ground-water flow and aquatic habitats"
Session 4 Conservation and management		
		<i>Chairman : Patrick Martin</i>
		<i>Reporter : Boris Sket</i>
11.40-12.00	B. Sket University of Ljubljana – Slovenia	"Some auxiliary criteria for the selection of groundwater sites for biodiversity conservation"
12.00-12.20	M.C. Bruno, V. Cottarelli University of La Tuscia – Viterbo- Italy	"Use of groundwater copepods as a tool for environmental management : the case of Everglades National Park, Florida, USA"
Lunch		
14.00-14.20	T. Datry, F. Malard, J. Gibert University Claude Bernard Lyon 1 – France	"Response of invertebrate assemblages to increased groundwater recharge rates in a phreatic aquifer"
14.20-14.40	B. Fiasca, T. Di Lorenzo, P. De Laurentiis, D.M.P. Galassi University of L'Aquila – Italy	"Hyporheic copepod assemblages as a tool for detecting man-induced perturbation on GW/SW ecotone."
14.40-15.00	T. Di Lorenzo, B. Fiasca, F. Stoch, D.M.P. Galassi University of L'Aquila – Italy	"Environmental quality of deep groundwater in the Lessinian mountains (Italy) : signposts for sustainability."
15.00-15.50	Report on the sessions	<i>5 reports</i>
15.50-16.00	J. Gibert	<i>Closing of the Symposium</i>
Break		

General introduction to biodiversity

THE STRUGGLE TO MEASURE SUBTERRANEAN BIODIVERSITY

Culver David C.

Department of Biology, American University, 4400 Massachusetts Ave., NW, Washington DC 20016 (USA)
dculver@american.edu

Since at least the late 19th century, speleobiologists have been interested in the number of species, especially the number of obligate cave-dwelling species in caves. It has been and continues to be a source of national and regional pride, as well of considerable biogeographic interest. Formidable problems face any large scale comparisons, especially those of inadequate sampling. However, recent advances in analysis of spatial patterns (Geographic Information Systems), the identification of points on the surface of the earth (Global Positioning Systems), and the ability to analyze these patterns (spatial and geostatistics) make some preliminary analyses possible.

Globally, troglobiotic and stygobiotic species richness appears greatest at mid-north temperate latitudes and among the mid-north temperate latitudes, greater in Europe. One, but certainly not the only cause the mid-temperate pattern, is the extent of cave development, both locally and regionally. While the ranges of most obligate subterranean species are quite small, the physical geography of a region beyond the range of many species seems to have an effect on diversity. This in turn suggests that there is more subterranean migration than previously thought. The ranges of species in two of the world's most diverse caves—Vjetrenica in Bosnia and Hercegovina and Mammoth Cave in Kentucky, USA—also lend credence to the idea of at least limited subsurface migration.

Sampling problems, including false negatives, unsampled caves, and undescribed species, continue to be important. While these problems will never entirely disappear, several approaches can minimize their impact, including statistical estimates of total species numbers and thorough sampling of a few caves. The effectiveness of these approaches will be evaluated.

WHY AND HOW TO TAKE CARE OF SUBTERRANEAN AQUATIC MICROCRUSTACEANS

Danielopol Dan L.¹ & Pospisil Peter²

1: Institute of Limnology, Austrian Academy of Sciences, Mondseestr. 9, 5310 Mondsee (Austria)

2: Reichmannng. 3/6, 1160 Wien (Austria)

Subterranean habitats harbour a diversified fauna of microcrustaceans mainly copepods, ostracods, syncarids. There are moral, scientific, economic and cultural arguments to protect against extinction groundwater organisms and/or to prevent the deterioration of their environment. The scientific interest of many subterranean crustaceans is related *inter alia* to the long evolutionary history of the groups to which they belong. A new ostracod group discovered during the PASCALIS project exemplifies this assertion. Additionally the study of some subterranean microcrustaceans allows to participate to debates related to evolutionary and/or biogeographical topics. For instance why there are a rather high number of species with wide geographical distribution beside many rare taxa with a restricted localisation? The case of several cyclopoid species will be discussed and the paradox of the generalist species solved with arguments derived from an organismic-ecological approach. Subterranean microcrustaceans, especially copepods, have in some cases also a practical value, e.g. they can be used as environmental tracers.

There are also moral arguments, many stygobites exist since a long period of time in an environment which is now disturbed by humans rapidly and in a brutal way.

We offer some practical ideas how to protect groundwater fauna and/or their habitats. In this context we emphasise the necessary involvement of scientists in debates related to environmental politics as well in the implementation of educational programmes for protection of groundwater domain and its life forms. We suggest that many places with a high number of stygobitic crustaceans (hotspot areas) could be viewed as "natural museums" with a cultural value. Hence they should be protected with priority. The example of integration of such an area within the Austrian National Park Danube Wetlands, closely located to Vienna, is discussed. The interest of the protection of the subterranean fauna within groundwater recharge places is emphasised.

BIODIVERSITY VALUES AND VALUE OF THE BIODIVERSITY CONCEPT

Joly Pierre

UMR CNRS Ecology of Fluvial Hydrosystems, University Claude Bernard Lyon 1, Bât Darwin C, 69622 Villeurbanne cedex (France) pjoly@biomserv.univ-lyon1.fr

Since the summits of Rio (1992) and Johannesburg (2002), public awareness has been heightened by the problem of massive biodiversity loss. However, the impacts of scientific alarms on public policies depend on the value attributed by public opinion to biodiversity. The "value" of a species is indeed a controversial issue since the relationship between man and nature comes under several conceptions. After analysing the historical and ethical roots of three main conceptions (anthropocentrism, ecocentrism and biocentrism), I show how they lead to different valuations of biodiversity. I then discuss some problems that result from each conceptual framework. This discussion highlights the usefulness of the biodiversity concept in the perspective of sustainable development and raises the question of the relationship between science and society since the classical virtuous circle that links science, technical powerfulness and economy no longer takes into account such an externality as biodiversity erosion. Finally, I show how the different conceptions could converge toward a synthetic approach of biodiversity conservation.

GLOBAL CHANGE AND BIODIVERSITY

Morand Serge

Centre de Biologie et de Gestion des Populations (CBGP) (UMR CIRAD-Agro.M-INRA-IRD). Campus International de Baillarguet. CS 30 016. 34988 Montferrier sur Lez Cédex (France) morand@ensam.inra.fr

Terrestrial, marine and aquatic ecosystems are all submitted to the multiple impacts of human activities. These activities, that can be estimated by a measure like the “Ecological footprint”, are characterized by several alterations affecting the use of biological resources, the functioning of ecosystems, and the climate. All environments are affected : deforestation, habitat losses, increases of urban areas, over-exploitation of renewable resources (fisheries), chronic pollutions, over use of water... Life is also directly submitted to new forms of exploitation such as the release of GMO. The globalisation of exchanges have increased the introductions of alien species, and environmental changes may favour their spread which may have dramatic impacts for ecosystem health, plant health and even human health. Human activities also profoundly influence the climate through the production of greenhouse gazes. Several aspects of biodiversity are affected by the global change (which includes the climate change) : area distributions of species, biological resources (such as fisheries), invading species. Scenarios should be proposed and may concern : the expected future distributions of species or biomes ; the adaptive (and co-adaptive) responses of organisms ; the impacts on biological resources ; the effects on plant, animal and human health ; the level and localisation of extinctions ; management of protected areas. The building of these scenarios need studies on : the characterisation of biodiversity (taxonomy) ; the role and functions of biodiversity ; a better understanding of interactions.

General presentation of PASCALIS

**THE PASCALIS DATABASE AND THE DIVERSITY OF STYGOBITIC
FAUNA IN EUROPE**

Deharveng Louis

Museum d'Histoire Naturelle, Origine, Structure et Evolution de la Biodiversité, Dpt Systématique et Evolution, 45 rue Buffon, 75005 Paris (France) deharven@mnhn.fr

The PASCALIS database includes distribution records of stygobitic and regular stygophilic species of the European countries covered by the project PASCALIS, i.e. Spain, Portugal (incomplete), France, Italy, Slovenia and Belgium.

In the mid-2004, the database had 14528 distribution records of which 12492 concerned obligate subterranean species. Out of this total, 10376 records of named stygobitic species were retained for mapping. The PASCALIS project contributed 3381 records, of which 1844 concerned named stygobitic species. It is considered that all stygobitic species of the PASCALIS countries of Europe are probably included in the database (1239 species). We derived some statistics from this large dataset, regarding species richness and rarity by countries and by zoological groups. Based on this analysis, several characteristic features of aquatic subterranean biodiversity in Europe are described.

**HOW TO ASSESS AND CONSERVE GROUNDWATER
BIODIVERSITY
THE PASCALIS EUROPEAN PROJECT IN THE INTERNATIONAL
CONTEXT : MAIN RESULTS**

Janine Gibert (PASCALIS coordinator)

Université Claude Bernard Lyon 1, CNRS - UMR 5023 Ecologie des Hydrosystèmes Fluviaux, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne (France)

The PASCALIS project (EVK2-CT-2001-00121) (2002-2004) is presented in the international context. It proposes to assess groundwater biodiversity and to elaborate recommendations in view of its conservation. The project is based on biodiversity assessment at regional and national scale in identifying biodiversity indicators. It includes actions to reinforce public awareness of the need for its protection and recommendations for its conservation at the European level.

For a better understanding of groundwater biodiversity patterns, a database compiles about 11000 distribution records of references to subterranean species from 6 countries: France, Italy, Belgium, Slovenia, Spain and the Canary Islands. 2200 species were recorded among which 1200 obligate subterranean species (stylobites). Furthermore, a comprehensive sampling strategy was proposed based on 6 selected regions. 1300 samples were collected to study environmental and biological attributes. The preliminary analyses of these samples show that the high sampling effort (192 sites per region) was insufficient for assessing the regional biodiversity, due to the rarity and the high rate of endemism (80 %). The lists of species identified for each region give an overview of the existing groundwater biodiversity and its partition. Permeability and size pore were the main variables explaining the structure of stygobiotic assemblages. Predictive models of species richness have been developed using environmental parameters, sets of indicator species, sets of higher taxa as indicators of groundwater biodiversity at the habitat level. A standard method to build conservation indices has also been proposed.

This project, of great scientific value, as research on groundwater biodiversity assessment and conservation, is carried out for the first time at the European level. It will also contribute to the 2010 target in offering applicable recommendations in terms of water management, legislation and procedures of biodiversity assessment and conservation.

**ADDITIVE PARTITIONING OF STYGOBIONT SPECIES RICHNESS
IN SOUTHERN EUROPE**

Malard Florian

Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622
Villeurbanne cedex (France) malard@univ-lyon1.fr

Determining how species diversity is generated over spatial scales is of paramount importance for selecting appropriate strategies for biodiversity assessment and conservation. We partitioned the richness of stygobiont fauna across nested spatial scales – aquifers, basins, and regions – using data collected in six European regions within the framework of the project PASCALIS. We also examined the local-regional patterns of species richness at 2 spatial scales using the alpha-beta-regional (ABR) approach. The between-region component made by far the highest contribution (i.e. 81%) to the stygobiont richness of southern Europe. The contribution of between-diversity increased monotonically with increasing spatial scale, reflecting the preponderance of rare species in groundwater invertebrate communities. Hierarchical cluster analysis and correspondence analysis corroborated these findings because they identified marked differences in community composition among regions. The ABR approach suggested that local richness was dependent upon regional richness in a manner consistent with proportional sampling but the contribution of alpha and beta richness apparently changed across spatial scales. The patterns of species composition and richness have strong implications for the assessment and conservation of stygobiont 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 stygobiont diversity in southern Europe is to protect multiple aquifers within different regions, thereby maintaining regionally distinctive species-rich assemblages.

DESIGN OF A NETWORK OF PRIORITY SITES FOR CONSERVING GROUNDWATER BIODIVERSITY IN EUROPE

Michel George¹ & Malard Florian²

1: Commission Wallonne d'Etude et de Protection des Sites Souterrains (CWE PSS), 21 Avenue Auguste Rodin, Bruxelles (Belgium) cwepss@swing.be

2: Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) malard@univ-lyon1.fr

In the face of the irretrievable loss of biodiversity world-wide, the identification of reserve network has become the cornerstone of most conservation agencies. Because the resources (e.g. space, funds) that can be devoted to the conservation of biodiversity are limited, it is increasingly thought of an effective reserve as network of sites that achieve defined objectives for the smallest possible cost. Whereas heuristic algorithms are commonly used for designing effective reserve networks in terrestrial and marine systems, the designation of priority groundwater sites is still essentially based on traditional selection methods. We examined the efficiency of four procedures used to select priority sites in representing groundwater species within a European reserve network. These 4 procedures consisted in selecting the hotspots of species richness (1), hotspots of endemism (2), most complementary sites (3); and most complementary sites with an additional constraint driving the selection of targeted species. The last two procedures were performed using a random search algorithm known as simulated annealing implemented under the free software Marxan (Ian Ball and Hugh Possingham). The data base assembled within the framework of the European program PASCALIS contained 10,366 occurrences of 881 stygobiont species in 6 countries (Belgium, France, Spain, Portugal, Italy, and Slovenia). These occurrences fell into a grid coverage containing 1097 cells having a surface area of 0.2x0.2 decimal degrees. The maximum size of the reserve network was fixed to 110 cells representing 10 % of the number of cells at which species were collected. The effectiveness of the four selection procedure was examined based on a series of criteria including the number of species and endemics, the sum of conservation indices of species, and the frequency of occurrence of species retained in the reserve network. Results showed that the heuristic algorithms provided the most effective solution. The 110 cells selected by the heuristic comprised 77 and 65 % of all species and endemics included in the original data base. We argue that selection procedures paying explicit attention to patterns of between-site complementarity at a European scale greatly improve the efficient representation of a large proportion of species and minimize the duplication of conservation efforts across regional and national agencies. A next important step is to shift from a grid cell selection approach to an aquifer selection approach that incorporates not only representation targets of species and minimum space requirement but also socio-economic costs related to the vulnerability of aquifers and strength of human activity in the catchment. The recognition of aquifers as biological conservation units is of particular importance for breaking the bridge between managers and ecologists and for promoting sustainable groundwater use strategies that maintain ecosystem service over time.

ASSESSING THE CONSERVATION VALUE OF GROUNDWATER SPECIES

Stoch Fabio

Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila (Italy)
fabio.stoch@faunaitalia.it

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 hydro geologists, 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, and 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 database and local biodiversity assessments. Maps of the mean values of each index and of a cumulative index for assessing the intrinsic conservation value of groundwater species in each grid cell were produced. The relationship between spatial patterns of conservation value and diversity (measured as species richness) of local assemblages were studied, and implications for selecting priority areas for conservation are discussed ; furthermore, the limitations to this procedure due to the high level of rarity and endemism of stygobitic species (more than 83 % of them can be defined as strict endemics) are also examined in detail.

BIODIVERSITY INDICATORS

Stoch Fabio

Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila (Italy)
fabio.stoch@faunaitalia.it

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, ecologists as well as natural resource managers need to develop predictive models of species richness using indicators. Environmental parameters, sets of indicator species as well as sets of higher taxa were used to develop successful regression models to predict groundwater biodiversity at the habitat level. The selection of indicators was performed using a model based on multiple regression analysis of any combinatorial set of indicators versus total species richness and selecting the most efficient model on the basis of information criteria. The most statistically efficient model is defined herein as the model that optimizes fitting error against model complexity (i.e. number of indicators retained).

Species richness of stygobionts was a significant function of latitude, pH, nitrates and dissolved oxygen. The most efficient model selected explained over 60% of the total variance of species richness; no area effect on biodiversity was detected, and habitat structure showed a low influence as well.

Sets of indicator species and higher level taxa were selected using statistically sound information criteria in multiple regression and Spearman's rank correlation of sets of indicators versus residual biodiversity (e.g. species richness excluding indicators). 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. 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 at the habitat level. The inclusion of this set of indicators in large scale assessments of stygobitic biodiversity in groundwaters is highly recommended.

CRYPTIC DIVERSITY OF SELECTED GROUNDWATER TAXA

Trontelj Peter¹ & Douady Christophe²

1: Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, PO box 2995, 1001 Ljubljana (Slovenia)

2: Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France)

Groundwaters were recently shown to harbor remarkably high numbers of sibling, or cryptic species. Ignoring them may mislead estimations of biodiversity and endemism, and obscure comparisons. Within the framework of the PASCALIS project, four groups of stygobitic organisms have been studied in terms of their cryptic diversity: the amphipod genus *Niphargus*, the cave salamander *Proteus*, the ubiquitous isopod *Asellus aquaticus*, and erpobdellid leeches.

The problems of cryptic diversity can be associated with two levels of genetic variation: (1) diversity due to cryptic speciation, and (2) diversity between phylogenetically separated, non-monophyletic lineages that are not distinguished by current taxonomy.

At least some indications of cryptic speciation were found in all analyzed taxa. It has no effect on the alpha diversity at the level of single drainages or other hydrographically interconnected units. It may have a strong positive effect on the gamma diversity and on the number of endemic species. Very tentatively, the increase of gamma diversity ranged from twofold for *Niphargus virei* in eastern France to sixfold for *Proteus* in the Dinaric region.

The second type of cryptic diversity was revealed for some *Niphargus* taxa, as well as the leech genera *Dina* and *Trocheta*. It is the consequence of convergent or parallel evolutionary change in non-sister groundwater taxa, causing an underestimation of gamma diversity.

Although hidden diversity is not a peculiarity of groundwater habitats, the overall species richness of epigeal ecosystems is much higher, and hidden diversity is likely to constitute a very small part of the total epigeal diversity. On the other hand, in groundwater ecosystems hidden diversity may account for most of its gamma diversity, as tentatively suggested by the *Proteus* case.

Our conclusions are mainly based on karstic groundwaters, which are often highly fragmented. Therefore they cannot be applied without caution to interstitial and alluvial types of groundwater.

Abstracts

DISTRIBUTION OF THE SPECIOSE GENUS *VESTALENULA*, ROSSETTI & MARTENS, 1998 (DARWINULIDAE, OSTRACODA), WITH THE DESCRIPTION OF A NEW SPECIES FROM THE ROUSSILLON REGION (SOUTH-EASTERN FRANCE).

Artheau Malvina

Laboratoire Dynamique de la Biodiversité. UMR/CNRS 5271. UPS, 118 route de Narbonne
31062 Toulouse cedex 4 (France)

A new species of *Vestalenula* Rossetti & Martens, 1998, belonging to the *danielopoli*-lineage is described. It was found during the PASCALIS project when sampling 5 hyporheic sites on the Aude and Tech rivers (Roussillon region, South Eastern France). This new record of the genus brings to 20 the number of *Vestalenula* species worldwide. *Vestalenula* representatives are known from the Lower Miocene to present. The biogeography of this group will be reviewed in order to stress out the interest of the discovery of a new species in Southern France.

Most living species of *Vestalenula* occur in subtropical regions. *V. cylindrica*, *V. boteai* and *V. danielopoli* lived or are living in the Palearctic. *V. pagliolli* occurs in both hemispheres. The other 16 are recent species. 12 of them are living in the southern hemisphere. The Recent species occur in restricted areas and many live in semi-terrestrial habitats and/or interstitial habitats.

The morphological characters which enable the *Vestalenula* species to be successful in colonizing groundwater habitats will be discussed.

This work has been done during a stay in June 2004 at the Limnological Institute of the Austrian Academy of Sciences in Mondsee, Austria.

CLADOCERA AND CALANOIDA – TWO INTERESTING GROUPS OF STYGOBIONTS

Brancelj Anton & Mori Nataša

National Institute of Biology, Vecna pot 111, 1000 Ljubljana (Slovenia)

Crustacea are well represented in subterranean environment in terms of number of taxa, with Copepoda (Harpacticoida, Cyclopoida), Amphipoda and Isopoda being the most numerous. Exceptions among Crustacea are Anostraca, Conhostraca and Notostraca with no known stygobionts. Cladocera and Calanoida (Copepoda) are very well represented in most types of fresh-water bodies, each with about 500 taxa all over the world, but they have only few representatives in subterranean environment. Members of both groups were found relatively late in different types of groundwater. The first cladoceran recognised as stygobiont was discovered in 1973 (*Alona smirnovi*) and the first stygobitic member of Calanoida was found in 1942 (*Microdiaptomus cokeri*). So far, only six stygobionts and additional two might-stygobionts are known among Cladocera and eight stygobionts and two might-stygobionts among Calanoida. The number of new stygobionts from both groups increased in the last few years due to intensive sampling efforts in caves and gravel-beds.

Patterns of geographical distribution of representatives of both groups, their morphological and ecological adaptations to subterranean environment as well as appropriate sampling techniques will be discussed.

USE OF GROUNDWATER COPEPODS AS A TOOL FOR ENVIRONMENTAL MANAGEMENT : THE CASE OF EVERGLADES NATIONAL PARK, FLORIDA, USA

Bruno M. C. & Cottarelli V.

Università della Tuscia, Dipartimento di Scienze Ambientali Largo dell'Università 01100 Viterbo (Italy)

Everglades National Park (ENP) is an extensive subtropical wetland ecosystem marsh ; its hydrology changed radically from the 1950s as a result of the construction of a canal system, which drained water eastwards and southwards. The Rocky Glades is an area of higher elevation in the Eastern part of ENP, with short hydroperiod, characterized by a karstic landscape with thousands of solution holes, which are filled with water during the wet season (Jun.-Oct.), and are dry in the dry season (Nov.-May).

We collected copepods from different depths in the superficial aquifer in 2 sets of wells in the Rocky Glades; and in a third set of wells along the canals bordering ENP to the east. Objectives of the three studies were to assess : 1) stygoxenic and stygophilic species and their spatial and temporal distribution ; 2) how seasonal changes in groundwater levels influence the ability of Everglades surface copepods to disperse into groundwater to survive the drought ; 3) how local geological features influence faunal exchange between surface and groundwater.

Groundwater communities were dominated by surface copepods ; abundance and diversity decreased with depth, due to different limestone permeability, and copepods did not disperse far from the input location. In the Rocky Glades, surface copepods colonized groundwater during the dry season, with peaks at the beginning and at the end of the wet season. In canals bordering ENP, copepods colonized groundwater following groundwater seepage along canals.

Recommendation for the management included : 1) solution holes in the Rocky Glades act as an exchange area between surface and groundwaters : over drainage should be avoided to preserve ENP biodiversity; 2) the use of copepods as indicators of seepage could be a tool to assess the direction and the duration and the direction of surface and ground water exchange ; 3) the minimum target water levels for the Rocky Glades had been set at 45 cm below the surface. This level might be too low for copepods to migrate vertically into the aquifer, or to survive adverse physical and chemical conditions in groundwater.

**COI DATA INDICATE A COMPLEX EVOLUTIONARY HISTORY FOR
ITALIAN SUBTERRANEAN AND SURFACE POPULATIONS OF
PROASELLUS (CRUSTACEA, ISOPODA)**

Campanaro Alessandro, Ketmaier Valerio & Argano Roberto

Department of animal and Human Biology, University of Rome "La Sapienza", V.le dell'Università 32, 00185 Roma (Italy)
valerio.ketmaier@uniroma1.it

The water louse species of *Proasellus* are widely distributed in the surface and subterranean freshwaters of the Mediterranean region. We investigated patterns of genetic divergence and phyletic relationships in 40 populations of *Proasellus*, sampled in continental Italy and in Sardinia, sequencing a fragment of the mitochondrial gene coding for the cytochrome oxidase I (COI; 411 bp). The study includes 26 surface populations from continental Italy, two subterranean species from Central Italy, *P. acutianus* and a new (still undescribed) species (*P. n. sp.*), and 12 both surface and subterranean populations from Sardinia. Phylogenetic and network analyses revealed the existence of two well-supported clades. The first clade is characterised by a relatively low degree of genetic heterogeneity and groups all the surface populations from continental Italy and three surface populations from Sardinia. The second clade includes all the remaining populations from Sardinia, *P. acutianus* and *P. n. sp.* This group shows a high level of genetic heterogeneity, suggesting several different cladogenetic events and multiple independent invasions of subterranean environments. The placement in the same clade of all the subterranean populations, independently from their geographic origin, suggests that these taxa could be extant representatives of an ancient (Miocenic) wave of colonisation. Divergence time estimates, calculated using published COI rate for *Stenasellus*, indicate that the split between the two clades occurred around 30 myr ago and should be related to the tectonic events leading to the displacement of the Sardinia-Corsica micro plate from southern continental Europe. Previous morphological and allozyme data on the *P. coxalis*-complex are also discussed.

**PATTERNS OF ENDEMISM OF THE EASTERN NORTH AMERICAN
CAVE FAUNA**

Culver D. C.¹, Christman M.C.², Madden M.³, & Whites D.⁴

1: Department of Biology, American University, Washington DC 20016 (USA)

2: Department of Animal and Avian Sciences, University of Maryland, College Park MD 20742 (USA)

3: Environmental Studies Program, American University, Washington DC 20016 (USA)

4: Environmental Protection Agency, Corvallis OR 97333 (USA)

Over 250 species of obligate terrestrial cave-dwelling animals (troglobionts) are known from single caves in the eastern United States. We investigated their geographic distribution especially in relation to non-single cave endemic troglobionts. We related these patterns to taxonomic group, opportunities for dispersal, and geographic location.

We associated over 3000 records of over 450 troglobiotic species and subspecies with hexagons of 1000, 5000, and 10,000 km² in size. We found Moran's I, black-white joins, and cubic regression of endemics on non-endemics at all three spatial scales. For 5000 km² hexagons, we found the spatial autocorrelation of the residuals of cubic regression of endemics on non-endemics.

Differences among orders in percent single cave endemism were not significant except for Pseudoscorpionida which was higher (67%) than any other order. At all three scales, Moran's I and black-white joins were significant, indicating a clumped distribution of both single-cave endemics and other troglobionts. Spatial patterns were similar at all three scales and Moran's I was highest at 5000 km². The cubic fit of endemics to nonendemics was consistently better with less systematic error or residuals than was linear or quadratic models. Residuals showed a significant geographic pattern with excess endemics in more southerly locations.

There was both a non-spatial and spatial component to the pattern of single cave endemism. The non-spatial component was the association of high levels of single cave endemism with areas of high diversity of non-endemics. It may be that both are high because of high secondary productivity. Spatially, single-cave endemism is high in central rather than peripheral areas and in the southern part of the range. It is not higher in areas of more dissected limestone which would reduce migration rates. Regional spatial effects are important indicating that cave communities cannot be understood (or protected) in isolation.

**RESPONSE OF INVERTEBRATE ASSEMBLAGES TO INCREASED
GROUNDWATER RECHARGE RATES IN A PHREATIC AQUIFER**

Datry Thibault¹, Malard Florian² & Gibert Janine²

1: UR Hydrobiologie, Cemagref, 3 bis Quai Chauveau, C.P. 220, 69336 Lyon (France) datry@lyon.cemagref.fr

2: Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, UMR CNRS 5023, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) malard@univ-lyon1.fr, Janine.Gibert@univ-lyon1.fr

Low organic matter supply and reduced spatio-temporal heterogeneity severely constrain biodiversity in ground water. At the aquifer scale, spatial variation in the groundwater recharge rate is considered as a key factor generating groundwater patches with distinct food supply and spatio-temporal heterogeneity. We addressed the role of groundwater recharge in sustaining biodiversity in a phreatic aquifer by testing differences in the density and species richness of invertebrate assemblages among reference sites and sites artificially recharged with storm water. We also examined the vertical distribution pattern of invertebrate assemblages at a shallow water-table recharge site and a nearby reference site. Higher inputs of dissolved organic carbon (DOC) and spatio-temporal heterogeneity at recharge sites were expected to increase the density and richness of invertebrate assemblages because of a greater number of niches available and reduced likelihood of competitive exclusion. Groundwater recharge elevated DOC concentration in ground water and considerably increased spatio-temporal heterogeneity in physico-chemistry. The thickness of the vadose zone (VDZ) controlled DOC input in ground water at recharge sites but did not markedly attenuate spatio-temporal heterogeneity. The increase in density and richness of invertebrate assemblages at recharge sites was also controlled by VDZ, suggesting that organic matter supply was the primary factor determining biodiversity patterns in ground water. Invertebrate density increased and species composition shifted with increasing depth below the groundwater table at the shallow water-table sites. Some species including several stygophiles preferentially occurred in the vicinity of the water table, whereas others, including several stygobites, colonized deeper groundwater layers. The vertical distribution patterns of density and species might also be driven by food availability and resulting competitive processes among species.

**ENVIRONMENTAL QUALITY OF DEEP GROUNDWATER IN THE
LESSINIAN MOUNTAINS (ITALY): SIGNPOSTS FOR
SUSTAINABILITY**

Di Lorenzo Tiziana, Fiasca Barbara, Stoch Fabio & Galassi Diana M.P.

Dipartimento di Scienze Ambientali, University of L'Aquila, via Vetoio, Coppito, 67100 L'Aquila (Italy)

The present study deals with groundwater site conservation plans in the Lessinian Massif (Verona-Italy). The final aim is to provide baseline information, helpful for the sustainable management of groundwater sites, after an evaluation of their intrinsic biological value. Groundwater assemblages are analysed in both terms of ecological categorization and species composition in order to test their potential role in detecting integrated effects of human impacts on the groundwater body, and their sensitivity to changes in water quality from site to site, and over short-term temporal scale. Environmental parameters and species occurrence data are entered into a GIS software in order to visualize, explore, and analyse data at aquifer scale. Geo-statistical analyses are performed on a large data set from 150 wells to determine if patterns exist between the distribution of fauna, assemblage composition and environmental factors such as geological setting, aquifer typology, water quality and level of anthropogenic disturbance. The main data layer integrates groundwater copepod species ranges and environmental parameters layers. By their overlapping it would be possible to identify habitats of high copepod diversity in need of protection in a sustainable network of reference hot spot sites. Moreover, an eco-hydrogeological approach is presented on data subset, focusing on the assessment of potential relationships between aquifer intrinsic vulnerability parameters and inhabiting copepod assemblages. Statistical analyses reveal that an eco-hydrogeological approach in detecting aquifer vulnerability is more sensitive than the hydrogeological one alone.

ARE BAYESIAN INFERENCES A USEFUL TOOL FOR PHYLOGEOGRAPHERS ?

Douady Christophe & Lefébure Tristan

Plate-forme d'Ecologie Moléculaire des Hydrosystèmes Fluviaux & Equipe d'Hydrobiologie et Ecologie Souterraines,
Laboratoire d'Ecologie des Hydrosystèmes Fluviaux, Université Claude Bernard Lyon 1 - UMR CNRS 5023, France

Usage of Bayesian inferences to reconstruct phylogenetic relationships between organisms is exponentially increasing. This method has been widely accepted for both theoretical and pragmatic reasons. It is indeed quite satisfying to be able to take into account the prior knowledge on the probability of a change, to obtain in a single analysis an estimation of reconstruction reliability and to do this in a fraction of the time necessary to frequentist methods (i.e. Maximum likelihood inferences). Surprisingly, most phylogeographic analyses realized in the course of PASCALIS program revealed an important discrepancy in branch lengths between Bayesian and Maximum Likelihood methods. Branch lengths being one of the most important phylogenetic / phylogeographic information, after the branching pattern itself, we here investigate this behavior using 38 real data sets and 100 simulated ones. We report our first observations and discuss their consequences for phylogeographic usage of Bayesian inferences. Finally a new bioinformatic tool developed to allow user-friendly bootstrapping of Bayesian inferences is presented.

ASSESSMENT AND CONSERVATION OF AQUATIC LIFE IN THE SUBSURFACE OF THE PILBARA REGION IN WESTERN AUSTRALIA

Eberhard Stefan & Halse Stuart

Department of Conservation and Land Management (Western Australia)

The Pilbara region covers approximately 230,000 km² in the north-west of Western Australia where the present climate is arid to semi-arid. A diverse array of groundwater environments occurs within a range of geologic, geomorphic and topographic settings, including granular, karstic and fractured rock aquifers, springs and hyporeic environments associated with several large river drainage systems. Previous work established the presence of a diverse, endemic and scientifically interesting stygofauna (eg. Spelaegriphacea, tainisopid isopods of genus *Pygolabus*) that also included faunal elements with apparent Tethyan (eg. *Halosbaena* [Thermosbaenacea], *Haptolana* [Cirolanidae], *Stygiocaris* [Atyidae]) and/or marine affinities (e.g. melitid and bogidiellid amphipods, and *Halicyclops* [Cyclopidae]). Some of the Tethyan and marine faunal elements are shared with nearby karstic groundwaters at Cape Range and Barrow Island. However, in contrast to these coastal karst areas, the Pilbara stygofauna is also characterized by the presence of several major lineages of freshwater origin (eg. paramelitid amphipods, tainisopid and phreatoicid isopods, candonid ostracods, momonid and mideopsid acarines). In consideration of potential threats to stygofauna posed by mining operations in the Pilbara, the Department of Conservation and Land Management (CALM) is undertaking a regional-scale survey to provide a framework for the assessment and conservation of groundwater biodiversity. The four-year survey (2002 to 2006) aims to map regional patterns in biodiversity by sampling the range of groundwater environments that can be accessed via bores, wells, caves, springs and interstitial habitats. Faunal patterns will be related to abiotic environmental parameters such as geology, geomorphology, hydrogeology and water chemistry. The purpose of this paper is to present a brief overview of the Pilbara groundwater fauna and survey results to date, and to invite the collaboration of other researchers in this project, which requires taxonomic description of the many new taxa being discovered. To date about 40 stygobitic species have been described but at least another 60 species have been collected and, based on species-accumulation-curves, we predict the overall regional diversity to be at least 200 species. The occurrence of stygofauna in the Pilbara is not restricted to karstic habitats and rich assemblages (> 10 species collected in one borehole) also occur in granular riverine aquifers. Faunal elements of presumed marine origin (e.g. melitid amphipods) occur in groundwaters at high elevations (> 500 m above sea level) in the Pilbara craton, which has been continually emergent since the Proterozoic. This suggests that some stygofaunal elements have considerable capacity for dispersal, which presumably is facilitated by hydrologic connectivity (via interstitial permeability) along river drainage systems. Molecular genetic techniques (mtDNA) are being used to improve definition of species boundaries that may be obscured by high morphological variability within groups such as the Amphipoda.

**HIERARCHICAL PATTERNS OF OBLIGATE GROUNDWATER BIODIVERSITY
IN FRANCE**

Ferreira David, Malard Florian, Dole-Olivier Marie-José & Gibert Janine

Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622
Villeurbanne cedex (France)

The determination at different spatial scales of species diversity patterns is of paramount importance for prioritizing conservation efforts. Because beta diversity (i.e. the change in species composition between sites) is considered as a major contributor to high species diversity of a heterogeneous landscape, understanding its spatial patterns appears as critical for understanding the local to landscape patterns of global biodiversity. Because of its high level of endemism, groundwater biodiversity is expected to follow a spatialized hierarchical pattern so that nested areas of increasing size should become increasingly dissimilar in terms of species composition. Unfortunately, our present ability to identify groundwater biodiversity regions is severely limited by the lack of appropriate data. The analysis of species occurrence data in 20 x 20 grid cells did not enable to reveal the spatial pattern of biodiversity in France because of taxonomic and sampling biases among cells. Instead, we selected a subset of 55 aquifers that had received comparable sampling efforts. Reciprocal scaling and a UPGMA dendrogram (unweighted pair-group method using arithmetic averages) were used to examine differences in species composition among aquifers. Both methods distinguished between two clusters of aquifers belonging to the Rhône River and Garonne River basins that contained distinct sets of species (beta diversity > 80 %). The dendrogram divided these basins into smaller spatial units of increasing species similarity, thereby supporting the hypothesis of a spatial hierarchical organization of groundwater biodiversity. We argue that the most efficient strategy of data acquisition for identifying groundwater biodiversity regions would consist in determining the species composition of selected aquifers in regions that have been poorly investigated.

**HYPORHEIC COPEPOD ASSEMBLAGES AS A TOOL FOR
DETECTING MAN-INDUCED PERTURBATION ON GW/SW
ECOTONE**

Fiasca Barbara, Di Lorenzo Tiziana, De Laurentiis Paola & Galassi Diana M. P.

Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coopito, 67100 L'Aquila (Italy)

The comparative analysis of hyporheic copepod assemblages was carried out in order to focus on the effects of surface anthropogenic activities on groundwater/surface water ecotone. The streams under study are tributaries of the River Adige and are fed by the Lessinian regional aquifer (Verona, northern Italy). The hydrology is strongly influenced by meteoric events and the valleys under study show quite marked differences in land use and resources exploitation. The sampling sites were selected and sampled once according to the PASCALIS standardized sampling procedure. Each sampling site consisted of four biological and four chemico-physical replicates. Specific conductivity, temperature, dissolved oxygen, pH, calcium, nitrates and phosphates were measured. Moreover, the sediment volume per each sample was assessed and the granulometric composition of the substrate was estimated. Most of the species showing high ecological plasticity co-occurred in the hyporheic zone of all streams. The remaining ones were clearly partitioned. Descriptive analyses and multivariate statistical methodologies were used in order to compare chemistry, physical parameters and geomorphological conditions of hyporheic zones, to check the occurrence of differences in composition and structure of the taxocoenoses and possibly assess correlations between environmental features and copepod assemblages. Temperature, pH and concentration of nitrates, phosphates and dissolved oxygen seemed to play an important role in structuring the hyporheic copepod assemblages; hydraulic conditions and substratum properties, on the contrary, could be helpful to describe site-specific attributes, just related to a chemico-physical gradient along each altitudinal stream profile.

ALLOZYME AND HAPLOTYPE DIVERSITY IN *PILBARUS MILLSI*, A WIDESPREAD GROUNDWATER SPECIES OF AMPHIPOD FROM THE PILBARA, WESTERN AUSTRALIA

Finston Terrie¹, Johnson Mike¹, Eberhard Stefan² & Halse Stuart²

1: School of Animal Biology M092, The University of Western Australia, 35 Stirling Hwy., Crawley, (Western Australia 6009)

2: Department of Conservation and Land Management, P.O. Box 51, Wanneroo (Western Australia 6946)

Twenty-six species of stygobitic amphipods have been described from the Pilbara, Western Australia, based on variation in morphological characters. Many are known from single bores. The Pilbara is rich in iron ore, and thus, an understanding of species diversity and distributions is necessary both to manage resources and to conserve fauna. Previous allozyme analyses of amphipods from six catchments in the Pilbara suggested that with few exceptions, each catchment contained a unique suite of species. One species, however, *Pilbarus millsii*, appeared to be widespread, occurring in multiple catchments and drainage basins throughout the Pilbara. The present study examined specimens of *P. millsii* from ten catchments in the Pilbara, using 15 allozyme loci, and a partial sequence of the mitochondrial COI gene. Allozymic divergence (Nei's D) among populations ranged from 0.05 to 0.56, and was associated with catchment structure. Haplotype diversity at COI was low within catchments, but haplotypes were not shared among catchments. Using an estimate of the evolutionary rate of the COI gene of 2% divergence per million years, haplotypes from different catchments diverged between 4 and 12 mya. This pattern of diversity suggests that current catchment boundaries, which represent ancient features of the landscape, have produced allopatric differentiation among populations. *Pilbarus millsii* may comprise multiple morphologically cryptic species, or may be an example of morphological stasis in a genetically diverse, widespread species.

**POPULATION STRUCTURE OF STYGOBITIC ISOPODS IN THE
PILBARA, WESTERN AUSTRALIA**

Francis Cara J., Johnson Mike S. & Finston Terry

School of Animal Biology, The University of Western Australia, Stirling Hwy, Perth, 6014 (Australia)

The arid northern region of Western Australia contains one of the world's most diverse and unique assemblages of stygofauna. In the Pilbara, the occurrence of locally restricted fauna has become a major environmental issue, as it coincides with extensive mining industries. Conservation of this endemic and relictual fauna is hampered by limited knowledge of species diversity and distributions. An understanding of the connectivity of populations and mechanisms generating genetic diversity is urgently needed for the management of stygofauna in this region. The application of molecular markers may be the only way to reveal the true level of biodiversity in stygobitic fauna. Therefore, I am using a molecular phylogenetic approach to establish conservation priorities in the Pilbara region, focussing on isopod, copepod and ostracod species. As the first stage of this study, the population structure of stygobitic isopods within and between three catchments was examined using mtDNA cytochrome oxidase I (COI) sequences. Genetic variation was assessed within the context of local hydrogeological structure.

**MAPPING THE STYGOFAUNA OF THE STATE OF BADEN-
WÜRTTEMBERG, SOUTHWEST GERMANY**

Hahn Hans Jürgen & Fuchs Andreas

University of Koblenz-Landau, Campus Landau, Dept. of Biology Im Fort 7 76829 Landau (Germany) hjhahn@uni-landau.de

Between November 2001 and December 2002, 306 monitoring bores were sampled twice for stygofauna and hydro-chemistry with a phreatic net and a bailer, respectively. The bores were representative of the hydrogeological units and the natural geographic regions (naturraums) of the state of Baden-Württemberg, southwest Germany. All taxa were determined on species level, except turbellarians and nematodes. The aim of the study was to describe regional models of the stygofauna of Baden-Württemberg, and to make a contribution to our knowledge of subterranean biodiversity.

In total, 105 species were detected. The fauna were found to reflect the natural geographical and hydrogeological conditions of the state territory. In particular, syncarids and isopods displayed a restricted regional distribution, while most cyclopoids occurred all over Baden-Württemberg.

EMERGING KNOWLEDGE OF DIVERSITY, DISTRIBUTION AND ORIGINS OF SOME AUSTRALIAN STYGOFAUNA

Humphreys W. F.¹, Watts C. H. S.² & Bradbury J. H.².

1: Western Australian Museum, Francis Street, Perth (Western Australia 6000)

2: South Australian Museum, North Terrace (South Australia 5001)

The last decade has transformed understanding of the diversity, distribution and affinities of Australian stygofauna, especially in the more arid parts. Western Australia, in particular, is emerging to have an exceptional diversity of often unexpected lineages. These studies are still in their early stages, in terms of both geographic coverage, and taxonomic and systematic knowledge.

Australian amphipods are diverse and predominantly stygal, containing representatives of ancient freshwater lineages and those of relatively recent marine origin. The distributions match that of land areas supposedly not submerged and submerged, respectively, in the ocean since the Palaeozoic, as also is the the distribution of other freshwater lineages (Phreatoicoidea and Tainisopidea).

The chains of salt lakes (playas) in arid Australia are the base level of groundwater flow along the course of palaeodrainages constrained by Permian palaeovalleys. Groundwater calcretes form near salt lakes and contain diverse stygal communities (Dytiscidae, Amphipoda, Oniscidea, Gastropoda, Ostracoda, Copepoda, Bathynellacea) endemic to a given deposit. The fauna is best known from the Oniscidea (Scyphacidae: *Haloniscus*) and Dytiscidae (~85 species of Copelatinae, Hydroporinae: Bedessini, Hydroporini) for which both morphological and molecular data are available and their phylogenies do not map the palaeodrainage channels. Conversely, ancient lineages, such as crangonyctoids, also represented by discrete populations in the calcretes, do tend to map the palaeodrainages.

There is increasing evidence from arid Australia that elements of both the terrestrial and aquatic subterranean fauna are refugial in origin (Schizomida, stygal Oniscidea, Dytiscidae).

The Western Shield contains a number of ancient and Tertiary stygal lineages but the northern region (Pilbara) has a stygofauna distinct from the southern part (Yilgarn) despite having supposedly been a single land mass since at least the Palaeozoic. The distinction remains enigmatic.

**ECTINOSOMATIDAE (COPEPODA, HARPACTICOIDA) IN
GROUNDWATER : RADIATION OR MULTIPLE INVASIONS ?**

Huys Rony¹, Galassi Diana M. P.² & De Laurentiis Paola²

1: The Natural History Museum, Cromwell Road, London SW7 5BD (U.K.)

2: Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila (Italy)

The family Ectinosomatidae currently contains 20 genera and shows a high radiation in the marine environment. Only secondarily members of the family, belonging to the genera *Ectinosoma*, *Halectinosoma*, *Arenosetella* and *Pseudectinosoma*, entered genuine freshwater habitats, and to a lesser extent, groundwater. The recent discovery in groundwater in India added the new genus *Rangabradya* to the family. A sampling campaign in the porous aquifers of the Lessinian Mountains revealed the presence of an additional member of the family, the taxonomic position of which required a wider morphological analysis of phylogenetically informative morphological characters in order to evaluate the monophyly of some questionable genera. Based on this examination, the Lessinian species is assigned to a new genus of the family, raising the question whether the colonization of groundwater by ectinosomatids occurred only once in the evolutionary history of the family or alternatively by multiple invasion events at different times and via different routes. A phylogenetic background is presented in order to evaluate the most plausible colonization patterns in groundwater of different members of the family. At least three different groundwater lineages with a different colonization history can be recognized : the *Arenosetella*-lineage via the marine psammic environment, the *Bradya-Ectinosoma-Halectinosoma-Rangabradya*-lineage, which probably passed the limnicoid surface habitat, before entering the groundwater habitat, and the *Sigmatidium-Noodtiella-Lineosoma-Pseudectinosoma*-lineage, which colonized fresh groundwater from the epibenthic/epiphytic brackish environment via crevicular karstic habitats.

GROUNDWATER FLOW AND AQUATIC HABITATS

Jones William K.

Karst Waters Institute, P.O. Box 537, Charles Town, WV 25414 (USA)

Description of groundwater habitats are typically based on physical and ecological characteristics of the sample location where certain animals are collected. The most important ecological variables controlled by the nature of the subsurface pore spaces are limits to animal size, the residence time of the water, and the flux of nutrients. Most aquifers are a mixture of different size pore spaces and water chemistry and therefore contain different potential habitats so sampling results will be influenced by the scale of the sampling or capture method employed.

For consolidated rock aquifers, most of the total volume of water in storage is in the less permeable but high porosity intergranular pore space, but most of the flow is through fractures or conduits. These higher permeability flow routes also represent preferential routes for the flux of nutrients or energy through the aquifer. Wells or bore holes will generally have a restricted capture zone and may not intercept the more permeable but highly localized flow paths that actually control much of the ground water flow. The results from sampling the interstitial habitats at wells should give variable capture counts, but the population assemblages will be somewhat limited, at least with respect to physical size. At the other extreme of sampling scale, collecting at a large karst spring may represent a “lumped parameter” model ranging from laminar flow through the epikarst zone to open channel flow through caves and covering a potential capture area of tens of square kilometers. In general, collecting at areas of concentrated ground-water flow will yield higher animal counts and greater species diversity. Flow is concentrated at zones of higher permeability and along stratigraphic contacts with less permeable units. Soil moisture is stored and held under tension if below saturation. Sampling in the unsaturated zone is often controlled by precipitation events and may result is a sort of “piston” flow where old water is displaced by new event water.

**RIVERSCAPE DYNAMICS AND THE DISTRIBUTION OF THE HYPORHEOS IN A
GLACIAL RIVER-FLOODPLAIN SYSTEM**

Malard Florian¹ & Lafont Michel²

1 : CNRS - Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France)

2 : UR Hydrobiologie, Cemagref, 3 bis Quai Chauveau, C.P. 220, 69336 Lyon (France)

River-floodplain systems are dynamic riverscapes, the extent, composition, and configuration of which vary at several temporal scales. The biodiversity of a habitat not only depends on its environmental attributes but also on its age, shape, and position within the riverscape that controls the exchange of organisms with neighboring habitat patches. We developed a functional habitat classification that integrated the suitability, life span, and position of water bodies within the riverscape for testing hypotheses on biodiversity patterns in a pulsing glacial river-floodplain system. We expected that the pattern of biodiversity in the hyporheic zone of the floodplain would reflect the intersection between dissimilar life cycles and dispersal capacities among insects and permanent aquatic taxa and the suitability, life span, and position of habitats. Flooding resulted in a highly fragmented mosaic of habitats, so that newly created suitable habitats (i.e. clear water channels) were separated from source areas of colonizers by inhospitable habitats (i.e. turbid water channels). Because of their high dispersal capacities, insects colonized equally the hyporheic zone of suitable habitats that differed, however, in their life span and position within the riverscape. As turbid water expanded throughout the floodplain, insects became increasingly more abundant in newly created suitable habitat patches. Permanent aquatic taxa preferentially colonized hyporheic sediments of permanent suitable habitats but they failed to develop abundant populations in newly created suitable habitats, some of which had continuous flow for 8 months. The dispersal of permanent aquatic taxa within the riverscape was apparently constrained by the spatial arrangement of suitable and inhospitable habitats and the weakness of hydrological connections between hyporheic sediments and underlying ground water.

PHYLOGEOGRAPHY AND TAXONOMIC STATUS OF *NIPHARGUS VIREI* (SUBTERRANEAN AMPHIPOD)

Lefébure Tristan¹, Douady Christophe¹, Gouy Manolo², Trontelj Peter³, Briolay Jérôme⁴ & Gibert Janine¹

1: Laboratoire d'Ecologie des Hydrosystèmes fluviaux, UMR-CNRS 5023. Université Claude Bernard - Lyon I. Campus de la Doua. 6 rue R. Dubois., 69622 Villeurbanne Cedex (France)

2: Laboratoire de Biométrie et Biologie Evolutive, UMR CNRS 5558. Université Claude Bernard - Lyon 1. 43, bd. du 11 Novembre 1918. 69622 Villeurbanne Cedex (France)

3: Department of biology, Biotechnical faculty, Univ. of Ljubljana. P.O. Box 2995, S1001 Ljubljana (Slovenia)

4 : Développement des Techniques d'Analyse Moléculaire de la Biodiversité (DTAMB), Université Claude Bernard- Lyon 1. 69622 Villeurbanne cedex (France)

Extreme conditions are suspected to be responsible for morphological convergence, and so to bias the assessment of biodiversity in groundwater. This hypothesis was tested within the well-defined subterranean amphipod *Niphargus virei*. All our Bayesian and Maximum Likelihood analyses, based on two independent genes (28S and COI), revealed the same tripartite structure. *N.virei* populations from Benelux, Eastern and Western France appeared as independent evolutionary units. Molecular rates estimated via global or Bayesian relaxed clock suggest that this split is at least 16 MY old and accredit the cryptic diversity hypothesis. Surprisingly, the geographical distribution of these lineages pointed to evidence of recent dispersion through apparent vicariant barriers. Comparison of the molecular divergences between those lineages and between several thousand of different species of Crustacean give evidence that the three lineages of *N. virei* should be considered as different species. In consequence, we argue that future analyses of subterranean biodiversity, or more generally of biodiversity in extreme environments, should consider morphology as a potentially biased marker and DNA of promising help.

BIODIVERSITY OF BELGIAN GROUNDWATERS : THE MEUSE BASIN

Martin P., De Broyer C., Fiers F., Michel G., Sablon R. & K. Wouters.

Royal Belgian Institute of Natural Sciences, 29 rue Vautier, 1000 Bruxelles (Belgium)

The Meuse Basin in Walloon (Belgium) was intensively sampled during a field campaign in 2002-2003, in the framework of the PASCALIS project, according to a stratified sampling scheme and a standardized protocol. A total of 202 sampling sites were selected in four hydrogeographic basins belonging to the Meuse Basin, equally shared out among the saturated and unsaturated zones of the fractured medium (karst) and among the hyporheic and the phreatic zones of the porous medium. Selected physico-chemical parameters were measured in parallel. More than 173 species were found inhabiting this environment, representative of the Amphipoda, Cladocera, Copepoda, Hydrachnidia, Isopoda, Nematoda, Oligochaeta, Ostracoda and Tardigrada. Thirty-four stygobite species were identified. Among them, 25 and 26 were present in the karst and porous strata, respectively, and 8 to 9 were exclusive to each of the two strata. Species accumulation curves suggest that the total number of sampled stations is not satisfactory as regards the best estimate of species richness; more samples have to be taken into account in future studies. The bulk of stygobite taxa can be considered as ubiquitous species which seem to have wide ranges of tolerance for most environmental factors. These ecological dispositions probably facilitated recolonization of the Walloon karst, following the eradication of its fauna during the Quaternary (Ice Age). Most taxa were typically more frequent in highly permeable formations, with hard waters, characterized by moderate to low hydrological connectivity. No statistically significant differences were observable in species richness whatever the stratification scheme considered. A few species are exclusive to one of the considered zone, in accordance with their known biology. Most statistical analyses are not significant, however, as a possible result of exceedingly low number of occurrences for stygobite species. The possibility that a stratification pattern could emerge as a result of an increased sampling effort cannot be ruled out.

DISTRIBUTION OF GROUNDWATER INVERTEBRATES ALONG AN ENVIRONMENTAL GRADIENT IN A SHALLOW WATER-TABLE AQUIFER

Paran Frédéric¹, Malard Florian², Mathieu Jacques², Lafont Michel³, Galassi Diana⁴, & Marmonier Pierre⁵

1: Ecole Nationale Supérieure des Mines de Saint-Etienne, Centre SITE, 158, cours Fauriel
42023 Saint-Etienne Cedex2 (France) **Erreur! Signet non défini.**

2: Université Claude Bernard Lyon 1, UMR CNRS 5023, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11
Novembre 1918, 69622 Villeurbanne cedex (France)

3: UR Hydrobiologie, Cemagref, 3bis Quai Chauveau, C.P. 220, 69336 Lyon (France)

4: Dipartimento di Scienze Ambientali, Università di L'Aquila (Italy)

5: Université de Rennes 1, UMR CNRS 6553 Ecobio, Campus de Beaulieu, Bât. 14A, 263 Av. Général Leclerc, CS 74205,
35042 Rennes (France)

Ecologists have recently begun to explore the relationships between the composition of invertebrate assemblages and environmental gradients in groundwater of unconsolidated sediments. Hydrogeological attributes such as permeability expectedly drive biodiversity patterns in groundwater because they not only exert a direct influence on organisms but also control fluxes of nutrients and organic matter. We used the OMI analysis (outlying mean index) to examine the responses of multiple species to hydrogeological and physico-chemical variables in a shallow water-table aquifer along the Loire River, France, the water chemistry of which has been severely affected by gravel extraction and refilling with demolition materials. Hydrogeological attributes were obtained from a finite-difference groundwater flow model whereas physical variables and biological data (i.e. presence – absence of species) were collected during a single survey of 29 wells. Groundwater had elevated concentrations of dissolved organic carbon (DOC) and contained a high number of species, among which a majority was epigeal. Spatial variation in physico-chemistry of groundwater induced by the dissolution of buried demolition materials had no detectable influence on the distribution pattern of fauna. Rather, species were significantly distributed along an environmental gradient defined primarily by permeability and vadose zone thickness. These variables had in turn a detectable influence on groundwater temperature and concentrations of DOC and total dissolved solids. Our results emphasize the importance of integrating hydrogeological measurements within the framework of ecological studies for understanding distribution patterns of invertebrate assemblages in groundwater.

EPIKARST FAUNA FROM NORTH AMERICAN CAVES

Pipan Tanja¹ & Culver David C.²

1: Karst Research Institute ZRC SAZU, Titov trg 2, SI-6230 Postojna (Slovenia)

2: American University, Department of Biology, 4400 Massachusetts Ave. NW, Washington DC 20016 (USA)

Drip pools and trickles of percolation water in 17 caves in West Virginia were sampled for epikarstic species, using aspirators and fine-mesh nets designed to capture copepods and other minute invertebrates. A total of 20 genera of copepods were found. 11 different genera of harpacticoids and 9 of cyclopoids were recognized in the samples collected from pools. In addition to copepods there were also specimens of Nematoda, Gastropoda, *Troglochaetus* among Archiannelida, Oligochaeta, Acarina, Ostracoda, Bathynellacea, Isopoda, Amphipoda, Millipedia, Collembola, Coleoptera and insects larvae. Results of the correlation analyses indicate that there was correlation between copepod abundance and number of species on the one hand and the quantity of pumped water on the other hand.

For understanding of the structure and physico-chemistry of epikarst, the focus was specially on trickles rather than pools. We choose three sampling sites of the Organ cave system: the Lipps, Sively 2 and Sively 3 streams. In Lipps streams we had collection of 5 drips within 4 m of each other. In passages Sively 2 and Sively 3 we found 4 trickles at distances of up to 200 m. Sampling and water chemistry measurements were undertaken at the sites at approximately 10-day intervals. All together in drips we found 13 genera of copepods, from which 8 of harpacticoids and 5 of cyclopoids. Monitoring of trickles tens or hundreds of meters apart showed that there is statistically significant negative correlation between the distance between the drips and the similarity of the fauna. Due to horizontal movement of water in epikarst it is hard to say if each drip represents an isolated habitat or nearby drips come from the same habitat. The positive correlation of abundance and taxonomic richness in drips is consistent with faunistic exchanges between microhabitats in epikarst. The epikarst fauna will add significantly to the overall species richness found in Appalachian caves, and likely elsewhere in the U.S. as well.

**THE RELATIONSHIP BETWEEN OSTRACOD SPECIES DIVERSITY
AND WATER CHEMISTRY IN THE SUBSURFACE OF THE PILBARA
REGION, WESTERN AUSTRALIA**

Reeves Jessica

Department of Earth and Marine Sciences, Australian National University (Australia)

The Pilbara region, northwestern Australia provides an eclectic array of environments for the study of subterranean fauna. Although climatically regarded as semi-arid with annual evaporation outweighing precipitation by around 10:1, the Pilbara is located at the tropical fringe and receives rainfall from cyclonic events during the summer months. At such times, the strength of the water inundates roads throughout the floodplains and commonly causes damage to bridges.

The region may be divided broadly into the ranges, floodplain and coastal zones. The ranges date to the Early Proterozoic – Archaean and include the metasedimentary Hamersley Ranges in the central Pilbara, and the predominantly volcanic Chichester Ranges to the north. These units overlie the Archaean greenstone and granite, which outcrop to the northeast of the region. The Fortescue and Ashburton Rivers form floodplains either side of the Hamersley's, and the Robe, Yule and De Grey Rivers extend as broad deltas toward the coast. The significant aquifers in the region include recent valley-fill alluvium and colluvium, chemically deposited calcretes and pisolites associated with former watercourses, and fractured-rock dolomite, banded-iron formations and granite. Groundwater in the region is typically fresh to brackish and bicarbonate-dominant, although Na-Cl-rich waters are common in both the coastal and arid eastern margins.

This paper presents analyses from the first two seasons of the Pilbara Stygofaunal survey (see Eberhard and Halse, this volume). The distribution of ostracods is highlighted as an example of the faunal diversity. Around 20 species of the family Candoninae have recently been identified from this region, and at least a further 20 taxa are yet to be described. The evolution of the groundwater through the aquifer is detailed in terms of major-ion and stable-isotope geochemistry of the waters, in conjunction with measured physical parameters, and related to processes of weathering, evaporation and solute evolution. The relationship between these patterns and species distribution is discussed.

THE BLIND MISLEADING THE BLIND: FALSE NEGATIVES AND ESTIMATIONS OF SUBTERRANEAN BIODIVERSITY

Schneider K.^{1,2}, Culver D. C.¹, Hobbs III H. H.³ & Fong D.¹

1: American University, 4400 Massachusetts Avenue NW, Washington DC 20016 (USA)

2: University of Maryland, 1205 Biology-Psychology Building, College Park MD 20742 (USA)

3: Wittenberg University, PO Box 720, Springfield OH 45501 (USA)

As the cave habitat is difficult (and expensive) to sample, most of what is known about cave biodiversity is based on a fraction of the world's caves, and at that, predominately large, easily accessible caves. But how representative are these caves of local or regional biodiversity? How many species are missed by only focusing on a small subset of the underground realm? How inaccurate and/or misleading is our knowledge of subterranean biodiversity and biogeography? We set out to answer these questions by thoroughly investigating every cave in a high cave density and species rich area of West Virginia, U.S.A. We sampled 65 caves within a 20km² study site. As a result of intensive sampling that included visual censuses and trapping procedures, 18 obligate stygobionts and troglobionts were collected. Only 7 caves were needed to collect 95% of the species, and by sampling only the largest 7 caves, 89% of the species could be recovered. However, the species accumulation curve did not reach an asymptote, and estimates of species richness based on frequency of rare species predicted that nearly half of the species were not collected at all. Two years later (2004), these caves were revisited and resampled. Preliminary results show that many groups (isopods, pseudoscorpions, beetles, and diplurans, for example) were encountered in more caves than in the previous study. At the same time, they were often not recollected from previously recorded caves. Beetles (*Pseudanophthalmus* sp.), for example, were only collected from half of the 13 caves recorded in the previous study; yet they were collected from 9 additional caves. Clearly, these repeated visits reveal that false negatives are in fact an issue when quantifying subterranean biodiversity. This complete dataset, based on two years of intensive sampling, contains information across many caves and entire communities, and enables a powerful investigation of biases associated with estimations of species richness.

**SOME AUXILIARY CRITERIA FOR THE SELECTION OF
GROUNDWATER SITES FOR BIODIVERSITY CONSERVATION**

Sket Boris

Oddelek za biologijo, Biotehniška fakulteta, Univerza v Ljubljani, p.p. 2995, 1001 Ljubljana (Slovenia)
boris.sket@bf.uni-lj.si

If we are selecting a groundwater site for the biodiversity conservation, the main criterion will be the richness and uniqueness (i.e. endemism or phylogenetic and taxonomic singularity) of its fauna. This may be connected either by the site's biogeographical position or by a diversity of habitats included. However, considering other aspects will on the one hand bring additional benefits to the science and/or to the local culture, and on the other hand, it will facilitate the achievement of the legal protection. If the site (e.g. a cave system, a site of gravel deposits) is the type locality of some taxa, it is scientifically more important; the same if it is at the same time a paleontological or archaeological site. If it is particularly appropriate for scientific study, for the public education, if it is a historical place of any kind, or of a particular beauty, the need for its protection will be locally and nationally easier recognisable. Postojna-Planina Cave System will be used to demonstrate these principles.

SUBTERRANEAN BIODIVERSITY IN GREAT BRITAIN AND IRELAND: A PREDOMINANTLY POST-GLACIAL COLONISING FAUNA WITH POSSIBLE TRANS-GLACIAL ELEMENTS

Wood Paul J.¹ & Proudlove Graham S.²

1: Department of Geography, Loughborough University, Loughborough, LE11 3TU (UK.) p.j.wood@lboro.ac.uk
2: Department of Zoology, Manchester Museum, Manchester, M13 9PL (UK) g.proudlove@manchester.ac.uk

Most of the land area of Great Britain and Ireland was covered by ice during several of the Pleistocene glaciations. Only small areas to the south of these islands remained ice free but they would have been subject to permafrost conditions. If there were any subterranean biota before the Pleistocene they would have been made extinct during this time. Samples collected over the past 60 years show that there are few obvious troglobites and stygobites. Terrestrial communities are dominated by Collembola, Acari and Diptera. Aquatic communities are dominated by Copepoda, Amphipoda and Isopoda. Most subterranean species appear to be morphologically similar to epigeal species and we class them as troglaphiles and stygophiles. However it is possible that some species found in caves are ecologically and evolutionarily troglobitic and stygobitic, with no genetic exchange between epigeal and hypogeal populations. In general the fauna appears to be colonising subterranean habitats in these areas. There is a strong possibility that the stygobitic Amphipoda (*Niphargus* species and *Crangonyx subterraneus*) and Isopoda (*Proasellus cavaticus*), together with a syncarid crustacea *Antrobathynella stammeri*, survived the glacial periods in sub-glacial refugia such as caves and deep groundwater. The syncarid is found in central Scotland many hundreds of km north of the last (Devensian) glacial maximum. The distribution abilities of these animals is very poor and a sub-glacial refugium model is the best explanation for current distribution. We will provide distributional and genetic data for the current distribution of subterranean animals in Great Britain and Ireland.

Posters

**DIAPAUSING STAGES OF CYCLOPOID COPEPODS IN
SABNLINSKIJE CAVES (LADOGA LAKE AREA) AND IN WELLS
AND SPRINGS OF MONGOLIA**

Alekseev Victor

Zoological Institute of RAS, University emb. 1, 199034, St. Petersburg (Russia)

Life cycle of most invertebrates in open waters consists of combination of periods of growing and breeding with arrest of these basic functions due to diapause to avoid harsh conditions in environment. The adaptive function of diapause in crustaceans and other organisms involves in two directions : biorhythmical adaptations that determine the synchronization of an animal's life cycle with the rhythm of external conditions, and defensive adaptations that permit the organism to survive in oppressive or even lethal for active phases in unfavourable periods.

In subterranean environments, external conditions are much more stable than in open waters but some seasonal fluctuations in water and organic matter ability can happen too. Life cycle of crustaceans living in subterraneous environment is practically unknown because of real difficulties with regular sampling and tracing of their population dynamics.

A little is known on the mode of reproduction of stygobiont and interstitial crustaceans. Some Decapods living in caves breed and molt without diapause with regularity about 3 months as production of a hormone inhibiting these processes depends on light periodicity (Carlisl 1957). The most common development stage of tiny interstitial Copepod *Microcyclops afganicus* (Lindberg) found in deep wells of The Goby Dessert was the copepodid 4 well known as diapausing stage in many other cyclopids (Alekseev 1996).

In zooplankton samples collected by Dr. Peter Krylov 31 January 1991 in Sablinskije Caves (Leningrad district, 40 km to East from St. Petersburg, Russia) I found cyclopids of several species (*Megacyclops viridis*, *Acanthocyclops vernalis*, *Diacyclops bicuspidatus*) with characters usually found in diapausing stages. All these species had red colour drops of oil fused inside segments; their bodies were bent with abdomen covered to dorsal side of thoracosome; dominative stages was copepodid four. In open water environment, combination of these characters definitely indicated diapause state (Elgmork, 1996). In samples collected in the caves in summer time of this year, no cyclopids with such characters were found.

In surface waterbodies, these species spend winter time in diapausing state but for diapause induction cyclopids follow photoperiod and temperature seasonal changes that are not present in subterraneous environment. The Sablinskije caves have artificial origin and do not exist for more than 200 years, so all cyclopid species we found in their lakes, should have recent connections with surface populations. We can conclude that these cyclopid species, at least in the very beginning of their underground isolation seem like following internal seasonal biorhythm in diapause induction.

THE MEIOFAUNA FROM VOLCANIC LAKES OF LATIUM WITH PARTICULAR REFERENCE TO MICROCRUSTACEANS

Berera R., Bruno M.C., Pariciani L. & Cottarelli V.

Università degli Studi della Tuscia Dipartimento di Scienze Ambientali, Largo dell'Università s.n.c Blocco D, 01100 Viterbo (Italy)

The present work reports on faunistic and ecological data concerning the meiofauna investigated for a thirty-year period with particular reference to Copepods Harpacticoids. This long research allows, in some cases, to follow the community's evolution over time. Our investigation is focused on microcrustaceans communities of the lakes of Vico, Bracciano and Bolsena.

Regarding Vico Lake, we noticed a drastic impoverishment of the biocenosis, probably due to anthropic impacts; Bracciano Lake didn't change its taxocenosis, but in some sampling sites a numeric rarefaction of the populations was detected. In Bolsena Lake, the community appeared quite constant and characterised by a suitable diversity.

In the last five years and in sporadic way, the lakes of Martignano and Mezzano were studied. All the investigated lakes provided interesting data from a faunistic and taxonomic point of view and for the relative biogeographical considerations. We'll discuss briefly the endemism and rare species underlying the recent finding of a *Schizopera* sp, probably new species, collected in the psammon of the lakes of Bolsena and Mezzano. This taxon, unknown in the Italian lakes until now, is a talassoid one and its origin may be related to the phenomena of marine transgressions.

Finally, the finding of microcrustaceans belonging to Cyclopoida, Amphipoda and Syncarida will be reported and briefly discussed.

DISTRIBUTION ATLAS OF THE STYGOBIONT MOLLUSCS OF FRANCE

Bertrand Alain

Etude et Conservation des Mollusques Continentaux, Abéla 09320 Bousсенac (France)
abela@club-internet.fr

France has about 75 stygobiont molluscs which represent \pm 10% of the continental Mollusca fauna.

Depending on the authors these species belong to 1 family (Hydrobiidae) or 3 families (Moitessieriidae, Hydrobiidae and Amnicolidae) and the systematics is still particularly confused for some groups. This lack of knowledge is due to :

- the lack of well-marked anatomical features,
- the great intraspecific variety (or: the great variety of forms within the same species),
- the lack of understanding the phylogenetic meaning of the anatomical features,
- the high degree of similarity in the anatomical features (Wilke et al 2001).

The number of recorded taxa may not reflect reality as :

- the number of newly described species has been considerable in the last few years and the rate of discovery is sustained,
- several genera which today have a lot of taxa (e.g. *Bythiospeum*, *Islamia*), need apparently to be re-evaluated with modern tools and methods,
- more than 30% of the species considered as belonging to the actual fauna have never been collected or found alive.

Despite all these difficulties, all the recognized taxa in France have small distribution areas and all are endemic to the national territory or to biogeographical areas shared by 2 countries (Jura, eastern Pyrenees). This means that the stakes are very high and that we have a great responsibility in the conservation of the species and their habitats.

The main goals of this atlas are :

- to underline the patrimonial importance first of the stygobiont molluscs in the French fauna and then of the resulting stakes,
- to make the scientific world and the administrators aware that it is necessary first to study these molluscs and then to take them into account in the implementation of conservation policies,
- to provide the present state of our knowledge in bibliographical references and map distribution.

A NEW SUBTERRANEAN CATFISH, GENUS *RHAMDIA*, FROM BRAZIL (SILURIFORMES: HEPTAPTERIDAE) WITH NOTES ON ECOLOGY

Bichuette Maria Elina & Trajano Eleonora

Departamento de Zoologia, Instituto de Biociências da USP. C.P. 11461, 05422-970. São Paulo, SP. (Brasil)
bichuette@uol.com.br

A new species of *Rhamdia* was recorded from subterranean waters in Southwestern Bahia, Northeastern Brazil. This species represents the first record of the genus for the subterranean realm in Brazil and one of the largest subterranean fishes recorded until now (225.0 mm SL). From about 20 species of cave fishes registered in Brazil, five are heptapterids. Troglomorphic characters are present in this species, such as reduction of eyes and melanic pigmentation, showing high variability. The population of *Rhamdia* from Enfurnado Cave comprises thousands of individuals distributed along 3,000 km of the subterranean stream. We observed 12 individuals.m⁻² in a stream reach. High amount of organic matter is present in the cave, which shows that the food is not scarce and dissolved oxygen is really low (1.43 mg.l⁻¹). Adult individuals are distributed in the bottom, midwater and most in the surface. Juveniles were observed hiding under boulders. Type-locality of *Rhamdia* is not legally protected and the region is under treat of deforestation. This can lead this population to extinction, once the food came entirely from the surface (floods between October and March). Protection actions are urgent and for this we are describing this species. Besides, we are going to start a population study of two years, using mark-recapture methods, in the next year.

HABITAT DETERMINATION AND SUBTERRANEAN SINGULARITY

Camacho Ana I., Valdecasas Antonio G. & Rodriguez Jaime

Museo Nacional de Ciencias Naturales C/ Jose Gutierrez Abascal 2 - 28006-Madrid (Spain)

Historically, the aquatic subterranean environment has been clearly associated with caves. The main diagnostic characteristic being the absence of light. More recently, other habitats like the interstitial, anquialine and profound benthos of sea have been added.

The biological counterpart to a physical definition of the subterranean environment is to look for shared characteristics of organisms living in this realm and not presented anywhere else. These characteristics could be used to define the stygobiont (strictly subterranean) organism.

Both sides, the physical and the biological, have their bias and controversies.

In this work we look at another side of the “subterranean singularity”: the sampling gear reliance.

We studied the chemistry and biology of subterranean samples taken from the interstitial environment, wells, caves and springs with different methods: simple hand-nets, Karaman-Chappuis digging method and Bou-Rouch pipe.

Data analysis included multidimensional scaling ordination, and permutation test of similarity among samples.

It can be concluded that some subterranean habitats are more “subterranean” than others, and that a “subterraneanbility” gradient could be established among the sampling gear/habitat sampled combinations.

**IMPROVING THE SAMPLING STRATEGY TO ASSESS THE
GROUNDWATER BIODIVERSITY IN THE MERIDIONAL JURA
(France)**

Castellarini Fabiana, Dole-Olivier Marie-José, Malard Florian, Olivier Jean Michel & Gibert Janine

Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France)

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 was to refine the sampling strategy to assess the stygobiotic species richness in the meridional Jura. Random and stratified strategies were analyzed on a total of 247 sites. Decentred Correspondance Analysis (DCOA), Principal Component Analysis (PCA) and correlation analysis showed significant relation between the species richness pattern and geology, elevation and glacial events. The 52 % of biodiversity are partitioned within-clusters resulted from the stratified protocol and the 48 % belong to the between-cluster component. Sampling protocol following the stratification strategy and taking into account 3 clusters with 111 sites might estimate 92 % of the biodiversity observed in the meridional Jura.

EFFECTS OF BASE FLOW REDUCTION ON AQUATIC ECOSYSTEMS

Cecchi Giuliano, Formichetti Paolo & Mancini Laura

Laboratorio di Igiene Ambientale, Istituto Superiore di Sanità, Roma Viale Regina Elena, 299 00161 Roma (Italy)

Italian National Institute of Health (ISS) strongly supports the integrated study of the surface water ecosystems. Following this principle, one major subject of investigation is the interaction between surface water ecosystems and all surrounding systems, both physical and ecological ones. In order to apply this strategy, ISS and other institutions developed the “Fluvial Functioning Index” (FFI) which accounts for vegetation conditions of the banks and the territory, physical and morphological structure of the banks, structure of the wet river bed, biological habitat composition and biological characteristics of the periphyton, macrophytes and macrobenthos. In many studies carried out by ISS, the impact of groundwater status on surface ecosystems turned out to be a major issue of concern. In the Almone River, in the “Appia Antica” Regional Park, the lowering of the water table and the infiltration of poor quality water from surface (mostly collected waters) determined poor groundwater quality (assessed by microbiological indicators). In the “Doganella wetland”, located in the “Castelli Romani” Regional Park, heavy water abstraction for drinking water supply determined the progressive drying of the area which caused a negative ecosystem impact measured in terms of biodiversity reduction (ex. absence of Simuliidae Diptera Nematocera species). Last, in the “Topino” River, monitored by the Environmental Regional Authority of Umbria (ARPA), the pressure on surface aquatic ecosystem took the form of hydromorphological alterations such as riverbed straightening, artificialization and riparian vegetation removal, these alterations are likely to affect surface and sub-surface waters connection; in this site, a heavy impact was measured in terms of biological quality (low values for EBI) and ecological functionality (low values for FFI) despite good physiochemical quality and unaltered flow regime.

INTERSTITIAL FAUNA DYNAMIC IN AN ISLAND-BRAIDED FLOOD PLAIN (TAGLIAMENTO RIVER, ITALY)

Claret Cécile¹ & Tockner Klement²

1: Institut Méditerranéen d'Ecologie et de Paléoécologie (UMR CNRS 6116), Université Paul-Cézanne Aix-Marseille 3, Case 31, 13397 Marseille cedex 20 (France)

2: Department of Limnology, EAWAG/ETH, Überlandstrasse 133, 8600 Dübendorf (Switzerland)

Surface-subsurface exchanges, hydrological flow paths, and sediment characteristics (size and distribution) lead to a mosaic of habitat conditions in the interstitial compartment that influence density, diversity, and distribution of faunal assemblages. In riffle-pool sequences or gravel bars, floods that modify the extent and configuration of surface-subsurface exchange patches, resulted in variations in the composition and distribution of invertebrate within sediments at fine scale. However, at large scale interstitial fauna dynamics in response to flow and flood pulse are less documented.

The Tagliamento River (Italy) experienced rapid and frequent changes of water level. In this highly dynamic river, the combination of small scale vertical exchanges, and large scale disturbances should result in complex dynamics of interstitial animals at the floodplain scale. Monthly sampling of faunal assemblages beneath lentic and lotic water bodies distributed across an island-braided flood plain was used to investigate, at large scale, the response of invertebrate assemblages to changes in water level.

**DIVERSITY AND ORIGIN OF THE INTERSTITIAL ISOPOD
MICROCHARON (CRUSTACEA, MICROPARASELLIDAE) FROM THE
ROUSSILLON AND WESTERN LANGUEDOC REGIONS (FRANCE)**

**Coineau N.¹, Artheau M.², Bedos A.³, Boulanouar M.⁴, Boutin C.², Brehier F.⁵,
Deharveng L.³ & Giani N.⁶**

1. Université P et M Curie-Paris 6, Observatoire Océanologique de Banyuls, Laboratoire Arago, 66650 Banyuls/Mer (France)
2. Université de Toulouse, LaDyBio-UMR CNRS 5172, Bât 4R3, 118 route de Narbonne, 31062 Toulouse cedex 04, (France)
3. Muséum National d'Histoire Naturelle, ESA CNRS 8043, Laboratoire d'entomologie, 45 rue Buffon, 75005 Paris (France)
4. Université de Marrakech, Faculté des Sciences Semlalia, UFR Sciences de l'eau, Laboratoire d'Hydrobiologie, BP 2390, Marrakech (Morocco)
5. Alas, 09800 Balaguères, France
6. Université de Toulouse, EDH-Ecologie des Hydrosystèmes, Bat 4R3, 118 route de Narbonne, 31062 Toulouse Cedex 04 (France)

The interstitial genus *Microcharon* (Isopoda Microparasellidae) is highly diversified within the Mediterranean basin. In the framework of the PASCALIS project, several species new for science were discovered, whereas the distribution pattern of other species was extended. In the Roussillon and western Languedoc regions, endemic species inhabit the four study basins of the Tech, the Têt, the Agly-Verdouble and the Aude rivers. They belong to the western Mediterranean phylogenetic group of species. These stygobites are derived from marine ancestors which formerly lived in the littoral interstitial of the Tethys. The two-step model of colonization and evolution, the second phase of which explains both the establishment in fresh groundwater and vicariance during Tethys regressions, provides an understanding of their evolutionary history. The species from the Aude basin evolved from an ancestor which might have settled in fresh groundwater at the end of the Middle Ilerdian regression of the Pyreneo-Provençal gulf (=lower Eocene, 48 MY BP). In the Tech and Têt basins, the ancestors of the two-three extant representatives of the genus might have been left by the regression of the Pliocene marine embayment which covered the southern Roussillon. Other species might result from a much more older Tethys regression in the late Senonian (end of the Cretaceous) together with further vicariance evolution due to the Pyrenees orogenesis and passive drift downstream. The palaeobiogeographic history in the Verdouble basin within the Corbières is much more complicated since a large allochthonous part of this mountain slipped northwards from the Pyrenees. Nevertheless, a late Senonian gulf flooded the region. Finally, regressions of more or less old marine embayments may have played a major role in the evolution through vicariance in the genus *Microcharon*.

ENVIRONMENTAL GRADIENTS IN GROUND WATERS: MAIN FACTORS DRIVING THE COMPOSITION OF STYGOBIOTIC ASSEMBLAGES AT A REGIONAL SCALE

Dole Olivier Marie-José, Malard Florian & Gibert Janine

Université Claude Bernard Lyon 1, CNRS - UMR 5023 Ecologie des Hydrosystèmes Fluviaux, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne (France)

The relationships between the structure of stygobiotic assemblages and environmental / palaeogeographic attributes of ground waters were explored by using biological (presence – absence of 62 species) and environmental data (16 variables) collected in the meridional Jura (269 sites distributed within 4 hydrogeographic basins). An OMI (Outlying Mean Index) analysis was performed to identify the main factors driving community structure and to evidenciate the ecological preferences of taxa along environmental gradients. The average marginality of all taxa was highly significant indicating the strong influence of pore size and historical determinants (e.g., distance to the Würm glacier). Almost all taxa had their centroids displaced towards the most permeable geological formations (first axis of the analysis), i.e. the karst aquifer and coarse alluvium also characterized by a high oxygen content. Stygobiotic assemblages were enriched both with increased distance to the Würm glacier and decreasing elevation (second axis of the analysis). Land use variables and physico chemical parameters (Mg, Ca, Specific conductance, PO₄, NO₃-N) had low influence on the composition of stygobiotic assemblages of the meridional Jura.

**HYPORHEIC ZONE AS REFUGIUM FOR THE
MACROINVERTEBRATE FAUNA IN THE PO RIVER (NW ITALY):
FIRST DATA**

Fenoglio Stefano, Bo Tiziano, Cucco Marco & Malacarne Giorgio

Di.S.A.V., Università del Piemonte Orientale, Via Bellini 25 15100 Alessandria (Italy) fenoglio@unipmn.it

There is a growing interest in the vertical dimension of stream systems. The importance of the hyporheos as refuge for stream invertebrates, for example to escape unfavourable conditions, is an important topic in this field. In this study we analysed the role of hyporheos as refugium for the benthic macroinvertebrates in the high reach of the Po river. This area (near Saluzzo) is characterised by high streambed permeability and summer droughts. Aim of our study was to investigate the vertical distribution of macroinvertebrates within the substratum. We positioned 12 hyporheic traps in the streambed: each trap consisted in an outer structure containing three inside bags. The structures were buried in the streambed in June 2004 with a mini excavator Kubota and still remain in place. The inside traps were filled with clean sterile substrate, similar to the streambed composition, and placed at different depths: the top trap was positioned from 0 to 30 cm, the medium one from 30 to 60 cm and the bottom one from 60 to 90 cm. At the same time, we conducted quantitative samplings, using a Surber net, to evaluate structure, density and composition of benthic macroinvertebrate communities. In this preliminary poster we present our first data about distribution, stratification and abundance of stream benthos within the substratum during the dry season.

OBLIGATE GROUNDWATER FAUNA OF FRANCE: SPECIES DIVERSITY PATTERNS AND CONSERVATION IMPLICATIONS

Ferreira David, Malard Florian, Dole-Olivier Marie-José & Gibert Janine

University of Lyon1, UMR CNRS 5023 – Ecology laboratory of Fluvial Hydrosystems, Groundwater Biology and Ecology research team, Bât Forel, 43, Boulevard du 11 Novembre 1918,-69622 Villeurbanne cedex (France)

A data base of species occurrences was established on obligate groundwater fauna (stygobites) and used to examine taxonomic and geographic patterns at the scale of France. Studies carried out at the community level were selected to estimate the spatial distribution of sampling effort and revealed that biological exploration of groundwaters was mainly restricted to karst areas. Large unconsolidated aquifers situated in northern and western zones, the centre area, and the main fluvial corridors, remained quasi-unexplored. The total number of stygobite species and subspecies (381) is distributed within a high number of genera (100) and families (39). Conversely to the surface water biodiversity, obligate groundwater fauna is strongly dominated by the group of Crustacea. The species accumulation curve over time (sampling effort) did not reach a plateau, indicating that groundwater biodiversity was still largely underestimated. The contribution of stygobite richness to the biodiversity of continental freshwater was higher than previously expected and in some cases exceeded the number of obligate surface water species. Two measurements of the geographic range size (area of occupancy and latitudinal extent of occurrences) demonstrated, that despite the accumulation of data over time, the geographic range size of the majority of obligate groundwater species remained spatially limited supporting their high level of endemism. An optimal selection of sites has been processed by using complementary software to obtain a proposition combining the highest biodiversity within the lowest set of sites. This selection should be considered as a trial, a first element of discussion towards the integration of fauna into groundwater protection processes.

**DIVERSITY PATTERNS OF COPEPOD ASSEMBLAGES
(CRUSTACEA, COPEPODA) OF DIFFERENT CAVES FROM
SOUTHERN AND CENTRAL ITALY**

Gattone Enrico, Di Lorenzo Tiziana, Fiasca Barbara, De Laurentiis Paola & Galassi Diana M. P.

Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila (Italy)

This contribution presents the first results of an analysis on a biological data set collected in six distinct active caves, four of which located within carbonatic massifs, the remaining two in gypsum formations. Faunal samples have been collected following different methodologies for each habitat typology encountered. The taxonomic group of Crustacea Copepoda is often found to be the most relevant component, in both terms of abundance and species richness, in the communities of most groundwater habitats, and it seems quite likely that the taxonomic diversity of copepod assemblages closely matches the overall diversity of the entire communities under consideration, as they may represent a focal group *sensu* Hammond (1995), possibly allowing an extension of the results of this study to the groundwater biodiversity of the areas investigated as a whole for the purpose of the establishment of future conservation strategies. The biological data have been processed by PRIMER v5 software in order to measure the taxonomic diversity based on phylogenetic relatedness of the species within samples, starting by a matrix of presence/absence of species. Information about geology, palaeogeography, microhabitat heterogeneity, hydrological regime has been used to evaluate the major determinants affecting the different values observed in the taxonomic distinctness for each cave. Paleogeographical factors, habitat heterogeneity and the degree of isolation of the aquifer seem to be the most important parameters affecting the measured diversity patterns. Moreover, the taxonomic dispersion of the diversity might adequately integrate the information resulting from diversity indexes based only on species richness, evenness and abundance. This study is partially granted by EU PASCALIS Project.

THE STYGOBIONTIC FAUNA OF TUNISIA : PRELIMINARY RESULTS

Ghlala A.¹, Della Vale D.², Charfi F.¹ & Messina G.²

1 Faculty of Sciences of Tunis, University campus – 2092 Manar II, 1060 Tunis (Tunisia)

2 Istituto per lo Studio degli Ecosistemi (CNR), Firenze (Italy)

Ground water fauna studies in Tunisia have been initiated in 1923 when Seurat discovered in a thermal spring on the Southern part of the country, the first depigmented, anophtalmous thermosbaenacean crustacean, then described by Monot in 1924 as *Thermosbaena mirabilis*. After a long period of silence, in the seventies some research was done on the hyporeic of some oueds south of Tabarka.

Up to now the knowledge on groundwater fauna was restricted, besides *Thermosbaena*, to few species of gastropoda and crustacea, the most interesting being the Isopoda, Cirolanidae *Saharolana seurati* from the south of Tunisia and the Asellidae *Poasellus bagradicus* from the Western side of the country.

In 2003 was begun an intensive research study in the Northern part of Tunisia, which has given several interesting results and widened our knowledge. The present paper gives a contribution to the understanding of Tunisian stygobitic fauna. A list of the subterranean aquatic fauna revealed in the north of Tunisia is detailed.

Moreover some preliminary considerations about the morphology and phylogenetic relationships of the newly discovered *Typhlocirolana* from the Ichkeul Park and of the genus *Proasellus* whose presence is confirmed and enlarged by the present research efforts are given.

RELATIONSHIP BETWEEN MORPHOLOGICAL TAXONOMY AND MOLECULAR DIVERGENCES : EVIDENCES FROM CRUSTACEAN

Lefébure T.1, Douady, C. J.1 , Gouy M.2 & Gibert J. 1

1Laboratoire d'Ecologie des Hydrosystèmes fluviaux, UMR-CNRS 5023. Université Claude Bernard - Lyon I. Campus de la Doua. 6 rue R. Dubois. 69622 Villeurbanne Cedex, France.

2 Laboratoire de Biométrie et Biologie Evolutive, UMR CNRS 5558. Université Claude Bernard - Lyon 1. 43, bd. du 11 Novembre 1918. 69622 Villeurbanne Cedex, France.

Species level is usually recognized as the major unit of biodiversity. Thus biodiversity assessment is directly dependent on taxonomy. However, morphological traits that represent the base for current taxonomy are non-neutral, complex and potentially highly biased makers. As noticed by the supporter of the DNA taxonomy, molecular markers seem of promising help. Nevertheless, to be employ conjointly morphological taxonomy and molecular markers should first show an overall correlation. This analysis tests this hypothetical relationship for two mitochondrial genes (COI and 16S) within the diversified group of Crustacea. Our analysis revealed that, when appropriately measured, there is a general but poor correlation between molecular divergence and taxonomy. At low taxonomic level morphology and molecular divergence tend to corroborate each other. Therefore our analyses tend to suggest that comparable amount of molecular and morphological evolution accompanies the phenomenon of speciation. In this way molecular divergences may be used to delimit what is usually called species by taxonomists. At upper level (i.e., genus, family), we find almost no correlation between morphology and molecule. If corroborated in other groups this would strongly compromise the possibility of using the COI as a universal marker for identification of any taxonomic level.

MICROINVERTEBRATES OCCURRENCE IN GROUND-WATERS

Mancini L., Venanzi D., Volterra L., Pennelli B. & Formichetti P.

Istituto Superiore di Sanità, V.le Regina Elena 299, 00161 Roma (Italy)

Drinking waters in Italy are mostly taken from ground- or spring-waters (84%) and only in minor part come from surface waters (15%) or other sources (1%). In this study we investigated for the presence of microinvertebrates, and Nematodes in particular, in drinking water samples collected from pipelines in nine Italian regions. For each sample ten litres of water were filtered through cellulose membranes and the residues were fixed and then investigated for the presence of microinvertebrates. Among them nematodes were the most frequently collected. A relationship between biological risk and nematodes occurrence in drinking water was not straightforward, rather the presence of such fauna might be a reliable indicator of the vulnerability of water networks.

**BACTERIOLOGY QUALITY IN SPRING WATER AND POTENTIAL
RELATION WITH FAUNAL ASSEMBLAGES**

Marinelli G.¹, Volpe R.², Civisca A.¹, Di Pietro C.¹, Ciotti F.², Fiasca B.³, Gagliardi C.¹ & Galassi D. M. P.³

1: Public Health Department, University of L'Aquila (Italy)

2: Chemical Engineering Department, University of L'Aquila (Italy)

3: Department of Environmental Sciences, Via Vetoio, Coppito - 67100 L'Aquila (Italy)

The major aim of this contribution is to gain new insights on the potential relationships between bacteriological quality of the spring water and the potential role of the fauna inhabiting this environment, as vectors of bacteria. Thirty samples of groundwater from the Presciano spring system (central Italy) were analysed along a 1-year sampling period, following the *Bou-Rouch* methodology in asepsis condition. The water samples were bacteriologically analyzed. The traditional methodology was used for biochemical typification of species. The analysis of this data set evidenced *Pseudomonas fluorescens* as biological indicator of specificity. The following species were frequently found along the entire sampling time: *Aeromonas hydrophila* and *Pseudomonas putida*. The quantitative analysis showed values strictly conforming the standards for drinking waters. From a biological point of view, the groundwater fauna resulted composed by several stygobiont species mostly belonging to the taxonomic group Crustacea Copepoda. The harpacticoid *Nitocrella pescei* was the dominant species along the collected samples, and was subsequently processed in asepsis to evaluate the occurrence of bacteria in its gut content. The most common species found was *Acinetobacter lwoffii*. No other species, e.g. fecal bacteria, were observed, suggesting that the spring water fauna is not implicated as potential vector of coliform species, and that no correlation exists between their gut microbiology and water quality (MIUR funds).

DIVERSITY OF SUBTERRANEAN FISHES IN BRAZIL

Trajano Eleonora & Bichuette Maria Elina

Departamento de Zoologia, Instituto de Biociências da USP, C.P. 11461, 05422-970, São Paulo (Brazil) etrajano@usp.br

With 18 hypogean troglomorphic species (of which, six awaiting for formal description) and an increasing number of new information to be checked, Brazil is placed among the countries with the highest diversity of subterranean fishes. Most are catfishes belonging to the Heptapteridae, Trichomycteridae and Loricariidae. An electric fish and a phreatobic characiform previously thought to be a tetra characin (recent studies indicated that it cannot be assigned to any of the known families), have also been recorded. These fishes greatly differ in their degree of troglomorphism, from totally anophthalmic and depigmented species to those showing population variability in development of eyes and pigmentation. Troglomorphic species occur in different karst areas, situated in distinct phytogeographic domains (humid Atlantic forest, savannah-like Cerrado, semiarid Caatinga) and occupying different kinds of habitat (from open channel streams including base-level streams and vadose tributaries to the upper phreatic zone), and also in non-karst areas in the Amazon basin, without a taxonomic correlation. São Domingos karst area, Central Brazil, distinguishes as a hotspot of diversity, with seven troglobitic species, including the only confirmed case of syntopy in the country. For several species, allopatric differentiation related to paleoclimatic fluctuations leading to breakdown of the hydrological continuity due to the lowering of the phreatic level may be hypothesized. On the other hand, topographic isolation as a consequence of alluvial downcutting was proposed for troglobitic species living in upper vadose tributaries in the São Domingos karst area. Such diversity results from a combination of epigeal megadiversity (potentially colonizing species) and opportunities for isolation.

Addresses of participants

Alexeev Viktor, Zoological Institute of the Russian Academy of Sciences, University emb. 1, 199034 St Petersburg (Russia) alekseev@zin.ru

Amoros Claude, Université Claude Bernard Lyon 1, UMR-cnrs 5023? Ecologie des Hydrosystèmes Fluviaux, Bât. Darwin C, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) amoros@univ-lyon1.fr

Artheau Malvina, Laboratoire Dynamique de la Biodiversité. UMR/CNRS 5271. UPS, 118 route de Narbonne, F-31062 Toulouse cedex 4 (France) artheau@cict.fr

Berera Raffaella, Università degli Studi della Tuscia, Dipartimento di Scienze Ambientali, Largo dell'Università s.n.c Blocco D, 01100 Viterbo (Italy) berera@unitus.it

Bertrand Alain, Etude et Conservation des Mollusques Continentaux, Abéla, 09320 Bousсенac (France) abela@club-internet.fr

Bichain Jean-Michel, 12 impasse de l'épargne 08300 Rethel France jean-michel.bichain@educagri.fr

Bichuette Maria Elina, Departamento de Zoologia, Instituto de Biociências da USP. C.P. 11461, 05422-970 São Paulo SP. (Brasil) bichuette@uol.com.br

Bo Tiziano, Di.S.A.V., Università del Piemonte Orientale, Via Bellini 25, 15100 Alessandria (Italy) tizianobo@hotmail.com

Boutin Claude, Université de Toulouse, LaDyBio, Bât 4R3, 118 Route de Narbonne, 31062 Toulouse cedex (France) boutin@cict.fr

Bouger Guillaume, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France), bouger@univ-lyon1.fr

Brancelj Anton, National Institute of Biology, Vecna pot 111, 1000 Ljubljana (Slovenia) Anton.Brancelj@nib.si

Bruno Maria Cristina, Università degli Studi della Tuscia, Dipartimento di Scienze Ambientali, Largo dell'Università s.n.c Blocco D, 01100, Viterbo (Italy) mcbruno@care2.com

Cadilhac Laurent, Agence de l'Eau Rhône-Corse-Méditerranée, 14, rue Jonas Salk, 69363 Lyon cedex 07, France, Laurent.CADILHAC@eaurmc.fr

Camacho Ana I., Museo Nacional de Ciencias Naturales, Dpto. de Biodiversidad y Biología Evolutiva C/ Jose Gutierrez Abascal 2, 28006 Madrid (Spain) mcnac22@mncn.csic.es

Campanaro Alessandro, Department of Animal and Human Biology, University of Rome "La Sapienza", V.le dell'Università 32, 00185 Rome (Italy) alessandro.campanaro@uniroma1.it

Castellarini Fabiana, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) fabiana.castellarini@univ-lyon1.fr

Cecchi Giulano, Via Tiburtina 720, 00159 Roma (Italy) giulanocecchi@libero.it

Claret Cécile, Institut Méditerranéen d'Ecologie et de Paléoécologie (UMR CNRS 6116), Université Paul-Cézanne Aix-Marseille 3, Case 31, 13397 Marseille cedex 20 (France) c.claret@wanadoo.fr

Programme and abstracts of the Symposium on World Subterranean Biodiversity

Coineau Nicole, Observatoire Océanologique de Banyuls, Laboratoire Arago F-66650 Banyuls-sur-mer (France) n.coineau@obs-banyuls.fr

Creuzé des Châtelliers Michel, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) deschate@univ-lyon1.fr

Culver David C., Department of Biology, American University, Washington DC 20016 (USA) dculver@american.edu

Dacre Silvia, British Cave Research Association, Biological Recorder Department of Zoology, The Manchester Museum, University of Manchester, Manchester M13 9PL (UK) g.proudlove@umist.ac.uk

Dangles Olivier, IRBI, UMR CNRS 6035, Université de Tours, Parc Grandmont, 37200 Tours (France) olivier.dangles@univ-tours.fr

Danielopol Dan L., Institute of Limnology, Austrian Academy of Sciences, Mondsee str. 9, 5310 Mondsee (Austria) dan.danielopol@oeaw.ac.at

De Broyer Claude, Institut Royal des Sciences Naturelles de Belgique, Rue Vautier 29, 1000 Bruxelles (Belgium) claude.debroyer@naturalsciences.be

Deharveng Louis, Museum National d'Histoire Naturelle, ESA CNRS 8043, laboratoire d'entomologie, 45 rue Buffon, 75005 Paris (France) deharven@mnhn.fr

DeOliveira Rita, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France)

Di Lorenzo Tiziana, Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, I-67100 L'Aquila (Italy) tizianodilorenzo@hotmail.com

Dole-Olivier Marie-José, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) mjdole@biomserv.univ-lyon1.fr

Douady Christophe, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) christophe.douady@univ-lyon1.fr

Eberhard Stefan, Department of Conservation and Land Management, P.O. Box 51, Wanneroo, 6065 WA, Australia stefane@calm.wa.gov.au

Falkner Gerhard, Raiffaisenstrasse 5, 85457 Würth-Hörlkofen, Germany, Falkner@malaco.de

Falkner Margrit, Raiffaisenstrasse 5, 85457 Würth-Hörlkofen, Germany, Falkner@malaco.de

Fenoglio Stefano, Di.S.A.V., Università del Piemonte Orientale, Via Bellini 25, 15100 Alessandria (Italy) fenoglio@unipmn.it

Ferreira David, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) ferreira@univ-lyon1.fr

Fiasca Barbara, Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, I-67100, L'Aquila (Italy) barbarafiasca@hotmail.com

Finston Terry, School of Animal Biology, The University of Western Australia, Stirling Hwy, Perth, 6014 (Australia) tfinston@cyllene.uwa.edu.au

Flamen Caroline, FUNDP, Department of Geology, Rue Grafé 2, 5000 Namur, Belgium carolineflamen@yahoo.fr

Francis Cara. J., School of Animal Biology (MO 92), The University of Western Australia, Stirling Hwy, Perth, 6014 (Australia) cjfran@tartarus.uwa.edu.au

Foulquier Arnaud, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) foulquier@univ-lyon1.fr

Fuchs Andreas, University of Koblenz-Landau, Campus Landau, Dept. of Biology, Im Fort 7, 76829 Landau (Germany) fuchs@uni-landau.de

Galassi Diana M. P. Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, I-67100 L'Aquila (Italy) diana.galassi@aquila.infn.it

Gattone Enrico, Dipartimento di Scienze Ambientali, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila (Italy) gattonenrico@yahoo.com

Gibert Janine, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) janine.gibert@univ-lyon1.fr

Ghlala Adnène, Faculté des Sciences de Tunis, Campus universitaire El Manar II, 1060 Tunis (Tunisia) ghlala@yahoo.fr

Ginet René, Université Claude Bernard Lyon 1, UMR-CNRS5023, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex, (France)

Hahn Hans Jürgen, University of Koblenz-Landau, Campus Landau, Dept. of Biology Im Fort 7, 76829 Landau (Germany) hjhahn@uni-landau.de

Hervant Frédéric, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) hervant@univ-lyon1.fr

Humphreys Williams Frank, Western Australian Museum, Francis Street, Perth, Western Australia 6000. South Australian Museum, North Terrace 5001 (South Australia) humphw@museum.wa.gov.au

Huys Rony, The Natural History Museum, Cromwell Road, London SW7 5BD, U.K., r.huys@nhm.ac.uk

Joly Pierre., Université Claude Bernard Lyon 1, UMR-CNRS5023, Ecologie des Hydrosystèmes Fluviaux, Bât. Darwin C, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex, (France) pjoly@biomserv.univ-lyon1.fr

Jones William K., Karst Waters Institute, PO Box 537, Charles Town, WV (USA) wkj30@hotmail.com

Juget Jacques., Université Claude Bernard Lyon 1, UMR-CNRS5023, Ecologie des Hydrosystèmes Fluviaux, Bât. Darwin C, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex, (France)

Lafont Michel, UR Hydrobiologie, Cemagref, 3 bis Quai Chauveau, C.P. 220, 69336 Lyon, (France) lafont@lyon.cemagref.fr

Lefébure Tristan, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex, (France) Tristan.Lefebure@univ-lyon1.fr

Lips Josiane, 4, Av Allende, 69100 Villeurbanne (France) josiane.lips@free.fr

Malard Florian, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex, (France) malard@univ-lyon1.fr

Magniez Guy, Université de Bourgogne, Sciences de la Vie, Bât Gabriel, 6 bd Gabriel, 21000 Dijon, France, Guy.Magniez@u-bourgogne.fr

Mangin Alain, Laboratoire souterrain du CNRS, 09200 Moulis (France) mangin@dr14.cnrs.fr

Martin Dominique, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) d-martin@univ-lyon1.fr

Martin Patrick, Institut Royal des Sciences de Belgique, Biologie des eaux douces, 29. Rue Vautier, 1000 Bruxelles (Belgium) Patrick.Martin@sciencesnaturelles.be

Mathieu Jacques, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) mathieu@biomserv.univ-lyon1.fr

Mermillod-Blondin Florian, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) mermillo@univ-lyon1.fr

Meyssonier Marcel, École Française de Spéléologie 28, rue Soeur Janin 69005 Lyon (France) comite.speleo.rhone-alpes@wanadoo.fr

Michel Georges, Commission Wallonne d'Etude et de Protection des Sites Souterrains, CWEPS, 21, avenue Rodin, 1050 Bruxelles (Belgium) cwepss@swing.be

Morand Serge, Centre de Biologie et de Gestion des Populations (CBGP) (UMR CIRAD-Agro.M-INRA-IRD). Campus International de Baillarguet. CS 30 016. 34988 Montferrier sur Lez Cédex (France) morand@ensam.inra.fr

Mori Nataša, National Institute of Biology, Vecna pot 111, 1000 Ljubljana (Slovenia) natasa.mori@nib.si

Mulot José, Comité Spéléologie région Nord-Ardenne, 4 rue du Village, 80160 Thois jose.mulot@wanadoo.fr

Nogaro Géraldine, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) geraldine.nogaro@univ-lyon1.fr

Notenboom Jos, Islands Brygge 63C 5tv, 2300 Copenhagen (Denmark) josnotenboom@mac.com

Papin Annick, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) annick.papin@univ-lyon1.fr

Paran Frédéric, Ecole Nationale Supérieure des Mines de Saint-Etienne, Centre SITE, 158, cours Fauriel, 42023 Saint-Etienne Cedex2 (France) paran@emse.fr

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Pattee Eric, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Darwin C, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) ec.pattee@wanadoo.fr

Pipan Tanja, Karst Research Institute ZRC SAZU, Titov trg 2, 6230 Postojna (Slovenia) pipan@zrc-sazu.si

Prié Vincent, (Muséum National d'Histoire Naturelle de Paris) La Clastre, 34520 Saint-Maurice Navacelles (France) don.priado@wanadoo.fr

Proudlove Graham S., Department of Zoology, Manchester Museum, Manchester, M13 9PL, (UK) g.proudlove@manchester.ac.uk

Reeves Jessica, Department of Earth and Marine Sciences, Australian National University (Australia) jessica@ems.anu.edu.au

Rejec-Brancelj Irena, Služba za poročanje EU / EU Reporting Service, Agencija Republike Slovenije za okolje / Environmental Agency of the Republic of Slovenia Vojkova 1b, 1000 Ljubljana (Slovenia) Irena.Rejec-Brancelj@gov.si

Schmidt Susanne, SNSD Museum für Tierkunde, Königsbrücker Strasse 159, 01099 Dresden, Germany suse_semperula@yahoo.de

Schneider Katie, University of Maryland, 1205 Biology-Psychology Building, College Park MD 20742 (USA) katie2@umd.edu

Sket Boris, Vecna , Oddelek za biologijo, Biotehnika fakulteta, Univerza v Ljubljani, p.p. 2995, 1001 Ljubljana (Slovenia) boris.sket@bf.uni-lj.si

Stoch Fabio, Museum of Natural History, Viale le XXV Aprile 24, 34015 Muggia (TS) (Italy) fabiocop@tin.it

Trajano Eleonora, Departamento de Zoologia, Instituto de Biociências da USP. C.P. 11461, 05422-970. São Paulo, SP. (Brasil) etrajano@usp.br

Trontelj Peter, Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, P.P. 2995, 1001 Ljubljana (Slovenia) peter.trontelj@bf.uni-lj.si

Turquin Marie-José, Université Claude Bernard Lyon 1, Ecologie des Hydrosystèmes Fluviaux, Bât. Forel, 43 Bd 11 Novembre 1918, 69622 Villeurbanne cedex (France) turquin@univ-lyon1.fr

Valdecasas Antonio G., Museo Nacional de Ciencias Naturales, Dpto. de Biodiversidad y Biología Evolutiva, C/ Jose Gutierrez Abascal 2, 28006-Madrid (Spain) valdeca@mncn.csic.es

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