EDITORIAL



One step forward for subterranean biology

Oana Teodora Moldovan¹

I "Emil Racoviță", Institute of Speleology, Cluj-Napoca, Romania

Corresponding author: Oana Teodora Moldovan (oanamol@hasdeu.ubbcluj.ro)

Received 8 April 2013 | Accepted 16 April 2013 | Published 29 April 2013

Citation: Moldovan OT (2013) One step forward for subterranean biology. Subterranean Biology 11: 1–2. doi: 10.3897/ subtbiol.11.5280

When somebody takes over the leading of a journal or other duties linked to our research activities it is common-sense to speak about honour, privilege and great responsability. I do not deny all these grantees or duties that come with a leading position. Still, I would rather say that it is a sweet challenge the offer to be the editor-in-chief of Subterranean Biology. The feelings are similar to those following long and frustrating research months when eventually you come to a surprising and satisfying result. I am happy and almost exult at the idea of working for the SIBIOS - ISSB (Société Internationale de Biologie Souterraine - The International Society for Subterranean Biology) journal. Of course, the first reaction is merely immature and the pleasures offered by the professional activity are in fact hard to achieve. It will be difficult, much more difficult than somebody wants. And there is also the burden of an impressive historical heritage. In 1907, Emil Racovitza, member of an aristocratic Romanian family, published "Essai sur les problèmes biospéologiques", the first manifesto on biospéologie (originally in French, biospeleology in English), defining the scope and future directions of researches on cave animals. Together with his collaborators Racovitza also developed the enterprise called *Biopeologica*, which was the precursor of all future journals on subterranean fauna.

Looking at the journals that increased significantly in the last years, one may speculate that they may have gathered many of the papers dealing with subterranean biology topics only because they are "blessed" with an Impact Factor. We will struggle for the same blessing and *Subterranean Biology* deserves it. The papers and the information found in the journal and its predecessor *Mémoires de Biospéologie*, the quality

Copyright O.T. Moldovan. This is an open access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

and the amount of information on subterranean creatures makes me feel confident in the future of the *Subterranean Biology*. The outputs of the former editors-in-chief and the former editorial boards were without compromises on quality. We are thirsty for papers and we expect that, at least, once every three years each member of the Society will provide a good contribution to the journal. We are also inviting all the people that work in subterranean biology or related fields to contribute with the input of "fresh scientific blood" to the Society journal and hopefully become new members.

We announce a new format for *Subterranean Biology*. We offer a journal with a fully electronic format, with options for print on demand of reprints or whole issues for different purposes, with the content published simultaneously in several formats. The content can include free colour figures and multimedia and each article will be published when ready and issues will be completed upon publishing of the last article. Professional archiving in world's leading archives will be provided and there will be active and professional promotion and PR, with the widest possible dissemination of the published content as whole articles or parts of them to increase the online presence of the journal.

Probably, the next years will be the most difficult, but, with your precious help, it will hopefully be only for a short time. We need constant input of papers of good scientific quality. We propose to increase the types of contributions; young people are encouraged to submit their thesis abstracts in the journal and established researchers are invited to submit reviews and ideas for future research. Finally, we wish to make this journal more attractive to the large public by letting them known that subterranean biology is not only about those creepy creatures that live underground, but also that the underground world is deeply connected with the surface and the outside green turns into downwards black only in our eyes but not for the living beings.

RESEARCH ARTICLE



Detritus processing in lentic cave habitats in the neotropics

Marconi Souza Silva¹, Rafaelly Karina Sales Rezende¹, Rodrigo Lopes Ferreira²

I Núcleo de Pesquisa em Ciências Biológicas, Centro Universitário de Lavras (UNILAVRAS) Fundação Educacional de Lavras, Rua Padre José Poggel, 506 Centenário, Lavras, Minas Gerais, Brazil 2 Departamento de Biologia/Setor de Zoologia – Universidade Federal de Lavras. CP.3037, 37200-000 Lavras, MG, Brazil

Corresponding author: Marconi Souza Silva (marconisouza@unilavras.edu.br)

Academic editor: M. Cobolli | Received 27 September 2012 | Accepted 11 March 2013 | Published 29 April 2013

Citation: Souza-Silva M, Rezende KSR, Ferreira RL (2013) Detritus processing in lentic cave habitats in the neotropics. Subterranean Biology 11: 3–14. doi: 10.3897/subtbiol.11.5107

Abstract

Lentic cave habitats are almost always heterotrophic habitats where there are food and oxygen input from the surface. This hydrological exchange seems to be the key factor shaping most groundwater communities. Litter processing in cave water environments has not been experimentally studied as much as it has in lotic subterranean systems, although detritus is likely a critical resource for organisms inhabiting shallow groundwater habitats. The present study sought to evaluate the processing rates and the nitrogen and phosphorous dynamics in plant debris deposited in lentic habitats of two Neotropical limestone caves during 99 days. 84-10×10 cm² litterbags with mesh sizes of 0.04 mm² and 9 mm² were used. In each weighed litter bag, 50 green, intact plant leaf disks (± 2.0 gr/bag) were conditioned. At the end of the experiment, the average weight loss was only 17.4%. No macroinvertebrates were found associated to the debris, but significant differences in the processing rate in relation to the cave and mesh size were observed. The weight loss rate of the plant debris was considered slow (average 0.003 K^{day}). The amount of nitrogen and remaining phosphorous in the plant debris in the two caves showed variations over time with a tendency to increase probably due to the development of microorganisms which assimilate nitrogen and phosphorus. The slow processing rate of the plant debris can be due mainly to the fact that these lentic cave habitats are restrictive to colonization by shredder invertebrates. Furthermore, the abrasive force of the water, which plays an important role in the processing and availability of fragmented debris for colonization by microorganisms, is absent.

Keywords

Caves, decomposition, plant debris, nutrients

Introduction

The groundwater systems are heterotrophic habitats where food and oxygen availability are determined by importation from the surface. This hydrological exchange seems to be the key factor shaping most groundwater communities. Together with oxygen and organic matter, stygoxene and stygophile faunas also have access to the groundwater. If the food and oxygen supply are sufficiently high, these species are able to durably colonize the groundwater and compete with stygobites (Sket 1999; Hahn 2006).

The resource supply in cave ecosystems can vary greatly temporally in the same cave and among caves within a limited geographic area and in some cases can be similar to that in many surface stream types (Souza-Silva et al. 2012; Venarsky et al. 2012). Factors that contribute to the variability in organic matter abundance among cave ecosystems are unknown, but are likely to be a combination of landscape features (e.g. epigean standingcrop, doline topology, watercourse dynamics and plant community structure), cave structure (e.g. depth of cave and size of voids and fractures in the surrounding bedrock) and climate (e.g. precipitation and hydrology) (Souza-Silva et al. 2012; Venarsky et al. 2012).

However, there is a larger movement of leaves and trunks in the epigean environment as well as in the hypogean environment in periods of intense rain, when the water speed and flow are increased (Souza-Silva et al. 2011). Inside the caves, the plant fragments are deposited along the water courses constituting organic matter deposits that are slowly decomposed by detritivore invertebrates (Souza-Silva et al. 2012).

The decomposition or processing of debris occurs in successive degradation stages with the consequent release of nutrients such as nitrogen and phosphorous. These stages are influenced by the activity of physical-chemical agents such as current speed, temperature, pH, oxygen, dissolved nutrient content and the present plant or animal tissues, and also by the biological activity such as the meso- and microorganism actions (Allan and Castillo 2007).

Decomposition of leaf litter has been widely investigated in both aquatic and terrestrial environments (Gessner et al. 1999; Hattenschwiler et al. 2005; Sangiorgio et al. 2010), but few studies have addressed decomposition of leaf litter in aquatic environments characterized by low water velocity such as the river ponds (Chergui and Pattee 1990; McArthur et al. 1994; Baldy et al. 2002) or in very small isolated environments such as springs, pools and caves (Horton and Brown 1991; Eichem et al. 1993; Souza-Silva et al. 2007; Sangiorgio et al. 2010).

The present study aims to evaluate the processing rates of the coarse particulate organic matter and both nitrogen and phosphorous dynamics in plant debris exposed to decomposition in the lentic habitats in two Neotropical limestone caves.

Materials and methods

Study site

The study was carried out from April to July of 2009 in the Brega and Santuário limestone caves (Brega - 419447.15E, 7742205.17S, elevation 725 m and Santuário

419312.50E, 7742176.54S, elevation 722 m), located in the municipal district of Pains, Minas Gerais, Brazil. The caves have permanent groundwater and only Brega cave receives also water from rain floods.

The Brega Cave had predominantly vadose genesis, presenting a horizontal development of approximately 760 meters and two opposite entrances. Santuário Cave had also predominantly vadose genesis and presents a horizontal development of approximately 700 meters and two entrances. At the end of the main conduits of the two caves are small perennial water holes feed by upwelling groundwater.

According to the classification of Köppen (Menegasse et al. 2002), the climate of the external area is the Cwa type: mild climate with hot, humid summer and dry winter. The average annual temperature is 20.7°C, July being the coldest month, with an average temperature of 16.3°C, and January the hottest month, with an average of 23.3°C. The local annual average precipitation is 1344 mm. The Brega and Santuário caves belong to the hydrographic sub-basin known as *ribeirão dos Patos* (530 km²), favored by the direct infiltration in the limestone fissures and the karstic absorption features such as dolines and sinkholes.

The local tropical climate is responsible for the seasonal variation of the hydric dynamics of the *ribeirão dos Patos*, significantly altering the surrounding landscapes. The wet season lasts from October to March, which according to Menegasse et al. (2002) corresponds to 81% of the annual precipitation. The great hydric volume of the *Patos* stream makes the increase of the water level present inside the caves possible, leaving a large part of their floors flooded.

Litter colonization and breakdown rates

Rates of litter breakdown in lentic environments were measured using bags containing 50 intact plant leaf disks (area = 63.6 mm²/each disk, \pm 2 grams). The bags with 0.04 mm² and 9 mm² meshes, measuring 10 × 10 cm² on the sides, enable the microinvertebrates colonization (Bärlocher 2005; Venarsky et al. 2012). 42 bags were used in each site, 21 with the smaller mesh size and 21 with the larger mesh size, totaling 84 litter bags in the two caves.

The leaves were collected from trees (Moraceae: *Ficus* sp.) located in the surrounding area before the fall of leaves, thereby reducing the possibility that the experimental material had been previously exposed to decomposition after falling on the ground.

Sample processing

Triplicates of each mesh size were removed (after intervals of 14, 35, 50, 64, 78, 85 and 99 days) to quantify the decomposition through the weight loss and to determine the composition of the associated invertebrate fauna. Before the incubation of the litter bags in the cave water, the initial weight and the initial nitrate and phosphate levels present in the foliar portions were measured. After each collection, the material was

washed with distilled water (Bärlocher 2005), the wash water and the removed disks were conditioned separately in Petri dishes and observed with the aid of a magnifying glass, to verify the presence of associated invertebrates.

The remaining plant material and the wash water were oven-dried at 100° C for 48 hours and later weighed. After the material was dried and weighed, it was sent to the Plant Nutrition Laboratory of the Federal University of Paraná, Brazil, where the analysis of remaining nitrogen and phosphorous in the fractions under decomposition was conducted. Total leaf nitrogen and phosphorus were also measured, using the microKjeldahl method on another portion of the leaf (Varley 1966).

To evaluate the influence of the moisture of the green leaves on the weight loss measurements, 50 green leaves were weighed (2.03 gr), oven-dried and reweighed (0.48 gr). Thus, the 76.3% plant disk weight loss is due to the moisture loss and not the mass loss. Water samples were collected from each cave on the sampling start date and analyzed for turbidity, dissolved oxygen, phosphorus and nitrogen.

Data analyses

The amount of organic matter remaining after exposure was expressed in percentage (%) of remaining dry weight [(FW × 100)/IW]. Where IW is the initial weight of the sample at time zero, FW is the final weight of the sample at time t+1. The decay processing rate (k^{day}) in each cave was described by the model Mt = M₀ e^{-Kt} (Oslon 1963; Bärlocher 2005), Where Mt is the weight at time t, M₀ is the initial weight; t is the exposure time of the sample.

To determine the processing speed (k/day) of plant debris we compared our results to data from the literature for aquatic environments and defined slow (≤ 0.005), moderate (0.006–0.10) and fast (0.10–0.15) processing (Table 1). To evaluate differences in the average final weight values among sites and among the dates of collection and in the same different mesh types, the *t* Test was used (Zar 1984).

Results

Litter colonization and breakdown rates

The plant disks exposed to decomposition presented slow processing speed, but varied with time and between the two exposure caves (Table 1, Figs 1 and 2). However, significant differences were observed in the average final weight between both the two caves and the two mesh sizes (Table 2).

In Brega Cave, during the first 14 days of exposure of the 0.04 mm² mesh an average remaining weight of 86% was observed. After 85 days of exposure the plant debris had an average remaining weight of 82% in relation to the initial mass (Fig. 1). In the

Habitat type	k ^{-day}	se	References
Brega cave water (mesh size 0.04 mm ²)	0.0025	0.0005	This study
Brega cave water (mesh size 9 mm ²)	0.002	0.004	This study
Santuário cave water (mesh size 0.04 mm ²)	0.003	0.000	This study
Santuário cave water (mesh size 9 mm²)	0.0032	0.000	This study
Cave pool	0.003-0.001	-	Galas et al. 1996
Ephemeral river pool	0.009	-	Maamri et al. 1997
Littoral zone, Lake	0.0058-0.0039	-	Pope et al. 1999
Chaney Lake	0.0025	-	Kelley and Jack, 2002
Cave stream	0.0158	-	Simon and Benfield 2001
Cave stream	0.003	-	Simon and Benfield 2001
Cave stream	0.043-0.598	-	Souza-Silva et al. 2011
Cave stream	0,03-0.05	-	Souza-Silva et al. 2007
Cave stream	0.0173	-	Souza-Silva et al. 2012
Cave stream	0.001-0.012		Venarsky et al. 2012
Cave stream (mesh size 10×8 mm)	0.004-0.012		Venarsky et al. 2012

Table 1. Decay process (k^{-day}) in this study compared to other published work from aquatic environments: Standard error (se).



Figure 1. Remaining weight of plant disks exposed to processing in the 0.04 mm² litterbag mesh size in the lentic habitats of Brega and Santuário caves. Mean, Box: Mean±SE, Whisker: Mean±SD



Figure 2. Remaining weight of plant disks exposed to processing in the 9 mm² litterbag mesh size in the lentic habitats of Brega and Santuário caves. Mean, Box: Mean±SE, Whisker: Mean±SD.

Table 2. Significant* ($p \le 0.05$) and non-significant differences in the final plant matter weight processing between caves and litterbag mesh sizes in lentic environments: Brega Cave (B), Santuário Cave (S), 0.04 mm² mesh (1), 9 mm² (2).

Cave	Initial	Mean	Mean	t-value	df	р	Std.Dev.	Std.Dev.
mesh	weight	1	2				Group 1	Group 2
B2 x. S2*	0.5	0.461	0.417	4.313	40	0.000	0.042	0.021
B1 x. B2*	0.5	0.430	0.461	-2.171	40	0.036	0.051	0.042
B1 x. S1	0.5	0.430	0.419	0.855	39	0.398	0.051	0.027
B1 x. S2	0.5	0.430	0.417	1.060	40	0.296	0.051	0.021
S1 x. B2*	0.5	0.419	0.461	-3.845	39	0.000	0.027	0.042
S1 x. S2	0.5	0.419	0.417	0.248	39	0.805	0.027	0.021

same cave, during the first 14 days of the 9 mm² mesh plant disk exposures, an average remaining weight of 88% was observed. After 85 days of exposure of the plant disks, the average remaining weight corresponded to 88% (Fig. 2).

In Santuário Cave, during the 14 days of exposure in the 0.04 mm² mesh, an average remaining weight of 83% was observed. After 85 days of exposure of the plant disks, the average remaining weight was 84% (Fig. 1).

In Santuário Cave from the 14^{th} to the 35^{th} days of exposure of the 9 mm^2 mesh plant disks, an average remaining weight of 80% was observed. From the 78^{th} to 85^{th}



Figure 3. Temporal values of nitrogen and phosphorous ($\mu g/kg$) measured in plant discs exposed to decomposition in lentic habitats of the Santuário and Brega caves.

days of exposure the average remaining weight was 78%. At the end of the 99 days of exposure, the average remaining weight corresponded to 82% (Fig. 2).

We can assume that for all of the mesh sizes a slow weight loss rate was registered. As such, we can also consider the low decay (K/day) values (average $0.003 \text{ K}^{-\text{day}}$) for the water of these two caves (Table 1).

There were no macroinvertebrates associated with the debris.

Dynamics of nitrogen and phosphorus in the plant disks

The water quality parameters evaluated for the two caves showed quite different values. In Brega Cave the values were as follows: turbidity (6.97 unt), dissolved oxygen (5 mg/l), phosphorus (1.2 mg/l) and nitrogen (0.8 mg/l). In Santuário Cave the values were as follows: turbidity (39.5 unt), dissolved oxygen (4.5 mg/l), phosphorous (1.8 mg/l) and nitrogen (1.4 mg/l).

The values for nitrogen and phosphorous in the plant disks in the two caves showed different values during the experiment. However, significant differences were not found in the average amount of nitrogen and phosphorous present in the debris of the litterbags of the two caves.

In Santuário Cave, the initial nitrogen value was 17.1 μ g/kg. After 14 days of plant disk exposure (April 18) this value reduced to 10.9 μ g/kg. After 99 days of exposure (July 12) the lowest value (7.2 μ g/kg) of the remaining nitrogen was registered (Fig. 3). In Bre-

ga Cave, the initial nitrogen value was also of 17.1 μ g/kg. With 14 days of exposure (April 18), the amount of nitrogen in the plant disks increased (28.4 μ g/kg). On the 99th day of exposure of the plant debris (July 12), the nitrogen value was of 26.9 μ g/kg (Fig. 3).

In Santuário Cave, the initial value of phosphorous was 1.44 μ g/kg. After 14 days of exposure of the plant debris (April 18) the lowest amount of this nutrient was registered, corresponding to 0.76 μ g/kg. After 85 days (June 28) there was a small reduction in the amount of phosphorous in the plant debris (Fig. 3). In Brega Cave, the initial phosphorous value was also of 1.44 μ g/kg. After 14 days of exposure (April 18) the lowest amount of this nutrient was registered, 0.92 μ g/kg. On the 99th of exposure (July 12), the amount of the remaining phosphorous in the plant debris showed a reduction to 1.20 μ g/kg (Fig. 3).

Discussion

Works related to processing of plant debris have not been undertaken in lentic cave habitats until present. Therefore, the data of this study are compared with organic plant matter processing studies conducted in underground and surface streams, ephemeral karst lakes and oligotrophic lakes (Table 1). Some studies revealed that decomposition had different speeds, from slow to fast, in subterranean streams (Table 1).

Organic matter decomposition depends on the action of organisms, physicalchemistry features and abrasive force of the water, all playing an important role in the organic plant matter degradation (Allan and Castillo 2007). Usually in streams, the processing is faster if there is a combination of abrasive physical, chemical and biological factors such as pH, currents and shredders that accelerate the decomposition (Suberkropp et al. 1976; Allan and Castillo 2007).

The microorganisms and aquatic shredder invertebrates activity significantly interferes in the organic matter decomposition coefficient, constituting an indirect measure of the use potential of the debris as a food resource (Dobson et al. 2003). Shredders invertebrates, such as some amphipods, plecopterans, trichopterans, and dipterans, are important for leaf breakdown in European and North American streams (Graça 2001; Wantzen and Wagner 2006). However, in the tropical areas the shredder action can be slower when compared to the temperate areas (Irons et al. 1994; Moretti 2005, Wantzen and Wagner 2006). This difference can be due to highest shredder abundance and low temperatures in the temperate streams and the high palatability of the organic plant debris (thin cuticle and few toxic compounds) that favor the shredder activity over that of the microorganisms (Wantzen and Wagner 2006). During the decomposition processes, bacteria, fungi and Protozoa colonize and proliferate on the plant debris substrate. They play an important role in altering the palatability of leaves for scavengers and in fragmentation of debris (Arsuffi and Suberkropp 1984).

The slow decomposition rates of the organic plant matter in the lentic environments of Brega and Santuário caves due mainly to the fact that these environments are spatially and trophically restrictive to colonization by shredder invertebrates. Furthermore, the abrasive force of the water, which plays an important role in debris processing and availability, is absent. Thus, lentic caves habitats can be vulnerable to organic matter enrichment when compared to surface aquatic environments or underground streams (Sket, 1999; Moretti 2005; Hahn 2006; Wantzen and Wagner 2006; Souza-Silva et al. 2011; Venarsky et al. 2012).

Caves and their microhabitats are often contaminated by nutrients resulting from human activities that are carried by water to the subterranean environment (Bowles and Arsuffi 1993; Simon and Buikema 1997; Gunn et al. 2000; Pronk et al. 2009; Souza-Silva et al. 2012). Many natural factors can also contribute to high input of organic matter in caves, such as landscape topology, surrounds plant community structure, cave depth, entrance size, fractures in the surrounding bedrock and climate (e.g. precipitation and hydrology) (Souza-Silva et al. 2012; Venarsky et al. 2012).

In cave aquatic habitats the quantity of organic matter is known as one factor that influences microorganisms and invertebrate fauna structure and physical-chemistry conditions (Simon and Buikema 1997; Wood et al. 2002; Graening and Brown 2003; Wood 2008).

The delay in the reduction of the amount of the nitrogen and phosphorous in the plant disks can be due to their low solubility and delay in colonization by microorganisms (Gessner 1991). On the other hand, the remaining nitrogen and phosphorous amount increase is probably due to subsequent colonization by microorganisms (Suberkropp and Klug 1976; Rogers and Debruyn 1998; Kelley and Jack 2002; Allan and Castillo 2007), knowing that water sources in caves provide a more suitable habitat for bacteria, fungi and protozoa communities (Mulec 2008; Sigalada -Regalado et al. 2011; Souza-Silva et al. 2012).

More researches are needed to understand how litter decomposition can be affected both by hydrology and biological communities. The understanding of the debris processing dynamics in the ecosystems and their influence on community structuring is fundamental for the biodiversity conservation in as much these processes represent the energy base of the systems.

Acknowledgements

The authors are grateful to John Holsinger for English corrections of the manuscript and two anonymous reviewers for their useful suggestions. We express our thanks to the Coordenadoria de Pesquisa do Centro Universitário de Lavras (UNILAVRAS). Leopoldo Ferreira de Oliveira Bernardi, Mr. Brega (proprietário da fazenda), Rosinei (Coelho), Conselho Nacional de Pesquisa (CNPq), Fundação de Amparo a pesquisa do Estado de Minas Gerais (Fapemig), Centro Nacional de pesquisa e conservação de cavernas (CECAV).

References

Allan DJ, Castillo MM (2007) Stream Ecology Structure and function of running Waters Second Edition, Published by Springer, P.O. Box 17, 3300 A.A Dordrecht, The Netherlands. 436 pp.

- Arsuffi TL, Suberkropp K (1984) Leaf processing capabilities of aquatic hyphomycetes: interspecific differences and influence on shredder feeding preference. Oikos 42: 144–154. doi: 10.2307/3544786
- Baldy VE, Chauvet J, Charcosset Y, Gessner MO (2002) Microbial dynamics associated with leaves decomposing in the mainstream and floodplain pond of a large river. Aquatic Microbial Ecology 28: 25–36. doi: 10.3354/ame028025
- Bärlocher F (2005) Leaf mass loss estimated by litter bag technique, in Methods to Study Litter Decomposition: A Practical Guide, Edited by Felix Manuel A.S. Graça, Bärlocher and Mark O. Gessner. Published by Springer, P.O. Box 17, 3300 A.A Dordrecht, The Netherlands, 329 pp.
- Bowles DE, Arsuffi TL (1993) Karst aquatic ecosystems of the Edwards Plateau region of Central Texas, USA: a consideration of their importance, threats to their existence, and efforts for their conservation. Aquatic Conservation: Marine and Freshwater Ecosystems 3: 317–329. doi: 10.1002/aqc.3270030406
- Chergui H, Pattee E (1990) The processing of leaves of trees and aquatic macrophytes in the network of the River Rhone. International Revue of Hydrobiology 75: 281–302. doi: 10.1002/iroh.19900750303
- Dobson MJ, Mathooko M, Ndegwa FK, M'erimba M (2003) Leaf litter processing rates in a Kenyan highland stream, the Njoro River. Hydrobiologia 519: 207–210. doi: 10.1023/B:HYDR.0000026592.50734.ea
- Eichem, AC, Dobbs WK, Tate CM, Edler C (1993) Microbial decomposition of Elm and Oak leaves in a karst aquifer. Applied and Environmental Microbiology 59: 3592–3596.
- Galas J, Bednarz T, Dumnicka E, Starzecka A, Wojtan K (1996) Litter decomposition in a mountain cave water. Archiv fuer Hydrobiologie 2: 199–211.
- Gessner MO (1991) Differences in processing dynamics of fresh and dried leaf litter in a stream ecosystem. Freshwater Biology 26: 387–398. doi: 10.1111/j.1365-2427.1991.tb01406.x
- Gessner MO, Chauvet E, Dobson M (1999) A perspective on leaf litter breakdown in streams. Oikos 85: 377–384. doi: 10.2307/3546505
- Graça MAS (2001) The role of invertebrates on leaf litter decomposition in stream-a Review. International Review of Hydrobiology 86: 383–393. doi: 10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D
- Graening GO, Brown AV (2003) Ecosystem dynamics and pollution effects in an Ozark cave stream. Journal of the American Water Resources Association 36: 1497–1507. doi: 10.1111/j.1752-1688.2003.tb04434.x
- Gunn J, Hardwick P, Wood PJ (2000) The invertebrate community of the Peak–Speedwell cave system, Derbyshire, England - pressures and considerations for conservation management. Aquatic Conservation: Marine and Freshwater Ecosystems 10: 353–369. doi: 10.1002/1099-0755(200009/10)10:5<353::AID-AQC413>3.0.CO;2-S
- Hattenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology and Systematics 36: 191–218. doi: 10.1146/ annurev.ecolsys.36.112904.151932
- Hahn HJ (2006) The GW-Fauna-Index: A first approach to a quantitative ecological assessment of groundwater habitats. Limnologica 36: 119–137. doi: 10.1016/j.limno.2006.02.001

- Horton RT, Brown AV (1991) Processing of green American Elm leaves in first, third and fifth order reaches of an Ozark stream. Journal of Freshwater Ecology 6: 115–119. doi: 10.1080/02705060.1991.9665285
- Irons, JG, Oswood MW, Stout RJ, Pringle CM (1994) Latitudinal patterns in leaf litter breakdown: is temperature really important? Freshwater Biology 32: 401–411. doi: 10.1111/ j.1365-2427.1994.tb01135.x
- Kelley RH, Jack JD (2002) Leaf litter decomposition in an ephemeral karst lake (Chaney Lake, Kentucky, U.S.A.). Hydrobiologia 482: 41–47. doi: 10.1023/A:1021209906661
- Maamri A, Chergui H, Pattee E (1997) Leaf litter processing in a temporary northeastern Moroccan river. Archiv Fuer Hydrobiologie 140: 513–531.
- McArthur JV, Aho JM, Rader RB, Mills GL (1994) Interspecific leaf interactions during decomposition in aquatic and floodplain ecosystems. Journal of the North American Benthological Society 13: 57–67. doi: 10.2307/1467265
- Menegasse LN, Gonçalves JM, Fantinel LM (2002) Disponibilidades hídricas na Província cárstica de Arcos-Pains-Doresópolis, Alto São Francisco, Minas Gerais, Brasil. Revista Águas Subterrâneas 16: 1–19.
- Moretti MS (2005) Decomposição de detritos foliares e sua colonização por invertebrados aquáticos em dois córregos na Cadeia do Espinhaço (MG). Dissertação apresentada à Universidade Federal de Minas Gerais, como pré-requisito do Programa de Pós-Graduação em Ecologia, Conservação e Manejo de Vida Silvestre, para a obtenção do título de Mestre em Ecologia, 63 pp http://www.icb.ufmg.br/pgecologia/dissertacoes/D155_Marcelo_da_Silva_Moretti.pdf
- Mulec J (2008) Microorganisms in hypogeon: examples from Slovenian karst caves. Acta Carsologica 37: 153–160.
- Oslon JS (1963) Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44: 322–331. doi: 10.2307/1932179
- Pronk M, Goldscheider N, Zopfi J, Zwahlen F (2009) Percolation and particle transport in the unsaturated zone of a karst aquifer. Groundwater 3: 361–369. doi: 10.1111/j.1745-6584.2008.00509.x
- Pope RJ, Gordon AM, Kaushik NK (1999) Leaf litter colonization by invertebrates in the littoral zone of a small oligotrophic lake. Hydrobiologia 392: 99–112. doi: 10.1023/A:1003537232319
- Rogers KH, Debruyn J (1998) Decomposition of *Paspalum distichum* L.: methodology. In seasonally inundated systems. Verhandlungen Internationale Vereinigung f
 ür Theoretische und Angewandte Limnologie (23): 1945–1948.
- Sangiorgio F, Glazier DS, Mancinelli G, Basset A (2010) How can habitat size influence leaf litter decomposition in five mid-Appalachian springs (USA)? The importance of the structure of the detritivorous guild. Hydrobiologia 654: 227–236. doi: 10.1007/s10750-010-0390-9
- Sigala-Regalado I, Mayén-Estrada R, Morales-Malacara JB (2011) Spatial and temporal distribution of protozoa at Cueva de Los Riscos, Querétaro, México. Journal of Cave and Karst Studies 73: 55–62. doi: 10.4311/jcks2009mb121
- Simon KS, Benfield EF (2001) Leaf and wood breakdown in cave streams. Journal of the North American Benthological Society 20: 550–563. doi: 10.2307/1468087

- Simon KS, Buikema AL (1997) Effects of organic pollution on an Appalachian cave: changes in macroinvertebrate populations and food supplies. American Midland. Naturalist 138: 387–401. doi: 10.2307/2426830
- Sket B (1999) The nature of biodiversity in hypogean waters and how it is endangered. Biodiversity and Conservation 8: 1319–1338. doi: 10.1023/A:1008916601121
- Souza-Silva M, Ferreira RL, Bernardi LFO, Martins RP (2007) Importação e processamento de detritos orgânicos em uma caverna calcária. Espeleo-Tema (19): 31–41.
- Souza-Silva M, Bernardi LFO, Martins RP, Ferreira RL (2012) Transport and consumption of organic detritus in a Neotropical limestone cave Acta Carsologica 41: 139–150.
- Souza-Silva M, Martins RP, Ferreira RL (2011) Trophic Dynamics in a Neotropical Limestone Cave. Subterranean Biology 9: 127–138. doi: 10.3897/subtbiol.9.2515
- Souza-Silva M, Liria CCS, Sampaio FAC, Ferreira RL (2012) Transitory aquatic taxocenosis in two neotropical limestone caves. Revista Brasileira de Espeleologia 1: 29–41.
- Suberkropp KF, Godshalk GL, Klug MJ (1976) Changes in the chemical composition of leaves during processing in a woodland stream. Ecology 57: 720–727. doi: 10.2307/1936185
- Suberkropp KF, Klug MJ (1976) Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57:707–719. doi: 10.2307/1936184
- Varley A (1966) Automatic Methods for the Determination of Nitrogen, Phosphorus and Potassium in Plant Material, http://pubs.rsc.org. doi: 10.1039/AN9669100119
- Venarsky MP, Benstead JP, Huryn AD (2012) Effects of organic matter and season on leaf litter colonization and breakdown in cave streams. Freshwater Biology 57: 773–786. doi: 10.1111/j.1365-2427.2012.02742.x
- Wantzen KM, Wagner R (2006) Detritus processing by invertebrate shredders: a neotropical–temperate comparison, Journal of the North American Benthological Society 25: 216– 232. doi: 10.1899/0887-3593(2006)25[216:DPBISA]2.0.CO;2
- Wood PJ, Gunn J, Perkins J (2002) The impact of pollution on aquatic invertebrates within a subterranean ecosystem-out of sight out of mind. Archiv fuer Hydrobiologie 155: 223–237.
- Wood PJ, Gunn J, Rundle SD (2008) Aquatic conservation: marine and freshwater ecosystems. Aquatic Conservation: Marine and Freshwater Ecosystems 18: 909–922. doi: 10.1002/ aqc.933
- Zar JH (1984) Biostatistical analysis, 2nd ed. Prentice Hall, New Jersey, 718 pp.

RESEARCH ARTICLE



Rana iberica (Boulenger, 1879) goes underground: subterranean habitat usage and new insights on natural history

Gonçalo M. Rosa¹, Andreia Penado²

I Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury, Kent, CT2 7NR, UK; Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Bloco C2. Campo Grande, 1749-016 Lisboa, Portugal 2 School of Life Sciences, University of Sussex, Brighton, East Sussex, BN1 9RH, UK; CIBIO/UP Jardim Botânico Tropical/IICT Tv. Conde da Ribeira, 9 1300-142 Lisboa Portugal

Corresponding author: Gonçalo M. Rosa (goncalo.m.rosa@gmail.com)

Academic editor: O. Moldovan | Received 19 March 2013 | Accepted 7 April 2013 | Published 29 April 2013

Citation: Rosa GM, Penado A (2013) *Rana iberica* (Boulenger, 1879) goes underground: subterranean habitat usage and new insights on natural history. Subterranean Biology 11: 15–29. doi: 10.3897/subtbiol.11.5170

Abstract

Reports of amphibians exploiting subterranean habitats are common, with salamanders being the most frequent and studied inhabitants. Anurans can occasionally be observed in caves and other subterranean habitats, but in contrast to salamanders, breeding had never been reported in a cave or similar subterranean habitat in Western Europe. Based on observations during visits to a drainage gallery in Serra da Estrela, Portugal, from May 2010 to December 2012, here we document: (i) first report of *Rana iberica* reproduction in cave-like habitat, representing the fourth report of an anuran for the Palearctic ecozone; (ii) oophagic habits of the tadpoles of *R. iberica*; and (iii) *Salamandra salamandra* predation on *R. iberica* larvae. These observations, particularly of *R. iberica*, highlight our lack of knowledge of subterranean ecosystems in the Iberian Peninsula.

Keywords

Subterranean habitats, amphibians, anuran reproduction, larval predation, oophagy

Introduction

Caves and other subterranean habitats contain a biodiversity that has long intrigued biologists, such as Darwin and Lamarck (Culver and Pipan 2009). Often limited in light, these places have revealed a great number of diverse and unique species (Romero 2009). The occurrence of amphibians in caves and associated subterranean habitats is not novel (e.g. Manenti 2008). Amphibians can use caves and other subterranean habitats for thermal refugia (Uhrin and Lesinsky 1997, Fenolio et al. 2005), to avoid predation (Manenti et al. 2009), as feeding habitats (Baumgart 1981, Resetaritis 1986, Uhrin and Lesinsky 1997) and as breeding sites (Diesel et al. 1995). A number of salamanders are troglobitic (obligate cavernicole species unable to survive outside low-light environments; Hoffmann 2002) restricted to cave habitats and exhibiting troglomorphic characters, such as degenerate eyes and depigmentation. Other amphibians use caves to complete some aspect of their life cycles and may or may not exhibit some level of troglomorphy. Anurans are occasionally observed in subterranean habitats, but no species are known to be obligate cavernicoles (Culver and Pipan 2009; Romero 2009).

In the Palearctic region, amphibians are relatively common in subterranean habitats; however only one species is obligate cavernicola (*Proteus anguinus* Laurenti, 1768). Other salamanders are known to use caves during part of their life cycles (e.g., Paradactylodon persicus (Eiselt & Steiner, 1970), Speleomantes spp. Dubois, 1984, Chioglossa lusitanica Bocage, 1864 and Salamandra salamandra (Linnaeus, 1758) (Sequeira et al. 2001, Kami 2004, Lanza et al. 2006, Manenti et al. 2009, Romero 2009, Ianc et al. 2012). Reports of anurans are frequent in Palearctic subterranean habitats (e.g., Mc-Clure et al. 1967, Baumgart 1981, Bonini et al. 1999, Bressi and Dolce 1999, Presetnik et al. 2002, Biswas and Shrotriya 2011), however breeding reports and limited to few species. During a regular monitoring of the amphibian community at Portofino Park (Liguria, north-western Italy) in 1996, Bonini et al. (1999) found tadpoles of Bufo bufo (Linnaeus, 1758) inside an artificial tunnel with a part of complete darkness, and kept on finding egg strings in subsequent years. These were then the first evidences of anuran reproduction inside subterranean habitat in this ecozone. In 1998, Gasparo and Stoch observed eggs of Rana temporaria Linnaeus, 1758 inside a natural cave (Kompoljska jama) in Dobrepolje (Slovenia) (Bressi and Dolce 1999). Additionally, in a visit to the Viršnica - Jamski cave system in Radenskem polju (Central Slovenia), Presetnik et al. (2002) discovered four egg-clutches and several adults of Rana dalmatina Bonaparte, 1840. Frogs were found more than 500m from the giant tunnel entrance, but as in the previous record, data on biology, phenology and cave habitat usage is also still largely missing.

Worldwide, breeding by anurans in subterranean habitats has only been confirmed for three additional species of leptodactylid frogs in the Neotropic ecozone: two of genus *Eleutherodactylus* Duméril and Bibron, 1841 inhabiting Caribbean islands (Diesel et al. 1995, Rogowit et al. 2001) and *Cycloramphus eleutherodactylus* (Miranda-Ribeiro, 1920) from Brazil (Lima et al. 2012). The Iberian brown frog (*Rana iberica* Boulenger 1879) is endemic to Portugal and north-western and central Spain. Although it is a typical mountain species, this frog can be common in coastal areas at low altitude. The species inhabits streams with cold and clear waters, rocky substrates, and abundant vegetation surrounding the margins (Loureiro et al. 2008). *Rana iberica* can also be found in other habitats, including small ponds, humid meadows and soaked fields. The breeding season occurs from November to March in the lowlands and from March to May in the highlands (Crespo and Cei 1971, Almeida et al. 2001, Salvador and García-París 2001, Ayres 2008). Females lay small clutches of eggs (between 100-450) with a gelatinous coat, beneath underwater stones or stuck to water-weed (Almeida et al. 2001, Ayres 2009). No records of this species inhabiting subterranean habitats are known.

Predators of *R. iberica* have been documented across all life stages. Adults are prey of water snakes (*Natrix maura* (Linnaeus, 1758) and *N. natrix* (Linnaeus, 1758)), *Vipera seoanei* Lataste, 1879, small carnivores such as *Neovison vison* (Schreber, 1777), *Genetta genetta* (Linnaeus, 1758) and *Lutra lutra* (Linnaeus, 1758) (Galán-Regalado 1988, Almeida et al. 2001; Morales et al. 2004), and even the barn owl, *Tyto alba* (Scopoli, 1769) (Rey et al. 2004). Larvae are vulnerable to several aquatic insects, trout, water snakes, and even the marbled newt, *Triturus marmoratus* (Latreille, 1800) (Almeida et al. 2001, Bosch et al. 2006). There are no records of predation by other salamander species to our knowledge.

Here, we report for the first time evidence of *Rana iberica* reproduction in a subterranean habitat, the fourth record of an anuran for the Palearctic ecozone. Furthermore, we discuss the natural history of *R. iberica*, including the report of a new predator of larvae and oophagic habits of its tadpoles.

Methods

Serra da Estrela Natural Park is located in north-central Portugal (40°20'N, 7°35'W) and is the largest protected area with approximately 89000 hectares (Fig. 1). It is one of the highest mountains (1993 m a.s.l. at Torre Plateau) in the Iberian Sistema Central. The climate is temperate-Mediterranean with Atlantic influences, presenting dry and warm summers and a wet season from October to May with frequent snowfall at higher altitudes (Daveau et al. 1977, Andrade et al. 1992, Jansen and Sequeira 1999). Rich in freshwater environments, Serra da Estrela comprises many permanent ponds and lakes of glacial origin, swamps, bogs, temporary pools, and a dense system of springs, brooks and rivers (Costa et al. 2004). The mean annual temperature is 3-4°C at the highest altitudes (Vieira and Mora 1998) and mean annual rainfall is greatest at the Planalto Superior (exceeding 2500 mm) and least in the northwest region (about 900 to 1000 mm) (Daveau et al. 1977, Jansen 2002).

Because of its geographic location, topographic and climatic conditions, Serra da Estrela has several ecosystems that are unique in Portugal. In addition, this area con-



Figure I. Serra da Estrela Natural Park and hypogean habitat used by individuals of *Rana iberica*: **A** entrance of the underground spring **B** *Schistostega pennata* covering walls and floor of the drainage gallery **C** horizontal tunnel of drainage gallery. Photo A by Madeira M, **B** by Rosa GM, **C** by Laurentino T.

tains many rare and endemic species. This region is one of the most biodiverse in Portugal and is considered an Iberian Peninsula biodiversity hotspot (Teles 1970, Costa et al. 1998, Moreira et al. 1999, Costa et al. 2003). Among amphibians, Serra da Estrela harbors 13 species, including five species of salamanders and eight species of anurans (Brito-e-Abreu et al. 1994, Lesparre and Crespo 2008).

Several drainage galleries were created for water capture in the 1950s, even before the establishment of the boundaries of the Natural Park. Galleries are characterized by a horizontal tunnel that penetrates the side of a hill. Typically, galleries are of limited width (ca. 1 m) and height (1.5 m), and extending several meters into the hillside (Fig.

19

1A–C; see also Penado et al. in press). These tunnels are cool and humid and often contain a small stream. These artificial subterranean habitats are often used as a refuge for many species, and are particularly important for *Chioglossa lusitanica*, an Iberian endemic of conservation concern (Cabral et al. 2005, ICNB 2006).

During an initial visit to one drainage gallery (40°20'36.95"N, 7°42'50.23"W, 985 m a.s.l.; Fig. 1) near Sazes da Beira (Seia) gallery in May 2010, several (not quantified) *R. iberica* were observed within this subterranean habitat. This particular gallery extended 25 m with walls, as well as some patches of the floor, partially covered by goblin's gold (*Schistostega pennata* (Hedw.) Web. & Mohr) (Fig. 1B). It has a door that is partially closed (with no light penetrating beyond about 5 m, when the door is opened), providing a true dark zone to this habitat. In addition, a small, shallow stream (average of 5 cm water depth) with low flow ran the length of the gallery. Water pH of 7.1 measured on 24 April 2011.

The unusual sighting of *R. iberica* motivated a series of subsequent visits that started in 2011: every three months until November 2011 and monthly between December 2011 and December 2012 to understand the use of this artificial subterranean habitat by this species. We obtained data on the activity cycle and reproductive period of *R. iberica* by noting first the presence of individuals inside the gallery, secondary sexual characters (nuptial pads in males) and breeding behaviour (individuals in amplexus), and the presence and developmental stage of larvae. Based on previous data (Crespo and Cei 1971, Brito-e-Abreu et al. 1994, Lesparre and Crespo 2008), we also generated polar coordinates, allowing a qualitative comparison between the activity of the population inside the gallery and the population of the Planalto Superior region (1400-1993 m a.s.l.).

Results and discussion

Phenology and subterranean biology of Rana iberica

All life stages were observed in the gallery during the study period, particularly adults, which were observed every month of the year (Table 1; Fig. 2–4). Adults were observed active both during the day and night often standing on the ground or in crevices (Fig. 3B), swimming underwater or floating, seeking refuge in holes or even climbing up the walls (Fig. 3C) (inactivity being defined by lethargy, habitually observed in hibernating frogs outside the gallery, found hidden under rocks during winter months). Individuals were mainly found beyond the first 5 m from the entrance, where the most intense daylight penetrates. These individuals often presented the lichen-shaped spots on their backs that are typical of this region (Fig. 3A; Malkmus 2004).

In both years of this study, the breeding season occurred between December and April (Fig. 2). Unfortunately, the little available data on the activity of *R. iberica* in Serra da Estrela is confined to the higher elevations (Planalto Superior, area above 1400 m and up to the summit). Although the gallery studied is located about 400 m below

Year	Month	Post-metamorphics			T	
		Males	Females	Juveniles	Larvae	Clutches of eggs
2010	May	+	+	+	+	+
2011	May	+	+	+	+	+
	August	9	6	2	1	0
	November	3	1	1	0	0
	December	+	+	-	-	-
2012	January	7	6	0	31	6
	February	+	+	+	+	+
	March	6	4	0	64	4
	April	8	4	0	52	2
	May	7	3	4	71	1
	June	6	4	6	12	0
	July	+	+	+	+	-
	August	8	5	8	7	0
	September	5	3	1	2	0
	October	+	+	+	-	-
	November	1	0	1	0	0
	December	+	+	+	-	-

Table 1. Absolute number of *Rana iberica* individuals (post-metamorphics or larvae) and clutches of eggs found per visit (month) inside a drainage gallery at Serra da Estrela Natural Park, Portugal. In some months data is restricted to *detected* (+) and *not detected* (-).

this limit, we noticed obvious differences in the activity patterns of these populations (Fig. 2). Because of very low temperatures, individuals seem to hibernate or reduce activity between December and February in the Planalto Superior population. Additionally, Crespo (1974) show in controlled experiments, that between 2-5°C there is no spermatogenic activity, which must be a reflex of what is happening in the natural habitat in these cool months. The breeding season usually begins in March (Spring) (Brito-e-Abreu et al. 1994, Lesparre and Crespo 2008); Lesparre and Crespo (2008) reported that *R. iberica* is active year-round in low elevation populations of the Serra da Estrela. However the altitudinal limit of year-round activity is unknown and no data are available on the breeding period. Nonetheless, the gallery population in the present study shows a pattern overall similar to other R. iberica populations occurring at lower elevation, described by Galán-Regalado (1982). Year-round activity of the gallery population may be related to the stability in environmental conditions occurring in this artificial subterranean habitat, which includes less variation in air temperatures and high relative humidity (Romero 2009). This may allow R. iberica to be active throughout the year and also have an extended breeding period with continuous gametogenic activity. Yet, phenology studies in lower altitude populations in Serra da Estrela would allow a better understanding of the biology of these underground residents.

Adults were found frequently in mating attempts and in amplexus. Occasionally, more than one male was seen trying to mate with a female, which has also been re-



Figure 2. Polar coordinates representing the activity cycle and breeding period of *Rana iberica* in two different sites (inside the drainage gallery in Sazes and the whole area of Planalto Superior) in Serra da Estrela; Portugal. Dark brown areas: post-metamorphic phase; beige areas: larval phase; green areas: adults in breeding activity.

ported by Ayres (2008), which seems to be more common than previously thought. Amplexus was not limited to nighttime. Mating occurred primarily in water (Fig. 3D), but also was observed on land (Fig. 3E) on the margins of the stream and on rocks mainly on the floor of the gallery.

Clumps of eggs with a gelatinous coat were generally laid and stuck to submerged rocks in the stream (except for one case: see Fig 5A and description below) and recently hatched tadpoles could be observed stationary above the egg mass for about two weeks (Fig. 4 A–B). Tadpoles were pale grey in coloration and became darker during the second week (Fig. 4C; Ayres 2009).



Figure 3. Adult individuals of *Rana iberica* found inhabiting a drainage gallery in Serra da Estrela, Portugal: A male with typical lichen-shaped pattern on the back B female hidden in a crevice of the gallery C male climbing up the wall D couple in axillary amplexus in water E axillary amplexus out of the water. Photos by Rosa GM.

Facultative oophagy

Oophagy was observed in this subterranean population of *R. iberica*. A clutch of eggs was probably laid in water, but got stranded when the water level presumably dropped. When we first noticed, the mass was already > 90% above the water surface (Fig. 5A), preventing normal development of the eggs. This situation turned the egg mass into a source of nutrients for free swimming tadpoles (at least three weeks old) from other clutches. Several tadpoles were observed feeding on the eggs



Figure 4. Egg and early life stages of *Rana iberica* inhabiting a drainage gallery in Serra da Estrela, Portugal: **A** egg mass stuck to underwater rock (28 January, 2012) **B** eggs' detail with new born tadpoles (one day old) **C** tadpole with dark pigmented colouration (Gosner stage 25; 11 March, 2012) **D** recently post-metamorphic individual (31 May, 2012). Photos by Rosa GM.

(Fig. 5B–E), a behaviour also reported for the first time for this species. Conspecific oophagy is a form of cannibalism (Miaud 1993) often associated with the intentional production of trophic eggs to feed young (e.g. Brust 1993, Gibson and Buley 2004), however this seems not to be the case. Nevertheless, facultative oophagy may be a more common and widespread habit of this species but also across other tadpoles of the same genus, that would take place when egg clutches are lost and larvae don't hatch.

Predation by fire salamander larvae

The gallery is also inhabited by other amphibians, including female *Salamandra sala-mandra gallaica* that use the underground spring to give birth to small larvae. During one visit to the gallery on the 29 April 2012, two predation events were recorded. Two *S. s. gallaica* larvae (post stage 46; Harrison 1969) were observed ingesting *R. iberica* tadpoles (stage between 26–30; Gosner 1960). In the first case, we observed a salamander larva slowing approach a tadpole and when the larva was less than a centimetre from the tadpole's flank (Fig. 6A), it lunged rapidly and seized the tadpole by the tail. A second salamander larva was found grasping a tadpole in its mouth with the hind



Figure 5. *Rana iberica* tadpoles feeding on lost clutch: **A** fresh egg mass of *Rana iberica* laid (mostly) above water surface (11 March, 2012) **B** group of tadpoles feeding on the dead eggs (29 April, 2012) **C**, **D** and **E** close ups of tadpole feasting on the eggs. Photos by Rosa GM.



Figure 6. *Salamandra salamandra gallaica* larvae predation upon *Rana iberica* tadpole on the 29 April (2012): **A** individual of salamander approaching tadpole instants before seizing it **B** salamander larval ingesting tadpole. Photos by Rosa GM.

part of the body (including the whole tail) already ingested (Fig. 6B) later that same day. These observations represent the first report of predation by Fire salamanders on Iberian brown frog. We believe that this is not a behaviour exclusive to subterranean habitats and that predation might occur more frequently in surface habitats shared by larvae of both species.

Final considerations

Anurans from the genus *Rana* appear to be the most common anuran visitors of caves and similar subterranean habitats, both in the Nearctic and the Palearctic regions (Resetarits 1986, Resetarits and Aldridge 1988, Bressi and Dolce 1999, Fenolio et al. 2005, Manenti 2008). Our observations represent the first record of *Rana iberica* reproduction in cave-like habitat and the fourth report of an anuran utilizing a cave or subterranean habitat during all stages of its life cycle for the Palearctic ecozone. It is unknown whether *R. iberica* exploits subterranean habitats elsewhere or why this particular population does so. Nearby breeding sites may have disappeared or experienced anthropogenic disturbance. For example, one of the artificial tanks was emptied during the summer of 2011 by the forestry services to be repaired, and frogs may have searched for alternative sites to lay their eggs, such as springs in drainage galleries.

Additionally, few other questions have been raised: is the presence of the species in the hypogean habitat temporal and accidental, or permanent? Does this apply to the entire population or only a part of it? Is the hypogean population in contact with the nearby epygean ones, and if so, where and when? A mark-recapture study (possibly by taking advantage of the characteristic dorsal pigment pattern of adult frogs) might help to shed some light on these questions, providing a better insight into the dynamics of this interesting population.

The Mediterranean region has experienced dramatic shifts in climate in the past (Luterbacher et al. 2006). This region has been identified as a hotspot of biodiversity but may face increased aridity in the future based on climate change projections (Giorgi 2006, Gao and Giorgi 2008) and intensive fire activity (Azul et al. 2010). The Iberian amphibian community may face huge ecological challenges (Bosch et al. 2007, Leadley et al. 2010, Carvalho et al. 2011) and artificial subterranean habitats, such as drainage galleries, may act as refugia for the survival of some populations.

Acknowledgements

We are grateful to Raoul Manenti, Truong Nguyen and Thomas Ziegler for the bibliographic support. A special thanks for everyone who supported the field study, particularly José Conde, Madalena Madeira, Telma Laurentino, Marta Sampaio and Diogo Veríssimo. Pedro Moreira for the insight about the frogs' activity. Jessica J. Scriven for the comments before submission. Finally, we are very grateful to both reviewers (Sebastiano Salvidio and anonymous) for their useful comments that significantly improved the manuscript.

References

Almeida NF, Almeida PF, Gonçalves H, Sequeira F, Teixeira J, Almeida FF (2001) Guia FAPAS Anfíbios e Répteis de Portugal. Porto: FAPAS.

- Andrade E, Mora C, Neves C, Vieira G (1992) Desportos de Inverno na Serra da Estrela. Contribuição para o estudo da sua viabilidade. Finisterra 53–54: 187–193.
- Ayres C (2008) Multiple amplexus in the Iberian brown frog *Rana iberica*. North-Western Journal of Zoology 4(2): 327–330.
- Ayres C (2009) Post-hatching behaviour of Iberian brown frog (*Rana iberica* Boulenger, 1879) tadpoles. Herpetology Notes 2: 141–142.
- Azul AM, Ramos V, Sales F (2010) Early effects of fire on herbaceous vegetation and mycorrhizal symbiosis in high altitude grasslands of Natural Park of Estrela Mountain (PNSE). Symbiosis 52: 113–123. doi: 10.1007/s13199-010-0103-1
- Baumgart G (1981) Observations sur l'hibernation de quelques amphibiens dans les anciennes mines vosgiennes: Salamandre tachetée (*Salamandra salamandra* Linné, 1758), grenouille rousse (*Rana temporaria* Linné, 1758) et crapaud commun (*Bufo bufo* Linné, 1758). Aquarama 58: 42–72.
- Biswas J, Shrotriya S (2011) Dandak: a mammalian dominated cave ecosystem of India. Subterranean Biology 8: 1–7. doi: 10.3897/subtbiol.8.1224
- Bonini L, Razzetti E, Barbieri F (1999) Cave breeding of *Bufo bufo* (Linnaeus, 1758) in Liguria (Nort Western Italy). In: Miaud C, Guyetant G (Eds) Current Studies in Herpetology. Proceedings of the 9th Ordinary General Meeting of the Societas Europaea Herpetologica 25–29 August 1998, Le Bourget du Lac, France. Le Bourget du Lac: SEH, 59–61.
- Bosch J, Carrascal LM, Durán L, Walker S, Fisher MC (2007) Climate change and outbreaks of amphibian Chytridiomycosis in a montane area of Central Spain; is there a link? Proceedings of the Royal Society B: Biological Sciences 274: 253–260. doi: 10.1098/ rspb.2006.3713
- Bosch J, Rincón, Boyero L, Martínez-Solano Í (2006) Effects of introduced salmonids on a montane population of Iberian frogs. Conservation Biology 20: 180–189. doi: 10.1111/j. 1523-1739.2005.00296.x
- Bressi N, Dolce S (1999) Osservazioni di anfibi e rettili in grotta. Rivista di Idrobiologia 38: 475–486.
- Brito-e-Abreu F, Moreira PL, Rosa HD, Paulo OS, Crespo EG (1994) Estudo da herpetofauna do Planalto Central da Serra da Estrela. Unpublished report of the Faculdade de Ciências da Universidade de Lisboa, Lisboa.
- Cabral MJ (coord.), Almeida J, Almeida PR, Dellinger T, Ferrand de Almeida N, Oliveira ME, Palmeirim JM, Queiroz AI, Rogado L, Santos-Reis M (Eds) (2005) Livro Vermelho dos Vertebrados de Portugal. Lisboa: Instituto da Conservação da Natureza.
- Carvalho SB, Brito JC, Crespo EG, Watts ME, Possingham HP (2011) Conservation planning under climate change: Toward accounting for uncertainty in predicted species distributions to increase confidence in conservation investments in space and time. Biological Conservation 144: 2020–2030. doi: 10.1016/j.biocon.2011.04.024
- Costa JC, Aguiar C, Capelo J, Lousá M, Neto C (1998) Biogeografia de Portugal Continental. Quercetea 0: 5–56.
- Costa LT, Nunes M, Geraldes P, Costa H (2003) Zonas importantes para as Aves em Portugal. Lisboa: Sociedade Portuguesa para o Estudo das Aves.

- Costa LT, Fidalgo JP, Neves R, Rufino R (2004) Lagoas do Planalto Superior da Serra da Estrela. Instituto da Conservação da Natureza / Centro de Zonas Húmidas.
- Crespo EG (1974) Observations sur l'influence de la temperature, en conditions expérimentales, sur le cycle spermatogenetique de *Rana iberica* Boul. Estudos sobre Fauna Portuguesa 2: 1–10.
- Crespo EG, Cei JM (1971) L'activité spermato-génetique saisonniere de *Rana iberica* Boul. du nord de Portugal. Arquivos do Museo Bocage (2ª Serie) 3: 37–50.
- Culver DC, Pipan T (2009) The biology of caves and other subterranean habitats. Oxford: Oxford University Press.
- Daveau S, Coelho C, Costa VG, Carvalho L (1977) Répartition et rythme des précipitations au Portugal. Memórias do Centro de Estudos Geográficos 3, Lisboa.
- Diesel R, Bäurle G, Vogel P (1995) Cave breeding and froglet transport: a novel pattern of anuran brood care in the Jamaican frog, *Eleutherodactylus cundalli*. Copeia, 1995: 354–360. doi: 10.2307/1446898
- Fenolio DB, Graening GO, Stout JF (2005) Seasonal movement patterns of Pickerel frogs (*Rana palustris*) in an Ozark cave and trophic implications supported by stable isotope evidence. Southwestern Naturalist 50: 385–389. doi: 10.1894/0038-4909(2005)050[0385:SMPO-PF]2.0.CO;2
- Galán-Regalado P (1982) Biología de la reproducción de *Rana iberica* Boulenger, 1879 en zonas simpátridas con Rana temporaria Linneo, 1758. Doñana, Acta Vertebrata 9: 85–98.
- Galán-Regalado P (1988) Segregación ecológica en una comunidad de ofidios. Doñana, Acta Vertebrata 15: 59–78.
- Gao X, Giorgi F (2008) Increased aridity in the Mediterranean region under greenhouse gas forcing estimated from high resolution simulations with a regional climate model. Global and Planetary Change 62(3-4): 195–209. doi: 10.1016/j.gloplacha.2008.02.002
- Gibson RC, Buley KR (2004) Maternal care and obligatory oophagy in *Leptodactylus fallax*: A new reproductive mode in frogs. Copeia 2004: 128–135. doi: 10.1643/CE-02-091R2
- Giorgi F (2006) Climate change hot-spots. Geophysical Research Letters 33: 1–4. doi: 10.1029/2006GL025734
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16(3): 183–190.
- Harrison RG (1969) Harrison stages and description of the normal development of the spotted salamander, *Ambystoma punctatum* (Linn.). In: Wilens S (Ed) Organization and Development of the Embryo. New Haven, London: Yale University Press, 44–66.
- Hoffmann L (2002) Caves and other low-light environments: aerophytic photoautotrophic microorganisms. In: Bitton G (Ed) Encyclopedia of environmental microbiology. New York, NY: John Wiley & Sons, 171–177.
- Ianc R, Cicort-Lucaci AŞ, Ilieş D, Kovács ÉH (2012) Note on the presence of Salamandra salamandra (Amphibia) in caves from Padurea Craiului Mountains, Romania. North-Western Journal of Zoology 8: 202–204.
- ICNB (2006) *Chioglossa lusitanica.* In: Plano Sectorial da Rede Natura 2000 (Vol.II Valores Naturais). Lisboa: Instituto da Conservação da Natureza e da Biodiversidade. Available at:

http//www.icn.pt/psrn2000/caracterizacao_valores_naturais/FAUNA/anfibios_repteis/ Chioglossa%20lusitanica.pdf. Access June 2012.

- Jansen J (2002) Guia geobotânico da Serra da Estrela. Lisboa: Parque Natural da Serra da Estrela / Instituto da Conservação da Natureza.
- Jansen J, Sequeira MPSM (1999) The vegetation of shallow waters and seasonally- inundated habitats (Littorelletea and Isoeto-Nanojuncetea) in the higher parts of the Serra da Estrela, Portugal. Mitteilungen des Badischen Landesvereins für Naturkunde und Naturschutz 17: 449–462.
- Kami HG (2004) The Biology of the Persian mountain salamander, *Batrachuperus persicus* (Amphibia, Caudata, Hynobiidae) in Golestan Province. Asiatic Herpetological Research 10: 182–190.
- Lanza B, Pastorelli C, Laghi P, Cimmaruta R (2006) A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). Atti del Museo Civico di Storia Naturale Trieste (Suppl.) 52: 5–135.
- Leadley P, Pereira HM, Alkemade R, Fernandez-Manjarrés JF, Proença V, Scharlemann JPW, Walpole MJ (2010) Biodiversity Scenarios: Projections of 21st century change in biodiversity and associated ecosystem services. Secretariat of the Convention on Biological Diversity, Montreal. Technical Series no. 50, 132 p.
- Lesparre D, Crespo EG (2008) A herpetofauna do Parque Natural da Serra da Estrela. Seia: CISE.
- Lima AMX, Araújo CO, Verdade VK (2012) *Cycloramphus eleutherodactylus* (Alto button frog): Calling among rocks and caves. Herpetological Bulletin 120: 39–42.
- Loureiro A, Almeida NF, Carretero MA, Paulo OS (Eds) (2008) Atlas dos Anfíbios e Répteis de Portugal. Lisboa: Instituto da Conservação da Natureza e da Biodiversidade.
- Luterbacher J, Xoplaki E, Casty C, Wanner H, Pauling A, Küttel M, Rutishauser T, Brönnimann S, Fischer E, Dominik F, Gonzalez-Rouco FJ, Garcia-Herrera R, Barriendos M, Rodrigo F, Gonzalez-Hidalgo JC, Saz MA, Gimeno L, Ribera P, Brunet M, Paeth H, Rimbu N, Felis T, Jacobeit J, DünkelohA, Zorita E, Guiot J, Türkes M, Alcoforado MJ, Trigo R, Wheeler D, Tett S, Mann ME, Touchan R, Shindell DT, Silenzi S, Montagna P, Camuffo D, Mariotti A, Nanni T, Brunetti M, Maugeri M, Zerefos C, De Zolt S, Lionello P, Nunes MF, Rath V, Beltrami H, Garnier E, Ladurie ELR (2006) Mediterranean climate variability over the last centuries. A review. Pp. 27–148 in Lionello P, Malanotte-Rizzoli P, Boscolo R (Eds) Mediterranean Climate Variability. Amsterdam: Elsevier.
- Malkmus R (2004) Amphibians and Reptiles of Portugal, Madeira and the Azores-Archipelago: Distribution and Natural History Notes. Ruggell: A.R.G. Gantner Verlag.
- Manenti R (2008) Amphibiens des sources et galeries drainantes en territoire prealpin, l'exemple du Mont Barro et du Mont de Brianza (Lombardie, Italie). Bulletin de la Société Herpétologique de France 128: 25–40.
- Manenti R, Ficetola GF, Bianchi B, De Bernardi F (2009) Habitat features and distribution of *Salamandra salamandra* in underground springs. Acta Herpetologica 4: 143–151.

- Miaud C (1993) Predation of newt eggs (*Triturus alpestris* and *T. helveticus*): Identification of predators and protective role of oviposition behavior. Journal of Zoology 231: 575–582. doi: 10.1111/j.1469-7998.1993.tb01939.x
- McClure EH, Lim BL, Winn SE (1967) Fauna of dark cave, Batu Caves, Kuala Lumpur, Malaysia. Pacific Insects 9(3): 399–428.
- Morales JJ, Lizana M, Acera F (2004) Ecología trófica de la nutria paleártica *Lutra lutra* en el río Francia (Cuenca del Tajo, Salamanca). Galemys 16(2): 57-77.
- Moreira PL, Almeida AP, Rosa HD, Paulo OS, Crespo EG (1999) Bases para a conservação da Lagartixa-da-montanha (*Lacerta monticola*). Estudos de Biologia e Conservação da Natureza 25. Lisboa: Instituto da Conservação da Natureza.
- Penado A, Sampaio M, Madeira M, Selfa J, Rosa GM (in press) Where to spend the winter in Serra da Estrela? The first record of *Diphyus quadripunctorius* (Mueller, 1776) hibernating in a cave habitat in Portugal. Entomological News.
- Presetnik P, Dular M, Koselj K (2002) Opazovanja dvoživk v jamah. Temporaria 8: 30-33.
- Resetarits Jr WJ (1986) Ecology of cave use by the frog *Rana palustris*. American Midland Naturalist 116: 256–266. doi: 10.2307/2425733
- Resetarits Jr. WJ, Aldridge RD (1988) Reproductive biology of a cave-associated population of the frog *Rana palustris*. Canadian Journal of Zoology 66: 329–333. doi: 10.1139/z88-049
- Rey JM, Esteban M, Sanchíz B (1994) Registros corológicos de anfibios españoles fundamentados en egagrópilas de lechuza común. Boletín de la Asociación Herpetológica Española 5: 4–8.
- Rogowitz LG, Candelaria CL, Denizard LE, Meléndez LJ (2001) Seasonal reproduction of neotropical, the cave coquí (*Eleutherodactylus cooki*). Copeia, 2001: 542–547. doi: 10.1643/0045-8511(2001)001[0542:SROANF]2.0.CO;2
- Romero A (2009) Cave Biology: Life in darkness. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511596841
- Salvador A, García-París M (2001) Anfibios Españoles. Talavera de la Reina: Canseco-Esfagnos.
- Sequeira F, Gonçalves H, Faria MM, Meneses V, Arntzen JW (2001) Habitat-structural and meteorological parameters influencing the activity and local distribution of the goldenstriped salamander, *Chioglossa lusitanica*. Herpetological Journal 11: 85–90.
- Teles AN (1970) Os lameiros de montanha do norte de Portugal. Agronomia Lusitanica, 31: 5–132.
- Uhrin M, Lesinsky G (1997) Mechanism of occurrence of amphibians in an underground spaces in Slovakia: preliminary data evaluation. Proceedings of the 12th International Congress of Speleology, La Chaux de fonds Switzerland 3: 325–327.
- Vieira GT, Mora C (1998) General characteristics of the climate of Serra da Estrela. In: Vieira GT (Ed) Glacial and Periglacial geomorphology of the Serra da Estrela. Guidebook of the field-trip of the IGU Commission on Climate Change and Periglacial Environments. Lisboa: CEG and Department of Geography, 26–32.

RESEARCH ARTICLE



Population size and dispersal patterns for a Drimeotus (Coleoptera, Leiodidae, Leptodirini) cave population

Akos Fejér¹, Oana Teodora Moldovan²

l Piața Abator 8-9/4, 400137 Cluj Napoca, Romania **2** "Emil Racovitza" Institute of Speleology, Romanian Academy, Clinicilor 5, 400006 Cluj Napoca, Romania

Corresponding author: Oana Teodora Moldovan (oanamol@hasdeu.ubbcluj.ro)

Academic editor: Achille Casale | Received 5 March 2013 | Accepted 13 June 2013 | Published 14 July 2013

Citation: Fejér A, Moldovan OT (2013) Population size and dispersal patterns for a *Drimeotus* (Coleoptera, Leiodidae, Leptodirini) cave population. Subterranean Biology 11: 31–44. doi: 10.3897/subtbiol.11.4974

Abstract

Drimeotus viehmanni (Coleoptera, Leiodidae) is abundant in the cave Peştera cu Apă din Valea Leşului (Western Carpathians) and was chosen for a mark-release-resight experiment. The aims of the experiment were to estimate the size of the population and to analyze the dispersal patterns inside the cave, for conservation purposes. During the three years' study, the observed abundance of *D. viehmanni* was significantly higher in summer compared to the winter season. The seasonal dynamics can not be explained by climate features such as temperature and air relative humidity which had low or no variation during all seasons. Few marked beetles were re-seen during the mark-resight experiment proving the existence of an important cave/subterranean population, which was estimated between 5,084 and 533,033 individuals. The marked individuals moved between neighbouring patches on a distance of 10 m over the same amount of time as on distances longer than 200 m. Dispersal inside the cave occurs during the winter months, which indicates non-continuous behaviour triggered by environmental features and involving only a negligible part of the population in the studied cave.

Keywords

Cave beetles, population dynamics, mark-resight, migration, cave climate, Carpathians, Romania

Introduction

Estimation and prediction of population sizes have been a challenge and a must of many ecological studies (Freckleton et al. 2006). There have been some endeavours to determine population growth, density regulation, and environmental variability (Hovestadt and Nowicki 2008). Moreover, in a world dominated by the need to protect vulnerable species (Crandall et al. 1999), the estimation of the population/species abundance became an important step towards the correct assessment of the need for rare species conservation.

Although the total number of cave species has been estimated to 50,000-100,000 (Culver and Holsinger 1992), few studies deal with estimations of cave populations and few mark-recapture experiments have been undertaken until present (Cabidoche 1966, Keith 1975, Peck 1975, Delay 1978, Hobbs 1978, Racovitza 1980, Hobbs 1981, Carchini et al. 1982, Tercafs and Brouwir 1991, Rusdea 1992, Carchini et al. 1994, Bernardini et al. 1996, Simon 1997, Knapp and Fong 1999, Willemart and Gnaspini 2004, Cooper and Cooper 2009, Venarsky et al. 2012). The aims of these studies were to census subterranean populations and to date the longevity of cave species. In most of them, seasonal variability has been documented and different explanations were proposed.

The use of the mark-recapture methods to evaluate subterranean beetle populations is problematic due to the difficult access to populations, and both long life and low reproduction rates of their representatives (Delay 1978). The estimated population of *Aphaenops loubensis* Jeannel, 1953 in one station of the Pierre-Saint Martin system (France) was of 100 to 400 individuals (Cabidoche 1966, Delay 1978). *Speonomus infernus* (Dieck, 1869) from the Saint Catherine Cave (France) has been estimated in one of the cave chambers at 300 individuals (Juberthie 1969, Delay 1978). Delay (1978) estimated the population of *S. longicornis* (Saulcy, 1872) from the Pigailh Cave (France) at 50,000 individuals. In Romania, the number of *Pholeuon proserpinae glaciale* Jeannel, 1923 in the Scărișoara Ice Cave was estimated at 33,000 individuals (Racovitza 1980).

The aim of our study was to analyse the patterns of distribution for a beetle population inside one cave and in correlation to topoclimate variations, and to estimate the cave population size by using the mark-resight method. The results on both population size and dispersal behaviour of a subterranean beetle population are the starting point in further studies about the impact of the touristic infrastructure on cave inhabitants.

We chose *Drimetous* s. str. *viehmanni* (Ienistea, 1955) (Coleoptera, Leiodidae, Leptodirini) from the cave Peştera cu Apă din Valea Leşului for the mark-resight experiment. *Drimeotus* s. str. endemic for the Pădurea Craiului Mountains (North-Western Romania) is represented by 14 species most of them endemic for one cave system or a complex of caves in one valley (Moldovan 2000). *D. viehmanni* is abundant inside the cave Peştera cu Apă din Valea Leşului all year round, with variations from summer to winter months (Racoviță and Şerban 1975). Previous researches found no correlation between population dynamics and the cave climate.

Methods and materials

Research protocol

The study on the cave population of *D. viehmanni* was carried out in the Peştera cu Apă din Valea Leşului, between March 2005 and November 2007, with a total of 25 visits in the cave. The time lap between two visits was no longer than one and a half month, with one exception during autumn 2006.

The Peştera cu Apă din Valea Leşului is located in the eastern part of the Western Carpathians (North-Western Romania) at an altitude of 700 m a.s.l. The cave has one main gallery in total length of 1265 m (Cocean 1995), with a permanent subterranean stream. Five stations (I -V) were established inside the cave (Fig. 1). The first station was located 160 meters from the entrance inside the cave and the others at distances between 385 and 420 meters inside the cave. They consist of a bait (salami) placed directly on the substrate and fixed with a rock, as described in other studies (Cabidoche 1966, Juberthie 1969, Delay 1978, Racovitza 1980). The number of the individuals present at each station has been counted monthly and the bait has been replaced at every counting time.

The cave microclimate is typical for a horizontal cave with one entrance; two different air flow systems, caused by temperature differences between cave and surface, are influencing the winter and the summer cave climate (Racoviță and Cocean 1977). The air temperature and the air relative humidity were measured in front of the cave (S), at the cave entrance (E) and in every station (I-V) (Fig. 1) at each visit. An Assmann type psychrometer was used for both the air temperature and relative air humidity measurements. The data have been transformed using an online converter (http://www.bom. gov.au/lam/humiditycalc.shtml).

Population size estimation

A mark-release-resight method was used for the estimation of the population abundance (N). During the first counting, every individual elytra was marked with nontoxic paint (as described in Delay 1978). The estimated population size was estimated using the Lincoln-Peterson index, which has high variability and considerably high error. To reduce this tendency to overestimate, Bailey (1951, 1952) proposed a corrected formula:

N = M(n + 1) / m + 1

where: N = the actual size of the study population, M = the number of individuals marked and released, m = the number of marked individuals in a sample of the population, n = the total number of individuals in the sample.

The mark-resight method is different from the traditional mark-recapture methods, in that it includes data on encounters of marked individuals and of the unmarked individuals. Individuals are marked prior to sampling which consists of sighting sur-



Figure 1. Peștera cu Apă din Valea Leșului (modified after Cocean 1995), with the position of the cave in Romania and the sites for climatic measurements (red) and fauna counting (green): I-V stations; S = surface; E = entrance.

veys instead of capture periods. The main advantage of this approach is that it is less invasive than the traditional mark-recapture (McClintock and White 2009).

328 individuals were marked the first time and 1065 individuals the second time. The individuals were marked in March 2005 at station II and in July 2005 at station III. Two different colours have been used, as follows: yellow at station II and blue at station III. The two points are on the opposite banks of the subterranean stream.

Statistical analyses

Non-parametric tests, Kruskal-Wallis (KW) and Mann-Whitney (MW), were used for the seasonal analysis of the air temperature, the relative air humidity and the number of individuals. All analyses were conducted using R 2.10.1. statistical software (R Development Core Team 2009).

Results

Seasonal dynamics

The amplitude of the air temperature in the Peştera cu Apă din Valea Leşului decreases inwards the cave, from 10.5°C at the surface to 8.3°C at station V (both are mean values for the period October 2006-November 2007). The temperature inside the cave (stations I-V) had no significant seasonal variation. There is no significant difference between the five cave stations (MW = 361, p = 0.13) in terms of air temperatures by comparing the winter months (December, January and February) to the summer months (June, July, August) (Fig. 2). The air relative humidity has larger variations outside the cave and at the entrance, while inside higher values were measured in all



Figure 2. Box-plot of the air temperature, during winter and summer months, at the surface (S), the entrance (E) and the five stations (I-V) for Peştera cu Apă din Valea Leşului.



Figure 3. Box-plot of the relative air humidity at the surface (S), the entrance (E) and the five stations (I–V) for Peştera cu Apă din Valea Leşului.

seasons (Fig. 3). The air relative humidity varied seasonally in all stations and there is a significant difference between data recorded at the five stations inside the cave (KW: $chi^2 = 10.5$, df = 4, p = 0.03).

During the study, the highest abundances of *D. viehmanni* were recorded at stations II and III (Fig. 4, Table 1). At station II, most individuals were counted in September 2007 (3,000 individuals), and there were only three occasions when the number of beetles was under 100 (November 2005, October 2006 and November 2007). At station III, the highest number of individuals was counted in June 2006 (1,700 individuals). In this station there were more than ten occasions when the total number of individuals was lower than 100, especially during the autumn-winter months. A reduced number of beetles was recorded at station I, the closest to the entrance. Here, the



Figure 4. Monthly variation of the number of individuals of *D. viehmanni* in the five stations (I–V) of Peştera cu Apă din Valea Leşului.



Figure 5. Box-plot of the number of individuals of *D. viehmanni*, in the winter and the summer months, in the five stations (I–V) of Peştera cu Apă din Valea Leşului.

maximum number was 65, and on five occasions *D. viehmanni* was absent. The stations IV and V were added later in our study and they had similar seasonal dynamics with a drastic increase in the number of individuals during the summer months (Fig. 4).

When comparing the number of individuals, a significant difference between the five stations (KW: $chi^2 = 30.2$, df = 4, p < 0.001) can be noticed (Fig. 5). Because station I had a low number of beetles, we compared only the other four stations, but the difference remained significant (KW: $chi^2 = 8.9$, df = 3, p = 0.03).
M	Sta	tion I	Sta	tion II	Stat	tion III	Stat	ion IV	Stat	tion V
Month	Total	Marked	Total	Marked	Total	Marked	Total	Marked	Total	Marked
Mar-05			328	328						
May	45	-	437	12	83	-				
June	50	-	30	1	700	-				
July	65	-	530	10	1065	1065				
August	15	-	475	3	350	9				
October	0	-	125	-	25	1				
November	15	-	150	-	30	-				
December	20	-	150	-	5	-				
Jan-06	30	-	120	-	40	2				
February	15	-	475	2	325	1				
March	25	-	900	1	1000	1				
April	47	-	1800	-	1200	2	500	1	600	-
June	40	-	0	-	1700	-	500	-	500	-
October	0	-	4	-	1	-	1	-	0	-
December	60	1	400	-	1	-	1	-	70	-
Jan-07	25	1	300	-	15	1	0	-	60	-
February	50	-	200	-	150	-	0	-	200	-
March	40	-	400	-	1000	-	0	-	350	-
April	15	-	1000	-	900	-	18	-	1100	-
May	1	-	350	-	400	-	70	-	200	-
June	0	-	1500	-	1000	-	400	1	1000	-
August	6	-	1300	-	700	-	600	-	600	-
September	0	-	3000	-	50	-	15	-	100	-
October	0	-	400	-	50	-	15	-	150	-
November	20	-	60	-	3	-	10	-	3	-

Table 1. Total number and number of marked individuals of *D. viehmanni* from Peştera cu Apă din Valea Leşului, during the period March 2005 – November 2007.

For a better understanding of the seasonal behaviour of the studied species, we separated the data recorded in winter from the data recorded during the summer months (Fig. 5). The results show an important difference between the two seasons in all the stations, except station I where more individuals were recorded in the winter months (MW = 101, p = 0.002).

Population size

Estimates of the population size in the Peştera cu Apă din Valea Leşului were obtained from the mark-resight data (Table 1). At station II, from the 328 individuals marked in March 2005, the highest number of recaptured individuals was 12, in May 2005. The re-seen marked individuals decreased from month to month and hereby in June 2005 there were 11 beetles marked from the total of 30 observed beetles. According



Figure 6. The evolution of the *D. viehmanni* abundance in two stations of Peştera cu Apă din Valea Leşului.



Figure 7. The migration routes of *D. viehmanni* inside Peştera cu Apă din Valea Leşului: yellow = mark for individuals at station II, blue = mark for individuals at station III.

to these data the estimated population size at station II varied between 5,084 and 147,764 (Fig. 6).

At station III, from the 1,065 marked beetles most individuals were observed in the next month (9 marked individuals). Afterwards, there were just one or two marked beetles at the bait. With the Lincoln-Petersen formula the lowest estimated size of the population at station III was of 13,845 and the highest was of 533,033 individuals (Fig. 6).

Only 5 marked beetles were found in a different station (Table 1). One yellow marked individual was observed after one and a half year at station I. A blue marked individual was observed one year and five months later at the same station. A third

one (yellow marked) was observed at station III after one year and seven months from the marking. Two individuals marked with blue were observed in station IV after 9 months and after almost 2 years, respectively. The covered distances were 225 m (between stations II and I), 260 m (between stations III and I), 35 m (between stations II and III) and 10 m (between stations III and IV), respectively. In their travel, the yellow marked individuals had to cross the stream between stations II and III (Fig. 7).

Discussion

The temperature in the studied cave oscillated from 8 to 9.4°C, higher than during the period 1972–1974 (Racoviță and Cocean 1977), when the temperature variations were between 7.6 and 8.4°C. This might suggest a warming of the cave climate with a possible ecological effect on the invertebrate populations from the Peştera cu Apă din Valea Leşului. The warming inside the cave follows the general raise of surface temperature in the last decades, knowing the fact that the mean cave temperature in deep zones is equal to the mean annual temperature at the surface.

The observed number of *D. viehmanni* was much higher during our study than during the period 1972–1974. In the previous study, Racoviță and Şerban (1975) recorded the maximum of 410 individuals in the corresponding station IV, and 150 individuals in the corresponding station V. In our study, the maximum abundances were of 600 individuals at station IV, and of 1100 individuals at station V. At this stage of our research we are not able to provide a reliable explanation for such an increase. It can be due to the total growth of the population of *D. viehmanni* in the entire system of Peştera cu Apă din Valea Leşului due to the natural dynamics of the species, or it can be the result of increased migrations from the surface habitats caused by environmental changes in the general context of climate warming. Another possible explanation lies in the changes of the surface, intensively deforested in the last two decades, with a consequent drop of humidity in soil/subsurface habitats.

The fluctuation of the number of individuals presented the same seasonality as in the cases of Pigailh Cave (Delay 1978), Sainte Catherine Cave (Juberthie 1969), Scărișoara Ice Cave (Racovitza 1980) and previous study of the Peștera cu Apă din Valea Leșului (Racoviță and Șerban 1975). A much higher number of individuals was recorded during the summer months compared to the winter ones. *D. viehmanni* is not influenced by temperature and air relative humidity because these parameters showed no significant difference between the two seasons in the same station, as was also suggested by Racovitza (1980). This author also suggested that the reproduction cycle cannot entirely explain the seasonal pattern of abundance. Therefore, we propose here another explanation that is the impact of surface seasons on the migrations between the voids network (including the cave) and the subsurface habitats. Colder months have high precipitation rates thus creating suitable conditions also in subsurface habitats, while during warmer months superficial habitats are drier and populations migrate towards deep habitats. *Drimetous* is known as a versatile genus and highly mobile in the entire subterranean environment, able to survive in subsurface humid habitats as well as in deep caves, probably preferring superficial habitats in wetter conditions.

The estimated population size presents high variability (between 5,084 and 147,764 individuals at station II and between 13,845 and 533,033 individuals at station III) inferred from the small number of recaptured beetles. The low resolution of this estimation is similar to that observed for Speonomus and Pholeuon representatives (Cabidoche 1966, Juberthie 1969, Delay 1978, Racovitza 1980). By considering the mean value for the entire cave population, we can estimate an abundance of 122,464 individuals, with a lower number in station II (45,135 individuals, similar to the estimation for other populations of Leptodirini; Delay 1978, Racovitza 1980), and higher in station III (199,794 individuals). The mark-resight method is subjected to high errors and the correct method would be to mark the individuals on every occasion they are counted, but this might be of negative impact on the survival of individuals. There are some assumptions of the simple Lincoln-Petersen mark-recapture (resight) methods: (1) the subset of selected population is representative of the entire population in terms of sighting probabilities; this is the fundamental assumption of the mark-resight method (McClintock 2013). It is impossible to know the population distribution in the entire subterranean system and to be sure that the estimation of the population size is due only to the cave population or it is also the contribution of other populations that live in the system. Given the high number we obtained it probably reflects the size of the entire population at the level of the entire system. It must be also emphasized that Peștera cu Apă din Valea Leșului is a special case among Romanian caves for the extremely abundant population inside the cave; (2) the population is close to addition and deletion; (3) marks are not lost or overlooked; (4) there are equal chances of "catchability" of animals. We assume that the number of births equals the number of deaths in this population. The finding of marked individuals 2 years after the marking can be the proof of a lasting paint and of survival of the individuals.

The metapopulation concept provides explanations for how species can survive in fragmented landscapes, such as subterranean habitats (Moldovan et al. 2012). Movement of individuals between spatially separated populations ensures the survival of the entire metapopulation and re-colonisation of the patches where local populations have gone extinct (Hanski and Gilpin 1997, Hanski 2001). Animal movement and the factors that can affect it must be considered for the conservation of highly fragmented populations and management of show caves. The number of migrants between habitat patches is expected to decrease with increasing patch isolation because of the dilution effect associated with the spread of individuals in space (Ims 1995), the mortality of individuals in time (Hanski 1999) and the limited dispersal ability of the beetles (Baur et al. 2005). The obtained results suggest that the beetles from the Peştera cu Apă din Valea Lesului moved between neighbouring patches on a distance of 10 m over the same amount of time as on distances longer than 200 m. The subterranean stream was not a barrier for beetles and proves the connection between cave sub-populations of D. viehmanni. Individuals of this species are able to cover large areas in long periods of time. The season of dispersal is in winter and migration of the few cave beetles indicates a behaviour which is not continuous but triggered by environmental features and involves only a negligible part of the population.

Although dispersal is critical for the population persistence, particularly for small populations (Den Boer 1979) and in fragmented habitats (Tilman et al. 1994, 1997, Hanski 1999), it seems that cave beetle populations are stenotopic if there are no environmental constraints. If they find a proper place with more or less continuous input of nutrients, migrations are occasional. The differences between patches in a heterogeneous system such as the subterranean environment could be explained by differences in habitat quality, by presence/absence of other species, and by patch size. In the case of the studied population of *D. viehmanni*, whith individuals that are always concentrated in few sectors of the cave, the differences can be given by the points of nutrients input. The patches where this cave species is extremely numerous are near natural narrow pits with contact with the surface (station III), where diffuse flow of water from the surface brings food underground.

Data on dispersal of subterranean beetles are rare, despite the theoretical and practical importance for the conservation of these highly endemic taxa. Knowledge of the movement rules can predict the dispersal capacity and patchwise migration rates in complex landscapes (Baur et al. 2005), such as subterranean environments. At local scales, the lack of migration of subterranean populations would isolate some of them and processes of speciation would occur. However, such processes are known to occur only at higher spatial levels of the subterranean heterogeneous environment. Subterranean populations can persist only if the dispersal rate is sufficient to maintain genetic variability against random genetic drift (Baur et al. 2005). Genetic markers may confirm the described pattern for cave beetles and further researches should concentrate on such studies.

Acknowledgements

We thank to Fejér Anna, Milik Csongor and Popa Flaviu for the field assistance. The suggestions of Mauro Rampini and Valerio Sbordoni improved the quality of the manuscript. OTM was supported by the Romanian Academy.

References

- Bailey NTJ (1951) On estimating the size of mobile populations from recapture data. Biometricka 38: 293–306.
- Bailey NTJ (1952) Improvements in the interpretation of recapture data. Journal of Animal Ecology 21: 120–27. doi: 10.2307/1913
- Baur B, Coray A, Minoretti N, Zschokke S (2005) Dispersal of the endangered flightless beetle Dorcadion fuliginator (Coleoptera: Cerambycidae) in spatially realistic landscapes. Biological Conservation 124: 49–61. doi: 10.1016/j.biocon.2004.12.011

- Bernardini C, Di Russo C, Rampini M, Cersaroni D, Sbordoni V (1996) A recent colonization of *Dolichopoda* cave crickets in the Poscola cave (Orthoptera, Rhaphidophoridae). International Journal of Speleology 25: 15–31. doi: 10.5038/1827-806X.25.1.2
- Cabidoche M (1966) Contribution à la connaisance de l'écologie des Trechinae cavernicoles pyrénéens. Thèse Faculté des Sciences Paris, 228 pp.
- Carchini G, Rampini M, Sbordoni V (1982) Congruence between mark-recapture and plot density estimates. International Journal of Speleology 12: 29–36. doi: 10.5038/1827-806X.12.1.3
- Carchini G, Rampini M, Sbordoni V (1994) Life cycle and population ecology of the cave cricket *Dolichopoda geniculata* (Costa) from Valmarino cave (Central Italy). International Journal of Speleology 23: 203–218. doi: 10.5038/1827-806X.23.3.6
- Cocean P (1995) Peșterile României, Editura Dacia.

42

- Cooper JE, Cooper MR (2009) Long-term mark-recapture studies of population sizes in the stygobiotic crayfishes (Decapoda: Cambaridae) of Shelta Cave, Alabama, USA. Subterranean Biology 7: 35–40.
- Crandall, KA, Posada D, Vasco D (1999) Effective population sizes: missing measures and missing concepts. Animal Conservation 2: 317–319. doi: 10.1111/j.1469-1795.1999. tb00078.x
- Culver DC, Holsinger JR (1992) How many species of troglobites are there? National Speleological Society Bulletin 54: 79–80.
- Delay B (1978) Milieu souterrain et écophysiologie de la reproduction et du developpement des coléoptères Bathysciinae hypogés. Mémoires de Biospeologie 5: 1–349.
- Deleurance-Glaçon S (1963) Recherches sur les coléoptères troglobies de la sous-famille de Bathysciinae. Annales des Sciences Naturelles, Zoologie 5: 1–172.
- Den Boer PJ (1979) The significance of dispersal power for the survival of species, with special reference to the carabid beetles in a cultivated countryside. Fortschrifte der Zoologie, 25: 79–94.
- Freckleton RP, Watkinson AR, Green RE, Sutherland WJ (2006) Census error and the detection of density dependence. Journal of Animal Ecology 75: 837–851. doi: 10.1111/j.1365-2656.2006.01121.x
- Hanski I (1999) Metapopulation Ecology. Oxford University Press, Oxford.
- Hanski IA (2001) Population dynamic consequences of dispersal in local populations and in metapopulations. Pp. 283–298 *in* Clobert J, Danchin E, Dhondt AA, Nichols JD (Eds) Dispersal, Oxford University Press, Oxford.
- Hansk I, Gilpin ME (1997) Metapopulation Biology. Academic Press, London.
- Hobbs HH III (1978) Studies of the cave crayfish, Orconectes inermis inermis Cope (Decapoda, Cambaridae). Part IV: Mark-recapture procedures for estimating population size and movements of individuals. International Journal of Speleology, 10: 303–322. doi: 10.5038/1827-806X.10.3.7
- Hobbs HH III (1981) Investigations of the troglobitic crayfish Orconectes inermis testii (Hay) in Mayfield's Cave, Monroe County, Indiana. International Journal of Speleology 11: 21–32. doi: 10.5038/1827-806X.11.1.4
- Hovestadt T, Nowicki P (2008) Process and measurement errors of population size: their mutual effects on precision and bias of estimates for demographic parameters. Biodiversity and Conservation 14: 3417–3429. doi: 10.1007/s10531-008-9426-0

- Ims RA (1995) Movement patterns related to spatial structures. Pp. 85–109 In Hansson L, Fahrig L, Merriam G (Eds) Mosaic Landscapes and Ecological Processes, Chapman and Hall, London. doi: 10.1007/978-94-011-0717-4_4
- Juberthie C (1969) Rélations entre le climat, le microclimat et les *Aphaenops cerberus* dans la grotte Saint Catherine (Ariége). Annales de Spéléologie 24: 75–104.
- Keith JH (1975) Seasonal changes in a population of *Pseudanopthalmus tenuis* (Coleoptera, Carabidae) in Murray Spring Cave, Indiana: a preliminary report. International Journal of Speleology 7: 33–44. doi: 10.5038/1827-806X.7.1.4
- Knapp SM, Fong DW (1999) Estimates of Population Size of Stygobromus emarginatus (Amphipoda: Crangonyctidae) in a Headwater Stream in Organ Cave, West Virginia. Journal of Cave and Karst Studies 61: 3–6.
- McClintock BT, White GC (2009) A less field-intensive robust design for estimating demographic parameters with mark–resight data. Ecology 90: 313–320. doi: 10.1890/08-0973.1
- McClintock BT (2013) Mark-resight models. Pp. 343–360 *in*. Cooch EG, White GC (Eds) Program MARK - gentle introduction, 13th edition, http://www.phidot.org/software/ mark/docs/book/
- Moldovan O (2000) Révision de *Drimeotus* s.s. Miller, 1856 (Coleoptera, Cholevidae, Leptodirinae) de Transylvanie (Roumanie) avec description de deux nouvelles espèces et clé de détermination des taxa. Zoosystema 22: 139–152.
- Moldovan OT, Meleg IN, Persoiu A (2012) Habitat fragmentation and its effects on groundwater populations. Ecohydrology 5: 445–452. doi: 10.1002/eco.237
- Peck SB (1975) A population study of the cave beetle *Ptomaphagus loedingi* (Coleoptera; Leiodidae; Catopinae). International Journal of Speleology 7: 19–32. doi: 10.5038/1827-806X.7.1.3
- R Development Core Team (2007) R: a language and environment for statistical computing Vers. 2.5.0. R. Foundation for Statistical Computing, Vienna.
- Racoviță G, Cocean P (1977) Recherches climatologiques dans la Grotte de Valea Leșului. Travaux de institut de Speologie "Emil Racoviță" 16: 183–201.
- Racoviță G, Şerban M (1975) Recherches sur la biologie de la population de coléoptères cavernicoles de la Peștera cu apă din Valea Leșului. Annales de Spéléologie, 30 : 351–363.
- Racovitza G (1980) Étude écologique sur les coléoptères Bathysciinae cavernicoles. Mémoire de Biospéologie 6: 1–199.
- Rusdea E (1992) Stabilisierende Selektion bei mikrophthalmen Höhlentieren: Untersuchungen zur tageszeitlichen Aktivitätsverteilung und Populationsdynamik von *Laemostenus schreibersi* Küster (Carabidae). Mémoires de Biospéologie 19: 1–110.
- Venarsky MP, Huryn AD, Benstead JP (2012) Re-examining extreme longevity of the cave crayfish Orconectes australis using new mark–recapture data: a lesson on the limitations of iterative sizeat-age models. Freshwater Biology 57: 1471–1481. doi: 10.1111/j.1365-2427.2012.02812.x
- Simon KS (1997) Effects of organic pollution on an Appalachian cave: changes in macroinvertebrate populations and food supplies. American Midland Naturalist 138: 387–401. doi: 10.2307/2426830
- Tercafs R, Brouwir C (1991) Population size of Pyrenean troglobiont coleopters (Speonomus species) in a cave in Belgium. International Journal of Speleology 20: 23–35. doi: 10.5038/1827-806X.20.1.3

- Tilman D, Lehman CL, Yin C (1997) Habitat destruction, dispersal, and deterministic extinction in competitive communities. American Naturalist 149: 407–435. doi: 10.1086/285998
- Tilman D, May RM, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. Nature 371: 65–66. doi: 10.1038/371065a0
- Turchin P (2003) Complex population dynamics. Princeton University Press, Princeton NJ.
- Willemart RH, Gnaspini P (2004) Spatial distribution, mobility, gregariousness, and defensive behavior in a Brazilian cave harvestman *Goniosoma albiscriptum* (Arachnida, Opiliones, Laniatores). Animal Biology 54: 221–235. doi 10.1163/1570756042484674

RESEARCH ARTICLE



New Zospeum species (Gastropoda, Ellobioidea, Carychiidae) from 980 m depth in the Lukina Jama – Trojama cave system (Velebit Mts., Croatia)

Alexander M. Weigand^{1,2,†}

l Department of Phylogeny and Systematics, Institute for Ecology, Evolution and Diversity, Biosciences, Goethe-University Frankfurt, Max-von-Laue Straße 13, 60438 Frankfurt am Main, Germany **2** Croatian Biospeleological Society, Demetrova 1, 10000 Zagreb, Croatia

+ http://zoobank.org/C15902F9-515F-4D53-94A7-59058492A569

Corresponding author: Alexander M. Weigand (weiganda@gmx.net)

Academic editor: Oana Moldovan Received 12 June 2013 Accepted 14 Au	gust 2013 Published 30 August 2013
	3FA8521E3

Citation: Weigand AM (2013) New *Zospeum* species (Gastropoda, Ellobioidea, Carychiidae) from 980 m depth in the Lukina Jama–Trojama cave system (Velebit Mts., Croatia). Subterranean Biology 11: 45–53. doi: 10.3897/ subtbiol.11.5966

Abstract

A new species of the eutroglobiont gastropod taxon *Zospeum* Bourguignat, 1856 is described. *Zospeum tholussum* sp. n. is characterized based on a population from the Lukina Jama–Trojama cave system (Velebit Mts., Croatia). A single living specimen occurred at 980 m depth. The species is morphologically related to *Zospeum amoenum* (Frauenfeld, 1856), but can be readily distinguished from the latter by the presence of a weak columellar fold and its dome-like structured 2nd whorl. DNA barcoding is capable to clearly delineate *Z. tholussum* from other *Zospeum* spp. as well.

Keywords

DNA barcoding, cryptic species, biospeleology, eutroglobiont gastropod, cave-dwelling species, microgastropoda

Introduction

Taxonomic research distinguishes eleven morphospecies (no subspecies considered) of the microgastropod taxon *Zospeum* Bourguignat, 1856 (Ellobioidea, Carychiidae) for the region of the Dinaric Alps (Bole 1974, Slapnik and Ozimec 2004, de Jong 2012). Their taxonomic history has been shaped by confusing and sometimes contradicting (sub-)species assignments, primarily as a result of the general problem to distinguish intraspecific and interspecific shell variability (Bole 1974), which for decades was the main source of taxonomic information. Recently, Weigand et al. (2011) implemented DNA barcodes to molecularly identify species (i.e. DNA barcoding) based on a fragment of the mitochondrial-encoded (mtDNA) Cytochrome C Oxidase Subunit I (COI) locus (Hajibabaei et al. 2007). In a subsequent study and by addressing the evolutionary history of Carychiidae, Weigand et al. (2013) revealed several morphologically unrecognized (cryptic) *Zospeum* species and demonstrated the suitability of molecular data for taxonomic purposes in this group.

All known *Zospeum* have lost visual orientation and are considered true eutroglobionts (see Sket 2008). Information on their biology is scarce, but species seem to possess a limited active movement (Slapnik 2001) and to prefer muddy and permanently wet subterranean microhabitats (Jochum et al. 2012), as generally found along the drainage system of a cave. Weigand et al. (2013) proposed that dispersal may primarily occur via passive transportation (e.g. by water or larger mammals).

Here, a new Zospeum population from the Lukina Jama-Trojama cave system situated in the Velebit mountain range of Croatia is characterized (Fig. 1). On the basis of molecular and conchological data, a new species from 980 m depth-Zospeum tholussum Weigand, sp. n.-is described.

Methods

Conchological measurements

Specimen shells were measured by taking individual images with 456 pixels corresponding to 1 mm. The total shell height (SH), shell width (SW), aperture height (AH), aperture width (AW) and the number of whorls were measured. Moreover, ratios for SH/SW and AH/AW were calculated.

Non-invasive DNA isolation, PCR and sequencing

Specimen shells of live-collected *Zospeum tholussum* sp. n. (single specimen) and *Zospe-um amoenum* (Frauenfeld, 1856) (two specimens) were kept intact by using a protocol for a non-invasive DNA isolation method originally described in Schizas et al. (1997) and partially modified after Böttger-Schnack and Machida (2011). Each specimen

was cleaned and then dried in a 0.2 mL PCR-tube by raising the temperature once up to 94 °C. A volume of 9 μ L ddH₂O and 1 μ L 10x PCR-buffer were added. The mixture was heated for 2 min at 94 °C; 1.3 μ L Proteinase K were added and the solution homogenized by gentle mixing. An incubation step was performed for 15 min at 55 °C followed by 10 min at 70 °C. The incubation was repeated once. 10 μ L of Gene Releaser (Bioventures Inc.) were added and the following thermocycler protocol used: 30 s at 65 °C, 30 s at 8 °C, 1.5 min at 65 °C, 3 min at 97 °C, 1 min at 8 °C, 3 min at 65 °C, 1 min at 97 °C, 1 min at 65 °C, 5 min at 80 °C and storage at 4 °C. The reaction mixture including the intact shell was centrifuged for 1 min using a table centrifuge. The clean phase containing the DNA was collected and transferred to another 0.2 mL PCR-tube. The shell was cleaned from the remains of the Gene Releaser chemicals by alternate rinsing in water and ethanol. 10–15 μ L of AE Buffer (DNeasy Kit, Qiagen) were added to the isolated DNA.

PCR conditions were the same as described in Weigand et al. (2011), sequencing was performed at Source BioScience (LifeSciences, Berlin, Germany) following the companies instructions.

DNA barcoding

DNA barcodes, chromatograms, images, geographic data and further information of the genetically analysed *Zospeum tholussum* (BOLD-ID BARCA210-13) and *Z. amoenum* (BOLD-IDs BARCA211-13 and BARCA212-13) specimens are deposited in the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007) in the project "Barcoding Carychiid Microsnails" [BARCA]. The obtained COI-sequences were compared with other *Zospeum* species in BOLD.

Results

Family Carychiidae Jeffreys, 1830 Genus *Zospeum* Bourguignat, 1856

Zospeum tholussum Weigand, sp. n. http://zoobank.org/A3B380AD-B918-451B-866D-7FA59642A733 http://species-id.net/wiki/Zospeum_tholussum Figs 1–4

Material examined. A single living specimen was collected on 31.07.2010 (leg. Jana Bedek), which is designated as the holotype specimen (BOLD-ID BARCA210-13) (Fig. 1, life image; Fig. 2, upper row). Eight shells comprise paratype specimens, of which one was partially broken and used to investigate the form of the columellar fold. Shells were collected on several days during the caving expedition from 29.07.



Figure 1. The Lukina Jama–Trojama cave system. Overview of the geographical position and 3D cave cross-section. In the latter, the region of collected shells (1) and the collection site of the living specimen of *Zospeum tholussum* (2) are indicated. The 3D cross-section was provided by D. Bakšić et al. (2010), Croatian Speleological Server, http://www.speleologija.hr/lukinajama. Photos were taken by J. Bedek.



Figure 2. Holotype and paratypes of *Zospeum tholussum*. The holotype (former living specimen) is marked with a solid line; five paratype specimens (shells) are surrounded by dotted lines.

Ind.	sh/sw	ah/aw	shell height	shell width	aperture height	aperture width
1	1.60	1.16	1.49	0.93	0.44	0.38
2	1.42	1.30	1.54	1.08	0.52	0.40
3	1.46	1.16	1.41	0.97	0.46	0.39
4	1.34	1.05	1.44	1.07	0.47	0.44
5	1.50	1.31	1.63	1.09	0.54	0.41
6	1.53	1.23	1.61	1.05	0.51	0.41
7	1.62	1.15	1.81	1.12	0.53	0.46
8 (holotype)	1.48	1.12	1.47	0.99	0.44	0.39
range	1.34–1.62	1.05-1.30	1.41-1.81	0.93-1.12	0.44-0.54	0.38-0.46

Table 1. Conchological measurements for *Zospeum tholussum*. Seven paratypes (Ind. 1–7) and the holotype specimen (Ind. 8) were measured. All measurements are given in millimeter. Additionally, ratios of shell height / shell width (sh/sw) and aperture height / aperture width (ah/aw) were calculated. The range of variability is given for all parameters.

- 03.08.2010 (Fig. 2). All investigated specimens are deposited in the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main, Germany (museum voucher SMF 341633).

Diagnosis. The general shape of *Zospeum tholussum* sp. n. resembles larger specimens of *Zospeum amoenum* (Frauenfeld, 1856) from which it can be best delineated by the dome-like structured 2^{nd} whorl (Fig. 2), the weak but present columellar fold (Fig. 3) and by means of their DNA barcodes (11.7 %–12.1 % genetic p-distance between both species).

Description. The eight conchologically investigated specimens (the holotype and seven paratypes) demonstrate moderate shell variability (Table 1). Shells are generally smooth, very thin and fragile. Fresh shells or those of living specimens are translucent, older ones adopt a milky white color. The number of whorls are 5–6, SH ranges from 1.41–1.81 mm, SW from 0.93–1.12 mm, AH from 0.44–0.54 mm and AW from 0.38–0.46 mm. The ratio of SH/SW is between 1.34–1.62 and AH/AW between 1.05–1.30. The 2nd whorl is remarkably enlarged possessing a height of 2/3–5/6 the height of the 3rd + 4th whorl. The columellar is only weakly developed (Fig. 3) and no teeth (i.e. parietalis, palatalis or columellaris) are present. The DNA barcode of the holotype, when compared with other DNA barcodes of *Zospeum pretneri* Bole, 1960 (5.6 %; BOLD-ID: BARCA120-10). This result is well above the barcoding gap of 3.2 % for Carychiidae, which is suitable to separate between-species (> 3.2 %, interspecific) and within-species genetic diversity (< 3.2 %, intraspecific) in *Zospeum* (Weigand et al. 2011, 2013).

Habitat. The single living specimen was found in an unnamed large chamber at 980 m depth (85 m long, 70 m wide) with lots of stones, rocks and sand (Fig. 1, right site). A temporal small stream of running water was present close to the collecting site. Air temperature was between 3.3–3.5 °C (depending on the measurement device),



Figure 3. Columellar fold views of *Zospeum tholussum*. The columellar fold of the broken paratype specimen shell is shown in clockwise rotation.

water temperature 5.1 °C and air humidity 100 %. Shells were observed beginning from 800 m depth till the bottom of the cave. Shells were generally found on layers of mud (Fig. 1, left site). The first 200 m of the entrance passage of Lukina Jama are permanently covered by varying levels of snow and ice.

Etymology. The Latin word *tholus* means dome or cupola and refers to the remarkable dome-like shape of the 2nd whorl.

Remarks. In addition to the newly described *Zospeum tholussum*, a second *Zospeum* species is present in the Lukina Jama–Trojama cave system (Fig. 4C). This species can be differentiated from *Z. tholussum* by the presence of a tooth, its general shell shape, more prominent columellar fold and absence of the characteristic dome-like structured 2nd whorl. Because only shells were found, no DNA barcodes are available for this species.

So far, Zospeum tholussum is only known from the Lukina Jama–Trojama cave system. However, this cave system is situated in the distribution range of the morphologically related species Zospeum amoenum (Frauenfeld, 1856), which inhabits caves of the West Balkan of North Slovenia, West Croatia, Bosnia and Herzegovina and South to Montenegro (Bole 1974). Absolon (1916) named but did not describe another morphologically related species - Zospeum troglobalcanicum. Since Weigand et al. (2013) revealed that Zospeum spp. with an inferred large distribution range are particularly prone to host additional, morphologically unrecognized species, some of the distribution records for Z. amoenum may very well refer to Z. tholussum. Moreover, the intraspecific genetic and conchological variability of the new species should be investigated in greater detail, thus to judge about the taxonomic information content of the diagnostic characters applied within this description.

The preliminary information on the habitat of *Zospeum tholussum* is in congruence with previous findings for *Zospeum*. Interestingly, the grazing-labyrinth-like structure, in which most of the shells were embedded (Fig. 1, left site), has been already observed during caving expeditions in Northern Spain (Jochum et al. 2012). Finally, the presence of a temporal stream at the collecting site of the living specimen further supports a passive dispersal scenario as suggested by Weigand et al. (2013).



Figure 4. Zospeum species. **A** Zospeum tholussum holotype **B** Zospeum pretneri from locus typicus, which demonstrates the lowest genetic distance to Z. tholussum **C** Second Zospeum sp. from the Lukina Jama-Trojama cave system.

Acknowledgements

I thank Jana Bedek and all members of the HBSD (Zagreb, Croatia) for their permanent biospeleological effort and the samples, Adrienne Jochum (Frankfurt, Germany) and Rajko Slapnik (Ljubljana, Slovenia) for their taxonomic assistance and Yasunori Kano (Tokyo, Japan) for his introduction to the method of non-invasive DNA isolation. The research on the adjustment of this method to carychiid microgastropods carried out at the AORI (University of Tokyo, Japan) was supported by the Japan Society for the Promotion of Science (JSPS). The 3D cross-section of the Lukina Jama–Trojama cave system was kindly provided by D. Bakšić et al. (2010), Croatian Speleological Server, http://www.speleologija.hr/lukinajama. The collecting permit for the Croatian Biospeleological Society (HBSD) was issued by the Croatian Ministry of Culture, dated 24 June 2010, no. 538-08-01-01/3-10-02.

References

Absolon K (1916) Z výzkumnýh cest po kraseh balkánu. Zlatá Praha 33. Bole J (1960) Novi vrsti iz rodu *Zospeum* Bourg. (Gastropoda). Biološki vestnik 7(1): 61–64.

- Bole J (1974) Rod *Zospeum* Bourguignat 1856 (Gastropoda, Ellobiidae) v Jugoslaviji. Slovenska Akademija Znanosti in Umetnosti. Razprave 17(5): 1–43.
- Böttger-Schnack R, Machida RJ (2011) Comparison of morphological and molecular traits for species identification and taxonomic grouping of oncaeid copepods. Hydrobiologia 666(1): 111–125. doi: 10.1007/s10750-010-0094-1
- Bourguignat JR (1856) Aménités malacologiques. Revue et Magasin de Zoologie pure et appliquée (2) 8: 499–516.
- de Jong YSDM (2012) Fauna Europaea version 2.5. Web Service available online at http:// www.faunaeur.org
- Frauenfeld G (1856) Die Gattung *Carychium*. Sitzungsberichte der Akademie der Wissenschaften, mathematisch-naturwissenschaftliche Klasse 19(1): 70–93.
- Hajibabaei M, Singer GA, Hebert PD, Hickey DA (2007) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends in Genetics 23(4): 167–172. doi: 10.1016/j.tig.2007.02.001
- Jeffreys JG (1830) A synopsis on the testaceous pneumonobranchous Mollusca of Great Britain. Transactions of the Linnean Society of London 16 (2): 323–392. doi: 10.1111/j.1095-8339.1829.tb00139.x
- Jochum A, Weigand AM, Slapnik R, Valentinčič J, Prieto CE (2012) The microscopic ellobioid, *Zospeum* Bourguignat, 1856 (Pulmonata, Ellobioidea, Carychiidae) makes a big debut in Basque Country and the province of Burgos (Spain). MalaCo 8: 400–403.
- Ratnasingham S, Hebert PD (2007) BOLD: The Barcode of Life Data System (http://www. barcodinglife.org). Molecular Ecology Notes 7(3): 355–364. doi: 10.1111/j.1471-8286.2007.01678.x
- Schizas NV, Street GT, Coull BC, Chandler GT, Quattro JM (1997) An efficient DNA extraction method for small metazoans. Molecular Marine Biology and Biotechnology 6(4): 381.
- Sket B (2008) Can we agree on an ecological classification of subterranean animals?. Journal of Natural History 42(21–22): 1549–1563. doi: 10.1080/00222930801995762
- Slapnik R (2001) Activity and movements of *Zospeum isselianum* Pollonera 1886 (Gastropoda, Pulmonata, Carychiidae) in a cave in the Kamniške-Savinjske Alps (Slovenia). Natura Croatica 10(3): 153–162.
- Slapnik R, Ozimec R (2004) Distribution of the genus Zospeum Bourguignat 1856 (Gastropoda, Pulmonata, Ellobiidae) in Croatia. Natura Croatica 13(2): 115–135.
- Weigand AM, Jochum A, Pfenninger M, Steinke D, Klussmann-Kolb A (2011) A new approach to an old conundrum DNA barcoding sheds new light on phenotypic plasticity and morphological stasis in microsnails (Gastropoda, Pulmonata, Carychiidae). Molecular Ecology Resources 11(2): 255–265. doi: 10.1111/j.1755-0998.2010.02937.x
- Weigand AM, Jochum A, Slapnik R, Schnitzler J, Zarza E, Klussmann-Kolb A (2013) Evolution of microgastropods (Ellobioidea, Carychiidae): integrating taxonomic, phylogenetic and evolutionary hypotheses. BMC Evolutionary Biology 13(1): 18. doi: 10.1186/1471-2148-13-18

RESEARCH ARTICLE



Aquatic biota of different karst habitats in epigean and subterranean systems of Central Brazil – visibility versus relevance of taxa

Luiza Bertelli Simões¹, Tânia Cristina dos Santos Ferreira², Maria Elina Bichuette¹

 Laboratório de Estudos Subterrâneos, Departamento de Ecologia e Biologia Evolutiva (DEBE), Universidade Federal de São Carlos (UFSCar). Via Washington Luís, km 235, 13565-905, Caixa Postal 676, São Carlos, SP, Brasil 2 Departamento de Biologia, FFCLRP. Av. Bandeirantes, 14040-90, Caixa Postal 3900. Ribeirão Preto, SP, Brasil.

Corresponding author: Luiza Bertelli Simões (lula_moluxca@hotmail.com)

Academic editor: O. Moldovan | Received 16 July 2013 | Accepted 15 September 2013 | Published 30 October 2013

Citation: Simões LB, dos Santos Ferreira TC, Bichuette ME (2013) Aquatic biota of different karst habitats in epigean and subterranean systems of Central Brazil – visibility versus relevance of taxa. Subterranean Biology 11: 55–74. doi: 10.3897/subtbiol.11.5981

Abstract

The karstic area of São Domingos, central Brazil, holds extensive drainage systems. In order to understand its biodiversity, various volumes of water were filtered with planktonic nets in stretches of subterranean and superficial rivers on five different occasions. We sampled four drips (152L), three calcite pools (368L), two subterranean rivers fed mainly by percolation water (6,395L), two subterranean rivers fed mainly by percolation water (6,395L), two subterranean rivers fed mainly by water coming from a sinkhole (4,175L) along different caves, one resurgence (158L), and four epigean rivers (101,690L). Physical and chemical variables were measured at some sites. Canonical Correlation Analysis was used to verify relationships between taxa and environment. The degree of similarity of the biota was assessed by cluster analysis (Sorensen, single linkage). There were records of exclusive taxa in epigean and subterranean samples, mainly in drips, which harbour the most unique fauna. The high richness of taxa presently recorded reveals the potential of the vadose zone biota in the tropical region, which was neglected in studies on Brazilian subterranean biodiversity. According to our results, the unsaturated zone tropical fauna may have different composition compared to that from temperate habitats. The studied communities were dominated by rotifers, while crustacean are predominant in the latter. The hypothesis can be clarified with the increase of long term studies and taxa identification at species level, besides the use of complementary sampling methods.

Keywords

Subterranean habitats, epigean rivers, aquatic biota, Rotifera, conservation, Neotropical region

Copyright Luiza Bertelli Simões et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Most of the subterranean habitats develop in karst systems, which are discontinuous geomorphic systems formed in soluble rocks and characterized by the presence of aquifers and conduits, with subterranean drainage (Dreybrodt 1988). These systems originate most caves that can reach hundreds of kilometres. The caves provide indirect human access to the hypogean environment in such a way that a large portion of information on subterranean ecosystems is derived from studies performed in them. Generally, the structure of karst systems consists in a sum of different horizons that change with depth. The vadose or unsaturated zone is the layer between the soil and the phreatic or saturated zone (Mangin 1994). The vadose zone is heterogeneous, with dissolution voids and fissures in which water percolates downwards by a multiphase process before reaching the phreatic zone (Ford and Williams 2007). In the upper section of the superficial layers of karstifiable rocks, a particular zone is formed - the epikarst (Mangin 1973). This consists of a heterogeneous system of openings in which surface water percolates through soil, retained for variable periods, potentially for years at a time (Jones et al. 2004).

The waters that refuel the epikarst can infiltrate through different voids (i.e. fractures, conduits or shafts), which vary in physical and chemical properties (Musgrove and Banner 2004, Williams 2008) (Figure 1). As such, epikarst acts as a transitional zone between epigean and subterranean environments, where organic matter and other resources are transported and redirected. The faunistic flow can also occur in these conduits where the environmental filters offer ideal conditions for a few species. Thus, the composition of unsaturated communities depends on different physical and chemical variables at each depth level (Pipan et al. 2008, Moldovan et al. 2012, Pipan and Culver 2012).

In these habitats an exceptionally rich fauna occurs, best described in some European karst areas (Pipan et al. 2008, Brancelj and Camacho 2009, Galassi et al. 2009, Meleg et al. 2011, 2012, Moldovan et al. 2012, Cottarelli et al. 2012) and in West Virginia (USA; Pipan and Culver 2005). The organisms that live in the unsaturated zone of the karst are occasionally carried by trickling water to the interior of caves. Local seasonality influences this process to the extent that the intensity of rainfall alters the volume of infiltration and the connectivity between surface and underground environment. This process is extremely variable and especially important to the understanding of the karst ecosystem functions (Bonacci et al. 2009).

The vadose zone fauna in temperate zones is mainly composed of small crustaceans, oligochaetes, nematodes, acari, and molluscs, less than 1 mm to several centimetres in body size (Gilbert et al. 2009). In general, stygobiotic biodiversity in Europe is dominated by Crustacea (Pipan et al. 2008, Brancelj and Camacho 2009, Meleg et al. 2011, 2012). Among these, copepods represent the most common group, with Harpacticoida outnumbering Cyclopoida in diversity and abundance (Moldovan et al. 2007, Meleg et al. 2011, Moldovan et al. 2012, Cottarelli et al. 2012). Their spatial and temporal distribution appears to be rather heterogeneous and has been related to a number of environmental parameters and cave characteristics, as topography, cave ceiling thickness and/or voids interconnectivity (Stoch 2000, Brancelj 2002, Pipan and Brancelj 2004a, 2004b, Sket et al. 2004, Camacho et al. 2006, Pipan et al. 2008,



Figure 1. Horizons in karst: 1 soil 2 karst terrain 3 limestone outcrop; 4 epikarst 5 aquifer in epikarst 6 drips 7 doline 8 cave 9 and 10 subterranean river at the base level 11 resurgence 12 epigean river A epikarstic zone B vadose zone C phreatic or saturated zone. (Ilustration: Pedro Pereira Rizzato).

Meleg et al. 2011). Nevertheless, studies on stygofauna in tropical regions are scarce, and non-existent in relation to the unsaturated zone horizons.

As the terrestrial hypogean fauna is more visible and can be easily assessed, it has been studied in more detail in Brazil (Trajano and Gnaspini 1991, Pinto-da-Rocha 1995, Trajano and Bichuette 2006, 2010). The aquatic diversity, however, is clearly underestimated, as sampling methods of this biota are not always standardized and/or adequate (Trajano and Bichuette 2006). Sampling of these organisms require the use of nets with micrometric mesh in habitats like pools fed by percolating water, as well as from sifting of sediment for interstitial species and use of vacuum pumps.

The unique long-term study focusing on Brazilian subterranean aquatic fauna was conducted for fish populations (Bichuette and Trajano 2004). For example, the catfish *Ituglanis epikarsticus* and *I. ramiroi* are rare in cave habitats, being found respectively in a single set of pools in São Mateus Cave and in a side pool in São Bernardo Cave, both located in our study area. However, when it comes to aquatic microfauna studies, the only records of copepods are restricted to six species from two caves in Goiás state, central Brazil, aside from the sparse references in general publications, which adds three other species (Pinto-da-Rocha 1995).

Many studies have demonstrated that organic carbon may arrive in the form of animals dripping into caves through the epikarst (Simon et al. 2003, 2007, Pipan 2005), acting as an important resource for the subterranean habitats. The assessment of local subterranean fauna is important for understanding the functioning of karst aquifers for protection and conservation purposes.

As the study of the unsaturated zone biota is a new challenge for subterranean biology in Brazil, the main goal of this study was to elucidate part of the richness and

distribution of the aquatic biota in different karstic horizons of São Domingos region and its surroundings, north-eastern Goiás state, central Brazil. Furthermore, we aimed to verify the correlation between this biota and some physical and chemical variables of the different habitats. The question of relevance *versus* visibility of this biota and its respective habitats was raised, considering that less visible species are yet important to the functioning of subterranean systems.

Material and methods

Study Area

The karst area of São Domingos and its surroundings represents one of the regional formations of the Bambuí geomorphologic unit, which constitutes the largest set of limestone areas favourable to caves development in Brazil. The region has an approximate area of 105, 200 km². In the region there are five large systems of caves, with up to 23 km of development (Figure 2).

Superficial rivers belonging to the Parana Basin (Alto Tocantins) penetrate the limestone layers after draining an extensive arenitic region, forming large cave systems (Karmann and Setúbal 1984, Auler and Farrant 1996). As part of these, there are upper tributaries in the vadose zone fed mainly by infiltrating waters from the surface.

The area is part of a state-level Conservation Unit (Terra Ronca State Park - TeR-SP), created 17 years ago but still with diverse land ownership problems. One aggravating factor is that some river sources that cross the cave systems are located outside the park. There are anthropic activities as livestock, extensive plantations and clandestine mining which on long-term are responsible for the adverse effects on local drainage systems, which include the subterranean domain.

The studied region belongs to the morphoclimatic Dominion of the Cerrados (Ab'Saber 1977), with a dry season between May and September, sometimes extending through October (Nimer 1979). Heavy showers characterize the rainfall regime during the rainiest periods (October through March), transporting a large amount of organic matter in the caves (Trajano and Bichuette 2010).

Sampling of aquatic invertebrates

The sampling area embodies subterranean systems and epigean rivers, sampled differently regarding frequency and volume since in some cases the availability of water was restricted. Abbreviations and characteristics of each sampled point are described in Table 1.

The region was sampled on five occasions: April and October 2011, and February, June and October 2012. The subterranean stretches included drips, subterranean rivers (base level) and water pools (Figure 3). The sampled caves and rivers are: Angélica, Bezerra, Buraco das Araras, São Bernardo, São Mateus and Revolucionários caves; the



Figure 2. Study area map with details of subterranean cave systems and epigean rivers at São Domingos karst area and surroundings, Goiás state, central Brazil. In dark gray – limits of Terra Ronca State Park (TeRSP). **A** São Domingos river **B** Angélica Cave **C** Bezerra Cave **D** São Mateus Cave **E** Buraco das Araras Cave **F** Terra Ronca I Cave (sinkhole of Lapa river) **G** Palmeiras river **H** São Bernardo Cave **I** Revolucionários Cave (this cave is located outside the limits of Terra Ronca State Park).



Figure 3. Subterranean river (**A** São Bernardo Cave) and drips (in blue) formed by infiltration water (**B** Angélica Cave – AngD01). Photography: a, Adriano Gambarini; b, Maria Elina Bichuette.

epigean Palmeiras, Angélica, da Lapa, São Domingos Rivers, and the Resurgence of Terra Ronca (Table 1). Revolucionários Cave and São Domingos River are outside the park area, but inserted in the same limestone lens. The Angélica Cave is the only locality sampled on every trip, as a long-term monitoring base.

Samples were taken with plankton nets (20µm mesh), which remained installed for periods varying from twenty minutes to three hours, according to the local con-

Table 1. Characteristics, volumes and months in which sampling was undertaken. Sampling sites in the karst area of São Domingos and surroundings (2011-2012). Caves: Angélica (Ang), Buraco das Araras (BAra), Bezerra (Bez), São Bernardo (SBer), São Mateus (SMat), Revolucionários (Rev); epigean rivers: Palmeiras (Palm), Angélica (Ang), São Domingos (SD); resurgence: Terra Ronca (RTR). D = drip; Ek = subterranean river fed mainly by percolation water; Ep = epigean; P =pool; S = subterranean. *Locality visited on the five occasions (segment of Angélica Cave, monitoring base); samplings were not performed during periods of inactive drips.

Points	Collection Occasions	Vol (L)	Characteristics
AngD01	Apr/2011; Feb/2012 *	83	Drips in the entrance zone of Angélica Cave. Water drips directly from the ceiling, with flow varying according to the season.
AngD02	Apr/2011*	3	Reduced dripping, with low flow rate, and ceasing during the dry season.
AngEk	Apr & Oct/2011; Feb, Jun & Oct/2012	3,596	River fed by percolation water, habitat of the small catfish <i>Ituglanis epikarsticus</i> Bichuette & Trajano, 2004. The water flows with moderate rate and little turbulence, and there is notable reduction in the river level during the dry periods.
AngEp	Jun/2012 & Oct/212	23,580	Epigean Angélica River, point close to the sinkhole.
BAraD	Oct/2011 & Oct/12	21	Water drips from ceiling speleothems.
BAraP	Oct/2011 & Oct/2012	45	Pool near the BurAraGot drip.
BAraEp	Oct/2012	6,000	Epigean Lapa River, entrance of Buraco das Araras Cave.
BezD	Jun/2012	45	Drips coming directly from the ceiling.
BezP	Jun/2012	113	Pool in the aphotic zone of Bezerra Cave. Water drips from the ceiling and remains puddled.
BezS	Feb & Jun/2012	2,603	Subterranean river in the aphotic zone of Bezerra Cave, base level.
SBerEk	Fev/2012	2.592	River in the aphotic zone of São Bernardo Cave.
SMatP	Feb/2012	210	Pool in São Mateus Cave. Water drips from the ceiling and forms a puddle.
RTR	Oct/2011	158	Lapa River at its epigean portion, resurgence of Terra Ronca Cave.
PalmEp	Apr/2011	110	Palmeiras River (epigean) near the sinkhole of São Bernardo Cave.
SDEp	Feb & Jun/2012	72,000	São Domingos River (epigean), near its sinkhole.
RevS	Jun/2012	1,545	Subterranean river in Revolucionários Cave.

ditions. Nets, installed under dripping water sites based on the device proposed by Brancelj (2004) were used to sample the fauna percolating through the unsaturated zone for similar periods. For the shallow stretches, the flow (used to calculate the filtered volume) was estimated from the velocity of the water surface. This velocity was determined by the time it took for a small plastic disc to travel a pre-determined distance. In the case of percolating water and pools, the volume was directly estimated using a graduated plastic container. The material was subsequently fixed (formaldehyde 4%) and packed. Afterwards, the samples were analysed in the laboratory of Limnology in the Biology Department of FFCLRP/USP (Ribeirão Preto campus), and the organisms were identified to the lowest possible taxonomic level.

Physical and chemical variables

The following physical and chemical variables of water were measured by using a Horiba multiprobe (model U50G): temperature (°C), pH, ORP redox potential (mv), conductivity (mS/cm), dissolved oxygen (mg/L), oxygen saturation (%), total of dissolved solids TDS (g/L), turbidity (NTU), depth (m) and salinity (%).

Data analysis

Relative taxa richness among sites was assessed. Furthermore, the richness of the taxa in epigean and hypogean environments was taken into account separately for comparison. Similarity in composition of aquatic invertebrates in the different habitats was analysed by cluster analysis (Sorensen, single linkage).

In order to study the species-environment relationship, Canonical Correspondence Analysis (CCA) was used, associating the measured environment variables with the matrix of taxa present at eleven sampled sites.

Finally, by means of simple linear regression (Pearson correlation), we verified the relationship between the sampling effort and the richness of taxa, as the volumes sampled in each segment varied. Analyses of similarity and CCA were performed using the software PAST (version 2.13) and R language (version 3.0.1), respectively.

Results

Thirty-six taxa were registered in all of the samples, belonging to different groups listed in decreasing order according to absolute/relative richness: Rotifera (23; 64%), Insect larvae (4; 11%), Copepoda-Cyclopoida, Calanoida and Harpacticoida (3; 8%), Cladocera (2; 5%), Dinoflagellata (1; 3%), Nematoda (1; 3%), Ostracoda (1; 3%) and Protozoa (1; 3%) (Table 2).

In decreasing order, the richest habitats (based on number of taxa present) were: AngEk (16); RTR (13); AngD02 (12); BAraP (10); BezS (9); PalmEp (8); AngD01, BAraD and AngEp (7); SBerEk, BezP, SMatP and RevS (5); SDEp and BAraEp (2); BezD (1). We summed up the richness of taxa from the samples of epigean and hypogean origin separately, totalling 21 and 30, respectively (Figure 4).

Some taxa were exclusive to one single sampling point: *Anuraeopsis fissa* and *Euchlanis* sp. (RTR); *Bosmina* sp. (PalmEp); *Anuraeopsis* sp. (AngEp); *Brachionus* sp. (SBerEk); Chaoboridae (AngEk); Simuliidae (BAraP), *Arcella costata* (AngD01), *Brachionus falcatus, Conochilus* sp., *Filinia* sp., *Kellicotia bostoniensis* and *Keratella cochlearis tecta* (AngD02). The group Calanoida was restricted to samples with epigean origin, and Harpacticoida was restricted to subterranean waters.

Cluster analysis for the 16 sites evidenced low similarity between them (S=0.32). The dendrogram also revealed that drips and epigean rivers are the most singular environments as they were more distant from other samples (Figure 5).

<u>а-</u>	ed	
M	nin	
São	terr	
er),	nde	
(SB	m =	
op	det	
rnaı	Ŋ	
Bei	Ŕ.	
São	RT	
2Z),	Ica	
(B	Ror	
erra	rra	
Bez	: He	
ca),	lces	
BAı	rgei	
as (resu	
Arar);1	
las /	(SL	
8	gos	
ura	min	
(), B	Do	
Ang	São	
ca (в), С	
géli	(An	
An	ica (
ves:	ıgél	
Ca	, Ar	
int.	(mh	
h pc	<u> </u>	
eac	eirae	
l in	lme	able
plee	ů.	пŢ
sam	ver	ed i
als	in ri	crib
idu	igea	des
vibr	e p	are
of iı	(v)	suc
oer -	s (F	iati
uml	ário	orev
d n	ion	abł
t an	oluc	oint
a lis	Sevi	g Pć
Тах:	t), I	plin
5	Ma	am
ble	is (S	a. S
Ta	teu	tax.

		Dri	ips			Pools		Su	bterran	ean rive	rs		Epi	igean rive	STS	
Sampling points	Ang 01	Ang 02	BAra	Bez	Bara	Bez	SMat	Ang Ek	SBer Ek	BezS	RevS	Palm Ep	Ang Ep	BAra Ep	SDEp	RTR
Taxa/ Sampled Volume (L)	83	3	21	45	45	113	210	3,802	2,592	2,603	1,545	110	23,580	6,000	72,000	158
Rotifera					3											
*Anuraeopsis fissa																-
Anuraeopsis sp.													1			
Brachionus falcatus		1														
Brachionus sp.									2							
Chonochilidae								2								
Collotheca sp.	4	4			2			1		2	2	3			18	15
Conochilus sp.		1														
*Euchlanis sp.																12
Filinia sp.		1														
Gastropus sp.	1															25
Hexarthra sp.			1											1		
Kellicotia bostoniensis		5														
Keratella cochelaris tecta		4														
Keratella cochelaris	4	1	1		2			2		4						
Lecanidae								3		2	2				18	
Lecane sp.	2															5
Lecane monostyla sp.								2								12
Ploimida			2					4					47			
Synchaeta sp.								1		2	1		46			
Testudinella sp.								1		2						

Trichocerca sp.		-								9			7		
Bdelloidea			3		3		2	7	2	4	3	3	7		55
Copepoda															
Cyclopoida adult		3	1		1			3				5			1
*Cyclopoida copepodito	1	1										2	46		
*Cyclopoida nauplius	-	2	1		-		2	6				3			6
Calanoida												4			2
Harpacticoida				2	4	2	1	1	2		1				
Cladocera									2			2			
Bosmina sp.												1			
Dinoflagellata															
Peridinium sp.						6		2							
Insecta	1	2	2			2	2	6	3	1					1
Chironomidae					1	4		4		4			7		
Chaoboridae								1							
Simuliidae					1										
Nematoda	5				8	2	1							4	2
Ostracoda															1
Protozoa															
Arcella costata	1														



Figure 4. Number of taxa recorded in 16 sampling sites. Total taxa for epigean (21) and subterranean habitats (30).



Figure 5. Similarity Cluster Analysis (Sorensen, single linkage) for 16 sampling sites from São Domingos karst area and surroundings, Goiás state, central Brazil. Abbreviations are described in Table 1.

In the resultant CCA (Figure 6), the taxa closest to the various environmental vectors are strongly associated with them. For this analysis, thirty-one taxa were recorded in 11 sampling points. The majority of hypogean taxa are close related to both high levels of conductivity and pH, and low ORP. The epigean taxa appear more related to



Figure 6. CCA for environmental variables and biotic data of 11 sampling sites from São Domingos karst area and surroundings, central Brazil (ORP= redox potential; T = temperature; OD= concentration of O₂; DO = oxygen saturation and cond=conductivity). Red abbreviations represent the taxa: Rot = Rotifera; An = *Anuraeopsis* sp.; Br.f = *Brachionus falcatus*; Br= *Brachionus* sp.; Col = *Collotheca* sp.; Con = *Conochilus* sp.; Fil = *Filinia* sp.; Gas = *Gastropus* sp.; Kel= *Kellicotia bostoniensis*; K.ct= *Keratella cochelaris tecta*; K.co = *Keratella cochelaris*; Le c= Lecanidae; L.sp = *Lecane* sp.; Bde = Bdelloidea; Cy= Cyclopoida; Cal = Calanoida; Har = Harpacticoida; Bos = Bosmina; Cla = Cladocera; Per = *Peridinium* sp.; Ins = Insecta; Chi = Chironomidae; Cha = Chaoboridae; Sim = Simuliidae; Nem = Nematoda; A.cos = *Arcella costata.* X words represent the sampling sites (described at Table 1): X1=AngD01; X2=AngD02; X3=BAraD; X4=BAraP; X5=AngEk; X6=SBerEk; X7=BezS; X8=PalmEp; X9=BAraEp; X10=AngEp and X11=SDEp.

high levels of OD and ORP, except for the SDEp site. In the same way, taxa near each other (Figure 6) have similar environmental preferences. The sampling point positions reflect the physical and chemical characteristics of each habitat.

Only six of the ten environmental variables that were measured held some importance in the distribution of the samples according to fauna: dissolved oxygen, oxygen saturation, redox potential, conductivity, pH and temperature (Table 3). Oxygen saturation and redox potential presented the highest correlation values with the two first ordination axes (0.7802 and 0.5588, respectively). The two first canonical axes represented 51% of the total variation (30% and 21%, respectively).

Pearson correlation did not show a significant relation between the volume of water filtered and the number of taxa recorded at each sampling point, since simple linear regression analysis presented a value of R = 0.10756.

Sampling point	pН	Cond (mS/cm)	OD (mg/L)	DO (%)	ORP (mv)	T (°C)
AngD01	7.40	0.81	3.17	37.92	179.00	23.00
AngD02	9.80	0.47	4.25	50.50	24.30	22.60
BAraD	8.20	0.49	12.80	145.90	210.20	22.30
BAraP	8.10	0.54	15.60	170.50	220.00	22.00
AngEk	7.50	0.49	4.48	102.63	215.90	23.90
SBerEk	6.30	0.45	3.50	42.73	203.90	24.50
BezS	7.50	0.09	5.55	66.03	300.00	23.00
PalmEp	7.60	0.01	6.03	75.75	68.70	26.20
BAraEp	7.00	0.02	11.55	197.70	204.40	25.30
AngEp	6.70	0.01	6.96	163.09	331.60	22.90
SDEp	7.00	0.02	2.91	36.80	20.00	26.50

Table 3. Physical-chemical variables of the 11 sampling points in São Domingos karst area and surroundings, central Brazil. Cond=conductivity, OD=concentration of O_2 , OD=saturation of O_2 , ORP= redox potential, T=temperature. Sampling points abbreviations are described in Table 1.

Discussion

Richness of the karstic waters of Goiás

In this preliminary approach, we studied the richness of taxa in habitats of karstic systems, which represents new data on tropical subterranean domain species. The fauna belonging to samples of percolating water were remarkable and responsible for the high number of recorded species in the studied habitats

Rotifers were the richest group in our study, representing more than half of the total richness recorded. The dominance of rotifers was associated with the increase in the trophic state of a system due to short life cycles and rapid reproduction, which favour this group in more dynamic, competitive and selective environments. Presently, it is known that rotifers are also dominant in several other aquatic environments, independent of the trophic state (Rocha et al. 1995). This group species have greater tolerance to different environmental conditions, and present short reproductive cycles and high renewal rate, providing an advantage for the formation of well-established populations (Neves et al. 2003, Segers 2008).

Diversity in subterranean water courses is frequently limited by competition, and especially limited by energetic resources and space (Culver 1994). In Brazilian karst, the subterranean aquatic microfauna certainly configures an important source in the diets of larger taxa, mainly for fish populations and aquatic invertebrate groups that inhabit the most inaccessible points of the unsaturated zone. Rotifers are considered a source highly rich in energy, as they make available a large quantity of renewable food through conversion of primary production in tissue that is easily assimilated by consumers (Esteves 1998). In dry periods, when the level of the phreatic zone tends to suffer brisk reductions, some fish become imprisoned in pools, without record of populations in base-level streams (e.g. populations of catfishes *Ituglanis epikarsticus* Bichuette & Trajano, 2004 of São Mateus Cave). Therefore, it is possible that rotifers

act as an essential component of the food chain, offering rapid renewal of nutrients for the other trophic levels.

The low number of Copepoda occurrences in our samples must be due to environmental factors and/or interspecific relationships in the communities. In dry periods, the water volume flowing through the system can decrease, as well as the access to aquatic communities from unsaturated zone habitats. In fact, some of our sampling points could not be efficiently accessed in all periods, in part because of the reduced local rain rates. Besides, sampling frequency could have also affected our results to the extent that it did not offer access when abundant taxa of these communities occurred (i.e. different life cycles). We also have to consider that benthonic fauna was not well assessed.

The fauna distribution depends on the scale of the karst system and generally species change in number from the surface to the saturated zone (Gibert et al. 2009). The most relevant factors to the composition of aquatic biota consist of physical and chemical variables of water, the thickness of rocks and the rainfall regime, as they directly influence waters properties and volume of the karst systems (Pipan et al. 2006, Kogovšek 2010, Meleg et al. 2011, Pipan and Culver 2012). The most relevant physical and chemical factors explaining the composition of aquatic biota were considered the thickness of rocks and the rainfall regime, as they directly influence waters properties and volume of the karst systems (Pipan 2006, Kogovšek 2010, Meleg et al. 2011, Pipan and Culver 2012). The environmental variables used in our correlation analysis have low significance in explaining the different faunistic distributions. Certainly, there are other more important factors that have not been considered, such as the dissolved organic carbon and concentration of ions (e.g., sodium, nitrate and potassium). Until present, few studies have considered the levels of carbon, phosphorous and nitrogen in the waters (e.g., Pipan et al. 2006, Simon et al. 2007), even if the dissolved organic carbon is an important limiting factor (Simon and Benfield 2002). Even if we did not measure these values, we can infer the availability of organic carbon in percolation water for the high number of taxa found in drips.

We observed that drips samples have the most unique fauna of all the other subterranean habitats. In our study, it is important to highlight that the water volumes filtered in drips were much smaller than the volume of water filtered in other habitats. However, we encountered elevated richness and occurrence of taxa exclusive to these samples. Thus, we recorded the greatest richness of taxa from the least voluminous samples (drips of Angélica Cave and epigean resurgence), while some of the most voluminous ones had inferior richness (São Domingos, Lapa and Angélica epigean rivers). The total of taxa encountered in drips outnumbered all others samples, emphasizing the complexity of communities from the unsaturated zones.

For the environmental parameters considered, we have observed that there was a separation between epigean and hypogean sites in the diagram generated by CCA as well as in the dendrogram of faunistic similarity (except for SDEp and RevS sites). Both demonstrated that there were clear differences between the biota of epigean and groundwater environments while distinctions of hypogean habitats among themselves are less evident. When comparing each sample individually, we noticed faunistic distinction between them. Epikarst and sinkholes are both responsible for supplying the aquatic hypogean realm, but they differ in their capacities to redirect and/or store water through the karst (Juberthie 2000). The small size of voids of the epikarstic and the vadose zone restricts the transport of particles and microorganisms, and also represents a much slower flow. However, the total area occupied by the unsaturated zone is much more ample and complex, permitting innumerable vertical and lateral connections. These factors interact in a heterogeneous way, generating various microhabitats and promoting the formation of distinct communities (Moldovan et al. 2012).

The waters that percolate the subterranean systems are filtered by soils on the karst surface and tend to suffer reduction of dissolved oxygen. Organic material, carbon, and diverse ions suffer reduction of dissolved oxygen, depending on the manner in which they are used and transformed through chemical processes by the interstitial and/or unsaturated zone biota. Water circulation in surface rivers promotes higher oxygenation rates than in the subterranean environment, which generally are formed by smaller and less dynamic or lentic systems (Malard and Hervant 1999, Pipan and Culver 2005).

Redox potential, the second most important variable in the analysis, has negative correlation to concentrations of oxygen, suggesting that reductive processes predominate in the absence of oxygen. Low levels of ORP can be associated with environmental pollution and/or high concentrations of ions, as occurs in waters with karstic origins (Drew and Hötzl 1999). The uncontrolled anthropic activities in the surroundings of the studied area can promote silting and pollution in drainages systems, including the subterranean domains. Most taxa recorded in subterranean habitats were rotifers, which appeared associated with alkaline pH and elevated conductivity. However, the other registered taxa were not strongly related to the measured parameters.

Fong and Culver (1994) found for some caves of the United States that the first order watercourses fed by percolation water have higher diversity than others of higher order. Generally, the water that drains directly from the ceiling can supply a better approximation of physical and chemical characteristics of the upper horizon. However, once in water pools, it frequently presents more different environmental conditions than in the epikarst.

Tropical versus temperate aquatic subterranean biota

Comparing the results obtained with studies on the unsaturated zone in temperate regions (Brancelj 2002, Pipan and Brancelj 2004a, Pipan et al. 2006; Moldovan et al. 2007, 2012, Meleg et al. 2011, Cottarelli et al. 2012), the tropical aquatic subterranean biota (mainly from the unsaturated zone) has a distinct composition. The richness demonstrated was very high compared to records from more extensive approaches, with a sampling effort along a couple of years (e.g., Pipan and Brancelj 2004a, Pipan et al. 2006). Furthermore, Copepoda was not the group with the highest diversity, as in samples of percolating water from many temperate regions, mainly in Europe. However, none of these studies have used nets with such thin mesh (smaller than 40μ m), so the richness of Rotifera may have been underestimated. We also have to emphasize that the sampling method we used could have underestimated the number of benthonic taxa, such as Harpacticoida, commonly find in samples of groundwater (Pipan et al. 2008, Moldovan et al. 2012).

The presence of exclusive taxa at some sites emphasizes the singularity of the studied systems. There are faunistic differences between sites belonging to the same limestone lens and geographically neighbouring. Many species inhabiting the void network of the unsaturated zone are known to have a linear distribution of only a few hundred metres (Pipan et al. 2006, Moldovan et al. 2012), and a discontinuous distribution, caused by different environmental condition (Brancelj 2002, Meleg et al. 2011, Moldovan et al. 2012).

The structure of the unsaturated zone can vary significantly in different regions according to its lithology, geomorphic history, as well as environmental parameters and seasonal variables (Klimchouk 2004). The wide variability of rainfall regime in tropical zones is expected to impact on the tropical subterranean fauna and to offer different models of distribution and diversity than in temperate karst zones.

Final considerations

Nowadays, there is an incessant increase in agricultural, livestock and mining activities in many karst areas in Brazil. In the surroundings of the studied area, cattle and extensive plantations of soybean and cotton can trigger pollution and/or siltation of these streams. Besides, there are many land ownership problems and clandestine mining, which cause deforestation and endanger the integrity of the subterranean environments.

The richness and distribution of aquatic species vary dependent on water quality, and the unsaturated zone biota can be used as a bio-indicator for the impact of polluting actions (Dole-Olivier et al. 1994, Malard et al. 1996, Di Lorenzo et al. 2005, Galassi et al. 2009). The epikarstic zone acts as a reservoir as well as a disperser of toxic substances, considering its ample vertical and horizontal drainage system (Loop and White 2001, Williams 2008). Therefore, it is expected to find variations in the unsaturated zone communities also depending of the degree of pollution. The variations encountered in biological communities can be associated with local activities, being a useful tool in controlling aggressive human practices in the environment. Most stygobionts have a narrow distribution range, the risk of species extinction is thus expectedly high in face of the increase of multiple anthropogenic pressures (Danielopol et al. 2003, Gibert et al. 2009). In our samples, we could not identify stygobiont species because it requires more accurate taxonomic and ecological approaches. However, it is important to highlight that we found many taxa with distribution restricted to just one or few of the studied habitats.

To efficiently assess this particular fauna, it is necessary to constantly monitor the subterranean habitats by means of frequent sampling during different periods of the year.

As such, not only can species richness be recorded but it also can be verified whether there are differences in their distributions and abundances according to seasonality. The use of combined sampling methods (e.g. micrometric mesh nets and vacuum pumps) is also recommended to efficiently access both the benthonic and planktonic fauna.

In Brazil, studies on diversity of aquatic biota in subterranean environments are few and data is still sparse (Pinto-da-Rocha 1995, Bichuette and Santos 1998, Souza-Silva et al. 2012). No such works have been done on the karst unsaturated zone communities. The high richness of taxa presently recorded reveals the potential of unsaturated zone in the tropical region. This preliminary faunistic study represents the first approach on fauna inhabiting the unsaturated zone of the Brazilian karst and also a novelty considering the Neotropic karst systems.

Acknowledgements

We are grateful to colleagues at the Laboratório de Estudos Subterrâneos/UFSCar for helping in the field collections (C. S. Fernandes; D. M. Neto; J. E. Gallão; P. P. Rizzato) and also to N. Negreiros for taxa identification. Statistical analyses were performed with the aid of G. H. Carvalho. We are grateful to the two anonymous reviewers and the editor for valuable suggestions which improved the manuscript and to Maria-Ana Laza for the English correction. Logistics were made possible thanks to the financial support of FAPESP to M. E. Bichuette (process nº 2010/08459-4) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), through a master's grant awarded to the first author (#132981/2011-4). All of the collections was performed in agreement with Brazilian state (authorization for scientific research in the conservation unit SEMARH nº 063/2012) and federal laws (SISBIO # 28992-1). We also thank PPGERN/UFSCar for the infrastructure used in the present study.

References

- Ab'Saber A (1977) Os domínios morfoclimáticos da América do Sul. Primeira Aproximação. Geomorfologia 52: 1–21.
- Auler A, Farrant AR (1996) A brief introduction to karst and caves in Brazil. In: Proceedings of University of Bristol Speleological Society 20(3): 187–200.
- Bichuette ME, Santos FS (1998) Levantamento e dados ecológicos da fauna de invertebrados da Gruta dos Paiva, Iporanga, SP. O Carste 10: 14–19.
- Bichuette ME, Trajano E (2004) Three new subterranean species of *Ituglanis* from Central Brazil (Siluriformes: Trichomycteridae). Ichthyological Exploration of Freshwaters 15: 243–256.
- Bonacci O, Pipan T, Culver, DC (2009) A framework for karst ecohydrology. Environmental Geology 56: 891–900. doi: 10.1007/s00254-008-1189-0
- Brancelj A (2002) Microdistribution and high diversity of Copepoda (Crustacea) in a small cave in central Slovenia. Hydrobiologia 477: 59–72. doi: 10.1023/A:1021043014879

- Brancelj A (2004) Biological sampling methods for epikarst water. In: Jones WK, Culver DC, Herman JS (Eds) Epikarst. Proceedings of the symposium October 1–4, 2003, Sheperdstown, West Virginia, USA. Karst Waters Institute Special Publ. 9: 99–103.
- Brancelj A, Camacho Pérez AI (2009) Groundwater Biodiversity in Europe. doi: 10.1111/j.1365-2427.2008.01972.x
- Camacho AI, Valdecasas AG, Rodriguez J, Cuezva S, Lario J, Lamp J, Sanchez-Moral S (2006) Habitat constraints in epikarstic waters of an Iberian Peninsula cave system. Annales de Limnologie International Journal of Limnology 42: 127–140. doi: 10.1051/ limn/2006009
- Cottarelli V, Bruno MC, Spena MT, Grasso R (2012) Studies on subterranean copepods from Italy, with descriptions of two new epikarstic species from a cave in Sicily. Zoological Studies 51: 556–582.
- Culver DC (1994) Species interactions. In: Gilbert J, Danielopol DL, Stanford JA (Eds): Groundwater ecology. Academic Press, San Diego, California, 271–285.
- Danielopol DL, Griebler C, Gunatilaka A, Notenboom J (2003) Present state and future prospects for groundwater ecosystems. Environmental Conservation 30: 104–130. doi: 10.1017/S0376892903000109
- Di Lorenzo T, Stoch F, Fiasca B, Gattone E, De Laurentiis P, Ranalli F, Galassi DMP (2005) Environmental quality of deep groundwater in the Lessinian Massif (Italy): signposts for sustainability. In: World Subterranean Biodiversity, Proceedings of an International Symposium: 115–125.
- Dole-Olivier MJ, Marmonier P, Creuzé des Châtelliers M, Martin D (1994) Interstitial fauna associated with the alluvial floodplains of the Rhône River (France). In: Gilbert J, Danielopol DL, Stanford JA (Eds) Groundwater ecology. Academic Press, San Diego, California, 313–346.
- Dole-Olivier MJ, Castellarini F, Coineau N, Galassi DMP, Martin P, Mori N, Valdecasas A, Gibert J (2009) Towards an optimal sampling strategy to assess groundwater biodiversity: comparison across six European regions. Freshwater Biology 54: 777–796. doi: 10.1111/j.1365-2427.2008.02133.x
- Drew DP, Orvan J, Pulido Bosch A, Salaga I, Sarin A, Tulipano L (1995) The characteristics of karst groundwater systems. In Cost action-65 Hydrogeological aspects of groundwater protection in karstic areas, Final report, European Commission, EUR 16547, Office for Official Publications of the European Communities, Luxembourg, 349–369.
- Drew D, Hötzl H (1999) Karst hydrogeology and human activities: impacts, consequences and implications. Rotterdam, Brookfield: Balkema.
- Dreybrodt W (1988) Processes in karst systems. Series in Physical Environment. Springer, Heidelberg, 288 pp. doi: 10.1007/978-3-642-83352-6
- Esteves FA (1998) Fundamentos da Limnologia. Interciência, Rio de Janeiro, Brasil, 790 pp.
- Fong DW, Culver DC (1994) Invertebrate fauna. In: Dasher GR, Balfour WM (Eds) The Caves and Karst of the Buckeye Creek Basin County West Virginia. West Virginia Speleological Survey, Maxwelton, 41–42.
- Ford DC, Williams P (2007) Karst hydrogeology and geomorphology. John Wiley & Sons. doi: 10.1002/9781118684986

- Galassi DM, Stoch F, Fiasca B, Di Lorenzo T, Gattone, E (2009) Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. Freshwater Biology 54: 830–847. doi: 10.1111/j.1365-2427.2009.02203.x
- Gibert J, Culver D, Dole-Olivier MJ, Malard F, Christman MC, Deharveng L (2009) Assessing and conserving groundwater biodiversity: synthesis and perspectives. Freshwater Biology 54: 930–941. doi: 10.1111/j.1365-2427.2009.02201.x
- Jones WK, Culver DC, Herman JS (2004) Epikarst. In: Proceedings of the symposium October 1–4 2003, Sheperdstown, West Virginia, USA. Karst Waters Institute Special Publ. 9, Charles Town, W.Va., 200 p.
- Juberthie C (2000) The diversity of the karstic and pseudokarstic hypogean habitats in the world. In: Wilkens H, Culver DC, Humphreys WF (Eds) Ecosystems of the World 30. Subterranean Ecosystems. Elsevier, Amsterdan, 791p.
- Karmann I, Setúbal JC (1984) Conjunto espeleológico São Mateus-Imbira: principais aspectos físicos e históricos da exploração. Espeleo-tema 14: 43–53.
- Klimchouk AB (2004) Towards defining, delimiting and classifying epikarst: its origin, processes and variants of geomorphic evolution. In: Jones WK, Culver DC, Herman JS (Eds) Epikarst, Special Publication 9, WV. Karst Waters Institute, Charles Town, 23–35.
- Kogovšek J (2010) Characteristics of percolation through the karst vadose zone. Ljubljana: ZRC Publishing.
- Loop CM, White WB (2001) A conceptual model for DNAPL transport in karst groundwater basins. Ground Water, 39: 119–127. doi: 10.1111/j.1745-6584.2001.tb00357.x
- Malard F, Plênet S, Gibert J (1996) The use of invertebrates in ground water monitoring: a rising research field. Ground Water Monitoring & Remediation 16: 103–113. doi: 10.1111/j.1745-6592.1996.tb00130.x
- Malard F, Hervant F (1999) Oxygen supply and the adaptations of animals in groundwater. Freshwater Biology 41: 1–30. doi: 10.1046/j.1365-2427.1999.00379.x
- Mangin A (1973) Sur la dynamique des transferts en aquifère karstique. Proceedings of the 6th International Congress of Speleology, Olomouc, CSSR, 6: 157–62.
- Mangin A (1994) Karst hydrogeology. Groundwater Ecology. Academic Press, Orlando, Florida, USA.
- Meleg IN, Moldovan OT, Iepure S, Fiers F, Brad T (2011) Diversity patterns of fauna in dripping water of caves from Transylvania. Annales de Limnologie - International Journal of Limnology 47: 185–197. doi: 10.1051/limn/2011014
- Meleg IN, Fiers F, Robu M, Moldovan OT (2012) Distribution patterns of subsurface copepods and the impact of environmental parameters. Limnologica 42: 156–164. doi: 10.1016/j. limno.2011.10.001
- Moldovan OT, Pipan T, Iepure S, Mihevc A, Mulec J (2007) Biodiversity and Ecology of Fauna in Percolating Water in Selected Slovenian and Romanian Caves. Acta Carsologica 36: 493–501.
- Moldovan OT, Meleg IN, Perşoiu A (2012) Habitat fragmentation and its effects on groundwater populations. Ecohydrology 5: 445–452. doi: 10.1002/eco.237
- Musgrove M, Banner JL (2004) Controls on the spatial and temporal variability of vadose dripwater chemistry: Edwards Aquifer, central Texas. Geochimica et Cosmochimica Acta 68: 1007–1020. doi: 10.1016/j.gca.2003.08.014
- Neves IF, Rocha O, Roche KF, Pinto AA (2003) Zooplankton community structure of two marginal lakes of the river Cuiabá (Mato Grosso, Brazil) with analysis of Rotifera and Cladocera diversity. Brazilian Journal of Biology 63: 329–343. doi: 10.1590/S1519-69842003000200018
- Nimer E (1979) Climatologia do Brazil. Fundação Instituto Brasileiro de Geografia e Estatística, Departamento de recursos naturais e estudos ambientais, Rio de Janeiro.
- Pinto-da-Rocha R (1995) Sinopse da fauna cavernícola do Brasil (1907–1994). Papeis Avulsos de Zoologia 39: 61–173.
- Pipan T, Brancelj A (2004a) Diversity and peculiarity of epikarst fauna: Case study from six caves in Slovenia (Europe). In: Jones WK, Culver DC, Herman JS (Eds), Proceedings of the Symposium on Epikarst, Shepherdstown, West Virginia, 1–4 October 2003. Karst Waters Institute Special Publication 9, Charles Town, WV, 119–126.
- Pipan T, Brancelj A (2004b) Distribution patterns of copepods (Crustacea: Copepoda) in percolation waters of the Postojnska Jama Cave System (Slovenia). Zoological Studies 43: 206–210.
- Pipan T (2005) Epikarst a promising habitat. Copepod fauna, its diversity and ecology: a case study from Slovenia (Europe). ZRC Publishing, Karst Research Institute at ZRC SAZU. Ljubljana, 101 pp.
- Pipan T, Culver DC (2005) Estimating biodiversity in the epikarstic zone of a West Virginia cave. Journal of Cave and Karst Studies 67: 103–109.
- Pipan T, Blejec A, Brancelj A (2006) Multivariate analysis of copepod assemblages in epikarstic waters of some Slovenian caves. Hydrobiologia 559: 213–223. doi: 10.1007/s10750-005-0920-z
- Pipan T, Navodinik V, Novak T, Janžekovič F (2008) Studies of the fauna of percolation water of Huda Luknja, a cave in isolated karst in northeast Slovenia. Acta carsologica 37: 141–151.
- Pipan T, Culver DC (2012) Convergence and divergence in the subterranean realm: a reassessment. Biological Journal of the Linnean Society 107: 1–14. doi: 10.1111/j.1095-8312.2012.01964.x
- Rocha O, Sendacz S, Matsumura-Tundisi T (1995) Composition, biomass and productivity of zooplankton in natural lakes and reservoirs of Brazil. In: Tundisi JG, Bicudo CEM, Matsumura-Tundisi T (Eds) Limnology in Brazil. Rio de Janeiro: 151–166.
- Segers H (2008) Global diversity of rotifers (Rotifera) in freshwater. Hydrobiologia 595: 49–59. doi: 10.1007/s10750-007-9003-7
- Sket B, Trontelj P, Žagar C (2004) Speleobiological characterization of the epikarst and its hydrological neighborhood: its role in dispersion of biota, its ecology and vulnerability. In: Jones WK, Culver DC, Herman JS (Eds) Proceedings of the Symposium on Epikarst, Shepherdstown, West Virginia, 1–4 October 2003. Karst Waters Institute Special Publication 9, Charles Town, 104–113.
- Simon KS, Benfield EF (2002) Ammonium retention and whole stream metabolism in cave streams. Hydrobiologia 482: 31–39. doi: 10.1023/A:1021257822591
- Simon KS, Benfield EF, Macko SA (2003) Food web structure and the role of epilithic films in cave streams. Ecology 84: 2395–2406. doi: 10.1890/02-334

- Simon KS, Pipan T, Culver DC (2007) A conceptual model of the flow and distribution of organic carbon in caves. Journal of Cave and Karst Studies 69: 279–284.
- Souza-Silva M, Liria CCS, Sampaio FAC, Ferreira RL (2012) Transitory aquatic taxocenosis in two neotropical limestone caves. Revista Brasileira de Espeleologia 2: 29–41.
- Stoch F (2000) New and little known *Parastenocaris* (Copepoda, Harpacticoida, Parastenocarididae) from cave waters in Northeastern Italy. Bollettino del Museo Civico di Storia Naturale di Verona 24: 195–206.
- Trajano E, Gnaspini P (1991) Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos táxons. Revista Brasileira de Zoologia, São Paulo, 7: 383–407. doi: 10.1590/S0101-81751990000300017
- Trajano E, Bichuette ME (2006) Biologia subterrânea: Introdução. Redespeleo, São Paulo.
- Trajano E, Bichuette ME (2010) Diversity of Brazilian subterranean invertebrates, with a list of troglomorphic taxa. Subterranean Biology 7: 1–16.
- Williams PW (2008) The role of the epikarst in karst and cave hydrogeology: a review. International Journal of Speleology 37: 1–10. doi: 10.5038/1827-806X.37.1.1

INSIDE SIBIOS



21st International Conference on Subterranean Biology, Košice, Slovakia, 2–7 September 2012

Ľubomír Kováč¹

I Faculty of Science, P. J. Šafárik University, Department of Zoology, Moyzesova 11, Košice, Slovakia

Corresponding author: Lubomír Kováč (lubomir.kovac@upjs.sk)

Academic editor: O. Moldovan Received 24 October 2012 Accepted 24 October 2013 Published 21 November 20

Citation: Kováč Ľ (2013) 21st International Conference on Subterranean Biology, Košice, Slovakia, 2–7 September 2012. Subterranean Biology 11: 75–78. doi: 10.3897/subtbiol.11.6490

Organizer: Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Košice, Slovakia

Organizing committee: Ľubomír Kováč, Andrej Mock, Peter Ľuptáčik, Igor Hudec, Marcel Uhrin, Vladimír Papáč, Zuzana Višňovská, Peter Hlaváč

Scientific committee: David C. Culver (USA), Louis Deharveng (France), William F. Humphreys (Australia), William R. Jeffery (USA), Remko Leijs (Australia), Oana T. Moldovan (Romania), Tone Novak (Slovenia), Pedro Oromí (Spain), Martin Plath (Germany), Valerio Sbordoni (Italy), Boris Sket (Slovenia), Eleonora Trajano (Brazil).

The Conference was held at the university campus in Košice City centre under the auspices of the International Society for Subterranean Biology. It hosted 116 participants from 20 countries: Brazil (16), Slovakia (14), Croatia (11), Slovenia (11), Czech Republic (10), Germany (8), USA (7), Australia (6), Italy (6), Romania (6), France (4), Bosnia and Herzegovina (3), Iran (3), Mexico (2), Poland (2), Spain (2), Switzerland (2), Great Britain (1), Portugal (1) and Serbia (1).

The Society supported altogether 10 young participants with travel grants: Kym Abrams (Australia), Jana Bedek (Croatia), Livia Cordeiro-Borghezan (Brazil), Teo Delič (Slovenia), Somayeh Esmaeili (Iran), Marjeta Konec (Slovenia), Ioana Meleg (Romania), Thais Giovanni Pellegrini (Brazil), Ana Sofia Reboleira (Portugal) and Erika Taylor (Brazil).

Copyright Lubomír Kováč. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



Figure 1. Conference participants. Photo: V. Kalinčáková.



Fong Dumnicka 15 Horst Wilkens 16 Luiza B. Simőes 17 Marcella P. Uchoa 18 Kym M. Abrams 19 Rachael A. King 20 Florian Malard 21 Ioana N. Meleg 22 Cornelia 64 Karel Tajovský 65 Somaveh Esmaeli-Rineh 66 Vahid Akmali 67 Branko Jalžić 68 Aleksandra Kilian 69 Jaroslav Svatoň 70 Roman Ozimec 71 Mladen Kučinić 72 Slavko Polak 73 Erika L. S. Taylor 74 Marcus P. A. Oliveira 75 Rodrigo L. Ferreira 76 Vladimír Papáč 77 Traian Brad 78 Alica Chroňáková 79 Edita Miková Valerio Sbordoni 8 Marina Cobolli 9 Monika Balogová 10 Dajana Hmura 11 Maja Hodžić 12 Ana Sofia P. S. Reboleira 13 Janice Muriel-Cunha 14 Elžbieta Komerički 32 Helena Bilandžija 33 Jana Bedek 34 Vladimír Košel 35 Jean-Jacques Geoffroy 36 Hans S. Reip 37 Elke Aden 38 Ulrike Strecker 39 Jaroslav Smrž 40 Michal Rendoš 41 Alena Nováková 42 Vladimír Šustr 43 José D. Gilgado 44 Alexander M. Weigand 45 Sanja Gottstein 46 David Eme 47 Lubomír Kováč 48 Gregor Aljančič 49 Magdalena Nžpărus 50 Zuzana Višňovská 51 William Jeffery 52 Thais G. Pellegrini 53 Iva Njunjić 54 Marko Lukić 55 Martina Pavlek 56 Lucie Jonas E. spengler 23 Maria Avramov 24 Lou Maurice 25 Peter Luptáčik 26 Remko Leijs 27 Thami G. Silva 28 Anđela Ćukušić 29 Stuart Halse 30 Tamara Ćuković 31 Ana volná 57 Beata Adamczyk 58 Giuseppe Messana 59 Oana T. Moldovan 60 Stefano Taiti 61 Maria E. Bichuette 62 Pedro Oromí 63 Lívia M. Cordeiro-Borghezan 96 Vlastimil Růžička 97 José G. Palacios-Vargas 98 lan Moody 99 Jason Corbett 100 Robert A. Susac 101 Barbara Zakrzewska 102 Maja Zagmajster 103 Ivica Rodica Plăiasu 89 Steven I. Taylor 90 Horton H. Hobbs III 91 Hans I. Hahn 92 Tania Pipan 93 Peter Tronteli 94 David Culver 95 Daniel W. 80 Peter Fenda 81 Marcel Uhrin 82 William F. Humphreys 83 Eleonora Trajano 84 Marie-José Turquin 85 Marcus V. Dominguez 86 Laura Epure 87 Barač, and 104 Valerio Ketmaier. Sketch: P. Ľuptáčik Gallão 88

"Subterranean realm – fragile ecosystems and unique biodiversity: towards new concepts" was a central topic of the event highlighting the necessity of complex study of subterranean environments. The scientific program was divided in seven symposia offered in advance:

- Subterranean Biodiversity
- Adaptation, Development, Physiology and Behaviour
- Phylogeography, Phylogeny and Evolution
- Microbiology and Geomicrobiology and Sedimentology
- Population and Community Ecology of Subterranean Organisms
- Subterranean Ecosystems
- Conservation of Subterranean Life

In total 70 oral presentations, 67 posters and 1 video document were presented during four conference days. Young participants were awarded by Scientific Committee of the Society: Alexander M. Weigand for the best oral presentation and Dajana Hmura for the best poster. Gregor Aljančič and Magdalena Năpăruş were awarded for oral presentation with important message to conservation of subterranean life. The other details on the conference are provided on the web page www.icsb2012.eu, including video records of the oral presentations.

Other events included welcome reception in Guest House Grand and Košice downtown visit by walk with guide. During the mid-conference excursion the participants visited two important show caves of Slovakia – Dobšinská Ice Cave and Demänovská Cave of Liberty. Museum of the Liptov Village in Pribylina displayed the typical folk architecture. The conference finished with the social dinner at House of Art.

General Assembly of ISSB elected new members of the Council Board and approved candidature of Mexico for organization of the next conference. It will be held on August 31–September 5, 2014 in Juriquilla campus of the Universidad Nacional Autónoma de México in Querétaro state. The main organizer is José G. Palacios-Vargas (UNAM).

See you in Mexico in 2014! On behalf of organizing committee of the 21st ICSB conference *Eubo Kováč*