The microscopic ellobioid, *Zospeum* Bourguignat, 1856 (Pulmonata, Ellobioidea, Carychiidae) makes a big debut in Basque Country and the province of Burgos (Spain)

Les microscopiques escargots ellobioides du genre *Zospeum* Bourguignat, 1856 (Pulmonata, Ellobioidea, Carychiidae) font leur début dans le Pays basque et dans la province de Burgos (Espagne)

Adrienne JOCHUM¹, Alexander M. WEIGAND¹, Rajko SLAPNIK², Jana VALENTINČIČ² and Carlos E. PRIETO³

¹ Institute for Ecology, Evolution and Diversity, Max-von-Laue-Str. 13, Goethe-University, 60438 Frank furt am Main, Germany
² Institute of Biology, Center for Scientific Research of the Slovenian Academy of Sciences and Arts, Novi trg 2, p.p. 306, 1000
Ljubljana, Slovenia

³ Department of Zoology and Animal Cell Biology, Faculty of Science and Technology, University of the Basque Country (UPV /EHU), P.O. box 644, 48080-Bilbao, Spain

Corresponding author: jochum@bio.uni-frankfurt.de

Abstract – Two thriving colonies and a new distribution of the minute troglobitic ellobioid snail, *Zospeum* Bourguignat, 1856 have been discovered in the Cordillera Cantábrica of northern Spain. This is the first report of *Zospeum* sp. in Arrikrutz Cave in the Aizkorri massif (Gipuzkoa, Basque Country). Another rich colony, including nest-like cavities comprising fecal strands of another *Zospeum* sp. was discovered in Cueva de Las Paúles in Sierra Salvada (Burgos Province).

Keywords - Ellobioidea, Carychiidae, Zospeum, troglobitic microsnails, endangered species, biospeleology

Résumé – Deux populations importantes et une nouvelle aire de distribution du minuscule escargot troglobie *Zospeum* Bourguignat, 1986 ont été découvertes dans la Cordillère cantabrique au nord de l'Espagne. La grotte Arrikrutz dans le massif d'Aizkorri (Gipuzkoa, Pays basque) présente une colonie de *Zospeum* jusqu'alors inconnue. Une autre importante colonie de *Zospeum*, incluant des cavités d'occupations comprenant des pelotes fécales, a aussi été découverte dans la grotte de Las Paúles de la Sierra Salvada (Province de Burgos).

Mots-clés - Ellobioidea, Carychiidae, Zospeum, escargots troglobies, animaux menacés, biospéologie

The authors, comprising an international team of malacologists from Germany, Slovenia and Spain, discovered a new distribution and colony of the troglobitic ellobioid, *Zospeum* Bourguignat, 1856 (Pulmonata, Ellobioidea, Carychiidae) in the Aizkorri massif, Gipuzkoa, Basque Country and a colony of a previously recorded (Altonaga *et al.* 1994) distribution in the Sierra Salvada in Burgos province. These unique finds included a first time view of a living colony of this minute (1.0 - 1.3 mm shell height) gastropod.

These glassy ellobioids belong to an ancient group of pulmonates that migrated onto land independently of the stylommatophoran gastropod clade (de Frias Martins 1996, Vermeij & Dudley 2000, Klussmann-Kolb *et al.* 2008, Dayrat *et al.* 2011). Although the Ellobioidea are primarily marine and demonstrate a mangrove ecology, this troglobitic lineage belongs to one of two groups to have settled land beyond the moist, humid conditions of the mangroves. To date, the ellobioid genus *Zospeum* has been found in karst caves along a mid-latitudinal belt of terrestrial biodiversity (Culver *et al.* 2006) along the $42^{\circ} - 46^{\circ}$ parallels, encompassing the Cantabrian Mountains, the Pyrenees, Southern Alps and the Dinaric Alps (Watson & Verdcourt 1953, Gittenberger 1980, Doll 1982, Slapnik & Ozimec 2004, Weigand *et al.* 2011). Recent finds have been sited in Guangxi caves of China (L. Deharveng, MNHN Paris, personal communication 2010) as well as in the Nodong Cave on "wet muddy walls near piles of small limestone fragments" of Gangweon Province, South Korea (Prozorova *et al.* 2010).

This expedition sampled 17 caves from Asturias to northern Navarra, including the Basque Country caves as well as those of northeastern Burgos Province (Figure 1). Some of these caves were already sampled for *Zospeum* years ago from which four unnamed species were found (Altonaga *et al.* 1994). The first colony and a new distribution of *Zospeum* were encountered in Cueva Arrikrutz



Figure 1 - Caves sampled for Zospeum in northern Spain

Red circle indicates caves explored (June 2011) containing *Zospeum* populations; **X** indicates caves explored (June 2011) not containing *Zospeum* populations; **White circle** indicates caves known to contain *Zospeum* populations but not sampled on this excursion.

within the Natural Park of Aizkorri-Araotz (municipality of Oñati). This cave is part of the Gesaltza-Arrikrutz karst system, which is located ca. 400 m above sea level beneath the Aizkorri massif and opened to the public in June 2007. Individual *Zospeum* sp. nov. 1 (Figures 2A, 2B and Figure 3A), like tonsil stones embedded within cavities of soft tonsil tissue, were found living in a fine layer of mud

constituting the cavity-laden, pillowy mesh-like pattern (Figure 2A) lining an upper wall of the cave. Associated with this colony were fine, yellowy fungal filaments (Figure 2B) on the surface of the mud as well as interlaced between the perforated pillowy matrix.

A second colony (Zospeum cf. suarezi, Figure 3C) was discovered in Cueva de Las Paúles, situated in beech forest within the karstified landscape of the Sierra Salvada comprising the Natural Monument of Monte Santiago. According to Altonaga et al. (1994), two species of Zospeum (Z. cf. suarezi and Z. sp. nov. 3) have been recorded in Cueva de Las Paúles. In congruence with Altonaga et al. (1994), two species were also found by this expedition team in Cueva de Las Paúles (Figures 3B, 3C). The prolific colony discovered in this cave constituted a fine, sediment matrix of minute spongy cavities, comprising furrows, vermiform fecal pellets, two apparent different kinds of fungi (yellow and white colored), empty shells and holes of circular nest-like structures (Figures 2C, 2D).

Despite the dry conditions in northern Spain during June 2011, nine caves demonstrated living populations of these sensitive carychiid snails. Empty shells indicated that *Zospeum* inhabited these

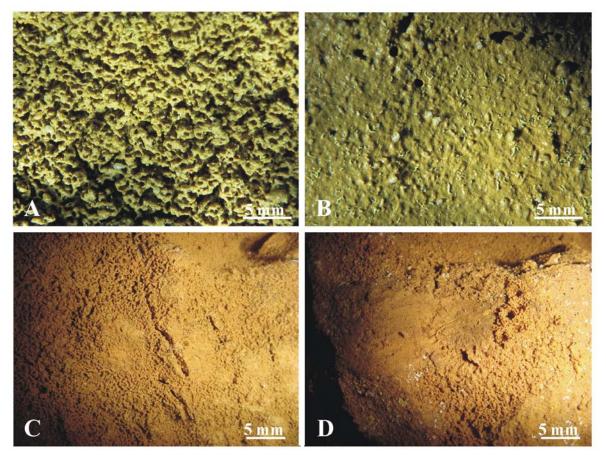


Figure 2 – Mud matrix comprising *Zospeum* colonies on cave walls

A. *Zospeum* sp. nov. 1 colony, yellow fungi and empty shells in Cueva Arrikrutz; **B.** *Zospeum* sp. nov. 1 nest holes, empty shells and yellow fungal filaments in Cueva Arrikrutz; **C.** *Zospeum* cf. *suarezi* colony furrows in Cueva de Las Paúles; **D.** *Zospeum* cf. *suarezi*, fecal pellet nests and fungal patches in Cueva de Las Paúles.



Figure 3 – Zospeum individuals found June 2011 in Spain
A. Zospeum sp. nov. 1 from Cueva Arrikrutz;
B. Zospeum sp. nov. 3 from Cueva de las Paúles;
C. Zospeum cf. suarezi from Cueva de las Paúles.

cave systems at a particular time. The population along the walls of Cueva Arrikrutz encompassed a vast tapestry of dried shells as well as a proportionately large number of living individuals within a few square centimeters. In a 200-gram sample of sediment, five live adult individuals and five live juveniles were collected. This sediment contained an additional, mainly translucent (a key indication of recent mortality) 450 empty adult shells and ca. 150 empty juvenile shells suggesting a 1.5 - 2.0% survival rate witnessed for the summer month of June. On the other hand, if emptied shells can maintain a fresh, translucent appearance for many years, these shells could well have accumulated through many generations. We attribute this spectacular find partly to easy accessibility to the upper level of the cave via a modern walkway constructed for tourists. Under normal speleological conditions, caves must be navigated more laboriously considering water levels, time of year and narrow passageways that otherwise complicate the already difficult location of living individuals in ephemeral populations of these cryptic microsnails (Figure 4).

Zospeum has up to now been scantily found as single individuals living on moist cave walls within their range and as shells reported in other caves in Spain (Gittenberger 1980, Escolà & Bech 1986).

It is presumed that the populations in Cueva Arrikrutz and Cueva de Las Paúles were most likely able to sustain themselves as colonies for many generations due to continuous, consistent environmental conditions such as temperature, humidity, airflow, water levels and the influx of organic material. Moreover, the patched fungal colonies associated with these populations may well serve as a food source or even comprise a symbiotic relationship in respect to the breakdown of fecal matter and the metabolic processes enabling the colonization of these minute snails. These pivotal findings enable new insights concerning their characteristic ellobioid colonization in mud and their adaptation to a troglobitic ecology.



Figure 4 – *Zospeum* hunt in June 2011

Acknowledgements - We are grateful for the financial support provided by Annette Klussmann-Kolb and the BiK-F Biodiversity and Climate Research Center of the researchfunding program, 'LOEWE - Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz' of the Ministry of Higher Education, Research, and the Arts for the State of Hessen. We also thank the Ministry of Higher Education, Science and Technology of the Slovene Republic (P1-0236 Biodiversity and Gradients) for their continued support of our research. We acknowledge Benjamin Gómez and Luis Javier Chueca for their collecting help in other caves of this expedition. We also thank Alexander Ule of Inguru Abentura for his expert assistance in navigating some very tight passages. We additionally thank the personnel at Arrikrutz Cave for their kind cooperation and interest in our work. We are grateful to Tim Pearce and the editor for their constructive input on an earlier version of the manuscript.

Literature cited

- Altonaga, K., Gómez, B.J., Martín, R., Prieto, C.E., Puente, A.I., & Rallo, A. 1994. Estudio faunístico y biogeográfico de los moluscos terrestres del norte de la Península Ibérica. Ed. Eusko Legebiltzarra -Parlamento Vasco (Premio Xabier María de Munibe), Vitoria: 505 pp.
- Culver, D., Deharveng, L., Bedos, A., Lewis, J., Madden, M., Reddel, R., Sket, B., Trontelj, P., & White, D. 2006. The mid-latitude biodiversity ridge in terrestrial cave fauna. *Ecography*, 29: 120-128.
- Dayrat, B., Conrad, M., Balayan, S., White, T.R., Albrecht, C., Golding, R., Gomes, S.R., Harasewych, M.G., & de Frias Martins, A.M. 2011. Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): New insights from increased taxon sampling. *Molecular Phylogenetics and Evolution*, doi: 10.1016/j.ympev.2011.02.014 1-13.
- Doll, W. 1982. Beobachtungen über Lebensweise und Fortpflanzung von Carychium tridentatum Risso im

Oberrheingebiet (Pulmonata: Ellobiidae). Archiv für Molluskenkunde, 112: 1-8.

- Escolà, O. & Bech, M. 1986. Molluscs recollits a cavitats subterrànies de Catalunya i Osca. *The Zoological Miscellany*, 10: 87-92.
- de Frias Martins, A.M. 1996. Relationships within the Ellobiidae. In: *Origin and Evolutionary Radiation of the Mollusca* (ed. Taylor J), Oxford University Press, The Malacological Society of London, London: 392 pp.
- Gittenberger, E. 1980. Three notes on Iberian terrestrial gastropods. *Zoologische Mededelingen* (Leiden), 55: 201-213.
- Klussmann-Kolb, A., Dinapoli, A., Kuhn, K., Streit, B., & Albrecht, C. 2008. From sea to land and beyond—new insights into the evolution of euthyneuran Gastropoda (Mollusca). *BMC Evolutionary Biology*, 8: 57.
- Prozorova, L., Noseworthy, R., Lee, J.S., Zasypkina, M. 2010. Korean cave malacofauna with emphasis on troglobitic carychiids (Pulmonata: Ellobioidea: Carychiidae). *Tropical Natural History* (Suppl. 3): 135.

- Slapnik, R., Ozimec, R. 2004. Distribution of the genus Zospeum Bourguignat, 1856 (Gastropoda, Pulmonata, Ellobiidae) in Croatia. Natura Croatica, 13: 115-135.
- Vermeij, G., Dudley, R. 2000. Why are there so few evolutionary transitions between aquatic and terrestrial ecosystems? *Biological Journal of the Linnean Society*, 70: 541-554.
- Watson, H., Verdcourt, B.J. 1953. The two British species of *Carychium. Journal of Conchology*, 23: 306-324.
- Weigand, A.M., 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: 255-265.

Soumis le	29 juillet 2011
Accepté le	18 septembre 2011
Publié le	12 octobre 2011