

Introduction

The underlying mechanisms and processes that prompt the colonization of extreme environments constitute major research themes of evolutionary biology and modern cave biology¹⁻⁴. The special adaptations required to survive in these extreme habitats (low food availability, hypoxic waters, permanent darkness), and the geographical isolation of caves nominate cave biodiversity as ideal study subjects to answer long-standing questions concerning the interplay among adaptation, biogeography, and evolution^{4,5}.

The present project aims to examine the phylogeographic patterns exhibited by two sympatric species of surface and cave-dwelling peracarid crustaceans (*Asellus aquaticus* and *Niphargus hrabei*), and in doing so elucidate the role of exaptations behind the colonization and successful adaptation to cave ecosystems.

Methods

Specimens of both crustacean species were sampled from freshwater cave and surface habitats in Hungary (Fig. 1), as well as in the neighboring countries of Austria, Romania, and Serbia⁶. Haplotype network reconstruction (TCS) and phylogenetic inference (using both Bayesian and Maximum Likelihood methods) were used in conjunction with population trees (inferred by multi-locus coalescent models) to elucidate the genetic structure, phylogeographic patterns, and divergence time estimates of *A. aquaticus* and *N. hrabei* surface and cave populations. The degree of monophyly of each population was then estimated using Genealogical Sorting Indices (*gsi*), and compared in a pairwise manner (*pwgsi*) using the phylogenies derived from the study.

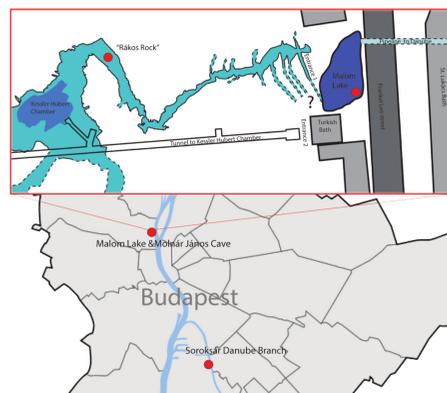


Fig 1. Schematic illustration of our sampling locations within Budapest, Hungary. Red circles indicating exact sites within Molnár János Cave (Rakos Rock) and surface environments (Malom Lake and Soroksár).

Results

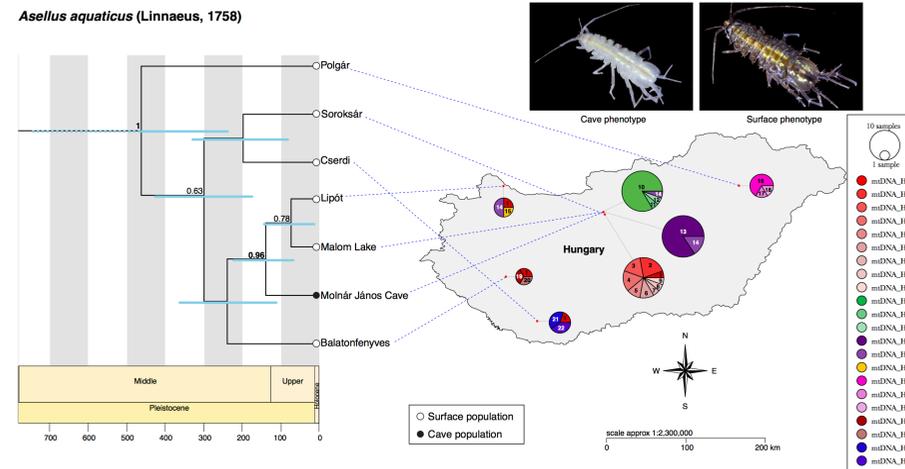


Fig 2. Divergence time estimates (x axis in thousands of years) of *Asellus aquaticus* populations (calculated with a multi-locus coalescent model in *BEAST; outgroups not shown) and the distribution of its populations with relative mtDNA haplotype frequencies throughout Hungary. Phylogenetic and population tree analyses support the inclusion of the cave phenotype as part of the species, but with evident population structuring and marked morphological differences as a result of the cave environment.

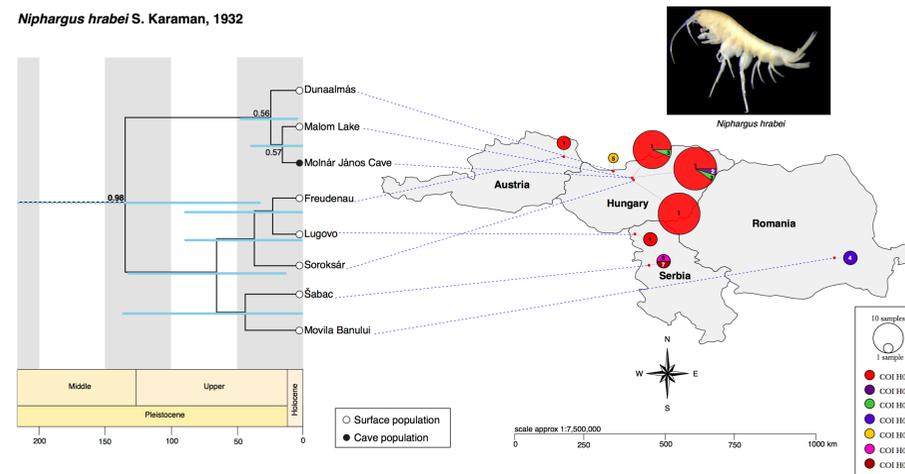


Fig 3. Divergence time estimates (x axis in thousands of years) of *Niphargus hrabei* populations (calculated with a multi-locus coalescent model in *BEAST; outgroups not shown) and the distribution of its populations with relative COI haplotype frequencies in our three main Hungarian sites and neighboring populations. Phylogenetic and population tree analyses do not support any evident genetic structuring between cave and surface populations.

Contrasting patterns were found between species, with *A. aquaticus* showing strong genetic differentiation between cave and surface populations and *N. hrabei* lacking any evidence of genetic structure mediated by the cave environment. Isolation by cave environment, rather than distance, likely drives the genetic structure observed between immediately adjacent cave and surface populations of *A. aquaticus*, a predominantly surface species with only moderate exaptations to subterranean life. For *N. hrabei*, in which populations exhibit a fully 'troglomorphic' phenotype, the lack of genetic structure suggests that subterranean environments do not pose a barrier for a surface-cave species.

Discussion

Under the scenario suggested by our divergence time estimates (Figs. 2–3), Molnár János Cave could have served as a thermal refuge for *A. aquaticus* during periods of climatic change throughout the Pleistocene. Being a moderately exapted species, *A. aquaticus*, colonized Molnár János Cave and was isolated from surface populations of adjacent Malom Lake and the Danube River eventually resulting in the emergence of troglomorphic phenotypes. Upon cessation of this physical isolation, it is possible that competitive exclusion prevented new surface populations from successfully invading in the cave. This mechanism would be in accordance with the observed patterns of genetic differentiation. On the other hand, *N. hrabei*, is a surface representative of a cave-dwelling genus and as such it displays high-levels of troglomorphic characteristics common amongst cave inhabitants. *N. hrabei* cave and surface populations appear to be panmictic and show no evidence of isolation by the cave environment nor of competitive exclusion in the cave.

Work in Progress

Further analyses are being conducted to elucidate the exact causes of the observed patterns, and to investigate the influence of exaptations and troglomorphy in shaping the observed phylogeographic patterns of these species.

For instance, we are presently undertaking a comparative analysis of their transcriptomes and methylomes to shed light on the adaptive divergence between cave and surface populations of these enigmatic crustaceans.

References

- Barr, T. C. & Holsinger, J. R. (1985) Speciation in Cave Faunas. *Annual Review of Ecology and Systematics* 16, 313–337.
- Juan, C., Guzik, M. T., Jaume, D. & Cooper, S. J. B. (2010) Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Molecular Ecology* 19, 3865–3880.
- Benvenuto, C., Knott, B. & Weeks, S. (2015) in *The Natural History of the Crustacea* (eds Thiel, M. & Watling, L.) 379–417, Oxford University Press.
- Pérez-Moreno, J. L., Iliffe, T. M., & Bracken-Grissom, H. D. (2016) Life in the Underworld: Anchieline cave biology in the era of speleogenomics. *International Journal of Speleology* 45, 149–170.
- Zhang, Y. & Li, S. (2013) Ancient lineage, young globites: recent colonization of caves by *Nesticella* spiders. *BMC evolutionary biology* 13, 183.
- Copilas-Cioceanu, D., Fišer, C., Borza, P., Balazs, G., Anghal, D., Petrussek, A. (2016) Low intraspecific genetic divergence and weak niche differentiation despite wide ranges and extensive sympatry in two epigean *Niphargus* species (Crustacea: Amphipoda). *Zoological Journal of the Linnean Society*, accepted.



This research was made possible by The Philip M. Smith Graduate Research Grant for Cave and Karst Research from the Cave Research Foundation and The Crustacean Society Scholarship in Graduate Studies.

