NATURA SLOVENIAE 21(1): 57-59
FIELD NOTE

Prejeto / Received: 6. 8. 2019
Sprejeto / Accepted: 8. 8. 2019

Discovery of subterranean amphipod *Niphargus stygius* (Schiødte, 1847) (Amphipoda: Niphargidae) in a cave drip pool with increased salinity

Najdba slepe postranice *Niphargus stygius* (Schiødte, 1847) (Amphipoda: Niphargidae) v jamski luži prenikle vode s povečano slanostjo

Maja ZAGMAJSTER, Teo DELIĆ,
SubBioLab, Department of Biology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana;
E-mail: maja.zagmajster@bf.uni-lj.si

With more than 430 species currently described, the subterranean genus *Niphargus* is the species richest genus of amphipods (Fišer 2019). Most of the species live in subterranean freshwaters (groundwaters) with rare cases of species that seem to have recolonized surface waters (Copilaş-Ciocianu et al. 2018). The genus is found in a wide range of habitats, yet the actual data on individual species tolerances to different abiotic parameters are few (Fišer 2019). Here we report on the finding of an individual of the species *Niphargus stygius* (Schiødte, 1847) in cave waters with increased salinity.

In winter 2012, we visited the cave Logarček (Cad. No. 28; Slovenian Cave Register 2019) near Laze pri Planini, close to Postojna (cave entrance coordinates and altitude: 14.26832 E, 45.86493 N, 498 m a.s.l.; Slovenian Cave Register 2019). The cave has a vertical extent of approximately 120 m, and the cumulative length of cave passages reaching nearly 5 km (Slovenian Cave Register 2019). The entrance of the cave is approx. 20 m deep shaft, leading to a fossil channel, which splits into two branches. Both the Northern and the Southern branches contain water lakes and siphons at the deepest parts (see Ilič 2003 for the cave map).

On 23. 2. 2012, upon our first visit to the cave, we found an active water drip, forming a water current and a series of small water puddles on the cave floor in a cave chamber opening from the Northern branch of the cave called »Podorna dvorana«. As the second author tried to drink the water from the water puddles, he noticed its unusually sour and salty taste. We found and collected one amphipod in one of the puddles filled with dripping water, and a sample of water was taken directly from the drip. We measured abiotic parameters of the water in the lab on the same day, when returning to the lab of the Department of Biology in Ljubljana, using portable multimeter CyberScan PCD650 (Eutech Instruments). The specific electric conductivity and salinity level were increased (Tab. 1) compared to normal values for freshwater, having salinity less than 0.5 ‰ (Venice system 1958).

We repeated the visit two weeks later, on 9. 3. 2012, when we checked only the Northern branch up to app. 600 m from the entrance, to the lake in »Skalni rov«. This time, there was no active water drip in »Podorna dvorana«, but the water remained in puddles on the cave floor. We collected the water from two different puddles, and measured abiotic parameters three days later (on 12. 3. 2012) in the lab. The salinity of the water in the puddles was lower than during our February sampling, but much higher than in the water taken from a cave lake in »Skalni rov«, about 250 m deeper in the cave (Tab. 1). At the time of the visit, there were no additional amphipods observed in any of the water puddles on the floor.

The animal collected during the February visit was kept alive in the same drip pool water in the laboratory, in the Speleobiological chamber at Department of Biology (Biotechnical Faculty, University of Ljubljana), at an approximate temperature of 10 °C. It remained alive in this water for about a month, and died between 26. and 30. 3. 2012.

Our accidental observations are interesting in two aspects. First, the water drip in »Podorna dvorana«, active for only a limited interval, appears to be occasionally salty. The chamber is positioned underneath the Vrhnika–Postojna motorway, with the ceiling only 13 m thick (Šebela 2000; Ilič 2003). During our first visit, the snow cover on the surface was melting due to sunny weather, and it is very likely that the increased salinity in the cave was caused by highly salted waste waters from the motorway above the cave. Similarly, increased levels of chlorides and specific electric conductivity in karst waters were observed

in a study of road waste waters from a section of the motorway near Postojna (Kogovšek 1993). Both parameters were directly connected to salting of roads. On our second visit, the snow was no longer present on the surface, and the water was no longer dripping into the puddles in »Podorna dvorana«. Noteworthy, in the deeper lake of »Skalni rov« of the Northern channel, a morphologically unusual form of cave hydrozoan *Velkovrhia enigmatica* was found during the same cave visits in 2012 (Zagmajster et al. 2013). The individuals had increased number of tentacles, which could be the effect of the increased salinity (Zagmajster et al. 2013, with references therein), although the lake with *V. enigmatica* showed normal freshwater salinity (Tab. 1). We suggest that waste waters from the motorway are neglected, yet temporary important pollutants of subterranean environments. The extent of this pollution and its impact on subterranean animals remain to be established.

Second, it is interesting to see that a freshwater *Niphargus* species was able to survive in the increased salinity in a cave nearly 50 km away from the sea coast. Some *Niphargus* species tolerate increased water salinity, but they were all found in anchialine (brackish) waters close to the sea (Sket 1977; Gottstein et al. 2012, Delić et al. 2017). Our discovery of *N. stygius* confirms its ability to survive in waters with increased salinity for a short time, but whether it survives in such conditions for a long time, remains unanswered. It is a species found in diverse groundwater freshwater habitats: cave streams and lakes, pools of dripping water, but also at springs and, consequently, in surface streams in the vicinity of springs (Delić 2017). Our observation suggests that the species may survive on a short term in suboptimal conditions (the individual was not fed during captivity), and that at least some individuals of this species are able to survive such increased salinity for a few weeks. However, we cannot rule out a possibility that other *Niphargus* individuals did not survive local pollution, and had been washed away from the cave puddles prior to the first visit. A controlled laboratory experiment is needed to resolve the species' tolerance to increased salinity in waters.

Table 1. Measurements of some abiotic water parameters from the cave Logarček near Laze in central Slovenia, taken in the »Podorna dvorana« chamber, and from the lake in »Skalni rov«, during two samplings in winter 2012. See Ilič (2003) for spatial positions of the chambers.

Tabela 1. Meritve nekaj abiotskih parametrov vode iz jame Logarček pri Lazah v osrednji Sloveniji, ki je bila vzeta v »Podorni dvorani« in v jezeru v »Skalnem rovu«, v dveh vzorčenjih pozimi 2012. Glej Ilič (2003) za prostorski položaj dvoran.

Date	Part of the cave	Conductivity (mS)	Salinity (NaCl ppt)	pH
23. 2. 2012	»Podorna dvorana« - dripping water	12.000	13.810	/
9. 3. 2012	»Podorna dvorana« - puddle 1 on the floor	7.031	7.527	7.50
	»Podorna dvorana« - puddle 2 on the floor	5.596	5.822	7.45
	Lake in »Skalni rov«	0.315	0.283	7.67

Acknowledgements

Field work was conducted with the help of Peter Trontelj, Marjeta Konec and Simona Prevorčnik. Species identification was confirmed by Cene Fišer. The work was supported by the Slovenian Research Agency, via the Research program P1-0184.

References

Copilaş-Ciocianu D. Fiper C., Borza P., Petrisek A. (2018): Is subterranean lifestyle reversible? Independent and recent large-scale dispersal into surface waters by two species of the groundwater amphipod genus *Niphargus*. Mol. Phyl. Evol. 119: 37-49.

Delić T. (2017): The role of ecological niche in speciation of the subterranean amphipods (genus *Niphargus*). Doctoral dissertation, University of Ljubljana, 116 pp.

Delić T., Švara V., Coleman C.O., Trontelj T., Fišer C. (2017): The giant cryptic amphipod species of the subterranean genus *Niphargus* (Crustacea, Amphipoda). Zool. Scr. 46(6): 740-752.

Fišer C. (2019): *Niphargus* - a model system for evolution and ecology. In: White W.B., Culver D.C., Pipan T. (Eds.), Encyclopedia of caves, Third Edition. Academic Press, London, San Diego, Cambridge, Oxford, pp. 746-754.

Gottstein S., Kršinić F., Ternjej I., Cukrov N., Kutleša P., Jalžić B. (2012): Shedding light on crustacean species diversity in the anchihaline caves of Croatia. Nat. Croat. 21: 54-58.

Illič U. (2003): Logarček trenutno dolg 4334 m. Internet site of Jamarski klub Železničar: http://www.ljudmila.org/jkz/html/logarcek_2003.html [accesed on: 10. 5. 2019].

Kogovšek J. (1993): Kakšna je sestava voda, ki odtekajo z naših cest? Ujma 7: 67-69.

Sket B. (1974): *Niphargus stygius* (Schiødte) (Amphipoda, Gammaridae) - die Neubeschreibung des Generotypus, Variabilitat, Verbreitung und Biologie der Art, I. Biol. vest. 22(1): 91-103.

Sket B. (1977): *Niphargus* im Brackwasser. Crustaceana, Suppl. 4: 188-191.

Slovenian Cave Registry (2019): Registry of Slovenian Caves, March 2019. Karst Research Institute ZRC SAZU, Postojna.

Šebela S. (2000): Karta ranljivosti krasa vzdolž avtocest v Sloveniji. Ann. Ser. hist. nat. 10(19): 127-132.

Venice system (1958). Symposium on the Classification of Brackish Waters. Oikos 9(2): 311-312.

Zagmajster M., Delić T., Prevorčnik S., Zakšek V. (2013): New records and unusual morphology of the cave hydrozoan *Velkovrhia enigmatica* Matjašič & Sket, 1971 (Cnidaria: Hydrozoa: Bougainvilliidae). Nat. Slov. 15(2): 13-22.