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Found after 60 years: the hows and whys of *Sphaeromides virei montenigrina* (Crustacea: Isopoda: Cirolanidae) rediscovery in Obodska pećina, Montenegro

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Abstract. Despite being the biggest isopod crustacean in Dinarides, little is known about the genus *Sphaeromides* and its taxonomical status. *Sphaeromides virei montenigrina*, one of the three subspecies, has been known only thanks to a female specimen found and described in the late 1950s. Despite the intensive search, almost 60 years were needed to discover additional specimens in the cave Obodska pećina, Montenegro. In this study we present a review of the possible reasons for this long lasting search.

Key words: Dinarides, *Sphaeromides virei*, *montenigrina*, stygobiont, Obodska pećina, Rijeka Crnojevića

Izvleček. Najden po 60 letih: razlogi za ponovno odkritje *Sphaeromides virei montenigrina* (Crustacea: Isopoda: Cirolanidae) v Obodski pećini v Črni gori – Čeprav so predstavniki rodu *Sphaeromides* največji podzemni raki enakonožci Dinarskega krasa, je o rodu le malo znanega, taksonomski status pa je še nedodelan. *Sphaeromides virei montenigrina*, eno od treh podvrst, ki je bila opisana v 50-ih letih prejšnjega stoletja, smo donedavna poznali le po samici, najdeni septembra 1956. Kljub pogostemu vzorčenju je bilo potrebnih skoraj 60 let, da je bilo odkritih nekaj dodatnih osebkov v jami Obodska pećina v Črni gori. V prispevku obravnavamo morebitne razloge za to.

Ključne besede: Dinaridi, *Sphaeromides virei*, *montenigrina*, stigobiont, Obodska pećina, Rijeka Crnojevića

Introduction

The biggest isopod crustacean living in the subterranean habitats of Dinaric karst is the well over 20 mm long and supposedly predatory *Sphaeromides virei* (Brian, 1923). This stygobiont, permanent resident of subterranean aquatic habitats, is known from more than 50 karst caves and springs connected to phreatic waters all along the Dinaric karst, from the extreme northeast of Italy to southern Montenegro (Sket 1964, own data). Beside the species from the Dinarides, several congeners have been recognized in different parts of southern

Europe – southern France and Stara planina in Bulgaria and eastern Serbia (Sket 2012). Range fragmentation of the genus is traditionally explained by historical events, i.e. the retreat of the Tethys Sea, and the genus itself is perceived to be a »Tethyan relict« without known marine ancestors (Baratti et al. 2010a).

Three subspecies have been recognized within the Dinaric species *S. virei*: *S. v. virei* (Brian, 1923), *S. v. montenigrina* Sket, 1957 and *S. v. mediodalmatina* Sket, 1964. While the type subspecies is found in caves in a belt along the northeastern coast of the Adriatic Sea, both latter subspecies are found within Montenegrin and Dalmatian mainland and inland, respectively (Sket 1964). Lately, specimens of *S. v. virei* and *S. v. mediodalmatina* were found living in syntopy in a spring cave Karišnica near Zadar, a fact raising questions about their taxonomic status (Sket 2012). The species status of both taxa was additionally supported by high molecular distances on cytochrome oxidase subunit I and 16S rRNA gene phylogenetic trees (Baratti et al. 2010b).

Contrary to both northern subspecies, *S. v. montenigrina* has been known only from the type locality, Obodska pećina in Montenegro (Sket 1957, Sket 1964, Sket 2012). Moreover, it is known from a single female specimen caught by the late Egon Pretner in September 1956, in a residual pool. In the ensuing year, it was described by Sket (1957), who reported on the repetitive unsuccessful searches for additional specimens few years later (Sket 1964). Despite regular visits and sampling – Sket visited the cave about ten times, while a couple of his colleagues visited it less often, all mainly in favourable hydrological conditions – no specimen was collected until this spring. Meanwhile, several malacostracan species were found (Culver et al. 2004), even one new species (Fišer et al. 2006), but not a single specimen of *Sphaeromides*.

In the light of a recent finding, this paper presents information on rediscovery of this elusive and mystic subspecies, and possible explanations for its almost 60 years long unsuccessful sampling.

Material and methods

Rijeka Crnojevića catchment, including Obodska pećina (42.351987 N, 19.004982 E) (Figs. 1, 2), is situated in southwestern Montenegro, at the southeastern part of the Dinaric karst. Owing to the intensive karstification by the end of Pliocene, 2.58 mya, there are no major surface watercourses in the area (Bonacci & Živaljić 1993). These activate only after a heavy rainfall or intensive snow melting. Largely disintegrated fluviokarst, present in the valley, is a remnant of the historical river Cetinjska Rijeka that flowed from Ivanova korita to Cetinjsko polje and across Dobrsko selo to Rijeka Crnojevića (Radulović 1989). Pliocene tectonic movements at the boundary of Cetinjsko polje interrupted hydrographic connections between the upper and lower parts of the river catchments, forcing the water to form exclusively underground water flow networks. Consequently, the main spring in the Obodska pećina is at 60 m a.s.l., while the permanent springs are situated along the river bed, below the level of the cave entrance. Although the entrance parts of the cave are mostly dry throughout the year, its maximum water discharge reaches up to 280 m^3s^{-1} (Bonacci & Živaljić 1993).

Figure 1. Entrance of Obodska pećina viewed from inside-out (photo: Primož Presetnik).
Slika 1. Vhod v Obodsko pećino, fotografiran iz notranjosti (foto: Primož Presetnik).

The cave entrance is a 12×14 m opening (Bonacci 1987) formed in Mesozoic stratified limestones and dolomites (Živaljević et al. 1971). The main gallery with dimensions reaching up to 35 m in diameter is oriented southwest and was partly formed by the water flow. The bottom of the gallery is covered by monumental limestone blocks, rock pebbles and sand as a result of ceiling breakdowns and water discharge, respectively (Fig. 1). In the dry season, the main gallery ends with a lake, 50 m long and 20 m wide. The lake ends with a siphon, leading to another cave gallery and further to unexplored submerged passages, approximately 340 m from the cave entrance (Ferk 2008).

In the time of our (T.D.) visit on 1. 5. 2015, the water level was slightly raised and the water discharge was high due to the heavy spring rainfall. The water in the terminal lake was muddy and the visibility was approximately 1 m. Since just a small portion of the lake could be checked from the rocks on the shore, we decided to try with snorkelling. Visual inspection started upstream along the right lake bank, continued to the siphon, and finished downstream along the left lake bank. The sampling was carried out with the permission No. UPI-341/6 given by the Montenegrin authorities.

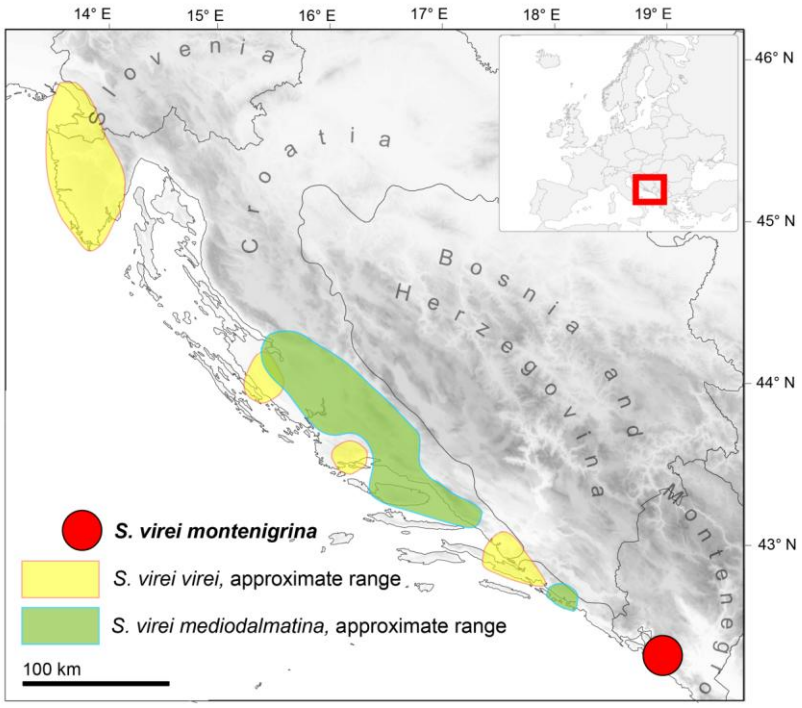

Figure 2. Approximate distribution ranges of the three *Sphaeromides virei* subspecies based on all known sites (Sket 1964, Sket, unpublished data) .
Slika 2. Približna razširjenost treh podvrst *Sphaeromides virei* na podlagi vseh znanih nahajališč (Sket 1964, Sket, neobjavljeni podatki).

Results and discussion

After almost 60 years, three specimens of *S. v. montenigrina* were found in the underground lake in Obodska Pećina, the only known locality of the subspecies (Figs. 1, 2). One specimen was found above the lake's inflow siphon, while the other two were collected close to the lake outflow siphon, within 25 cm distance. The specimens: i) 17 mm male, ii) 11 mm juvenile and iii) 10 mm juvenile, were immediately preserved in 70 % EtOH (specimens i and ii) and 96 % EtOH (specimen iii) and labelled. Specimens i and ii were used for morphological identification and possible male description, while the third specimen will be used to infer molecular relations within the genus *Sphaeromides* (see morphologically similar subspecies in Fig. 3). All the specimens are deposited in the Zoological Collection of the Biology Department, Biotechnical Faculty, University of Ljubljana, Slovenia.

Figure 3. *Sphaeromides virei mediodalmatina* from the river Ruda spring in southern Croatia (photo: T. Delić).
Slika 3. *Sphaeromides virei mediodalmatina* iz izvira reke Rude na jugu Hrvaške (foto: T. Delić).

There are several assumptions that could throw light on why this subspecies was found never again after 1956. We present only the most plausible ones. First, a (sub)population of *Sphaeromides* from Obodska pećina could live at the edge of the (sub)species range. Such marginal (sub)populations often exhibit sub-optimal habitats (according to suitability) and consequently, they often occur at low abundance and are more sensitive to habitat changes (Hanski 2012). According to Cukrov and Ozimec (2014), the nearest *S. v.* cf *montenigrina* population should be present in the cave system Vilina špilja – izvor Omble near Dubrovnik (Croatia). Subsequent identification of those specimens as *S. v. mediodalmatina* (not published) left Obodska pećina as the only known locality for *S. v. montenigrina*. That, however, doesn't exclude the possibility of more stable subspecies populations existing at yet unknown or inaccessible places.

Second, high water levels that are usually connected with lower visibility could also be a reason for repeated unsuccessful sampling. Although silty sediments are generally missing in Obodska pećina (Ferk 2008), visibility is reduced due to higher concentration of organic and inorganic matter in the water current. In the periods of high precipitation, the cave lake turns into a muddy river (Ferk 2008), which implies that a good time for sampling is only during the low-water periods. Low water and a small stream running through the cave were present also during the first discovery of the subspecies (Pretner, pers. comm.).

Obviously connected to the second is the third explanation considering an inappropriate sampling method. As mentioned, a dipnet sampling from the bank is possible only in a small portion of the lake, and only when the water is low and transparent. Therefore, cave diving, snorkelling or the deployment of baited traps seem to be more efficient methods. While only professionals are capable of safe cave diving or snorkelling, anyone can set the baited traps.

Fourth, the most discouraging reason is the devastating human impact on nature of the Rijeka Crnojevića catchment. The catchment area, variable at different spatial and temporal scales, covers only 76 km^2 of mostly Mesozoic limestones (Bonacci 1987, Bonacci and Živaljić 1993). It is the particularly threatened by the largest urban area within the catchment, the city of Cetinje. Urban and industrial development of the town in the 20th century wasn't followed by effective measures to protect and conserve the environment. The resulting aftereffect is a possible harmful impact of communal and industrial waste water from urban areas on the natural resources (Radulović 2012). According to Tomović (2008), up to 60% of wastewater is discharged into sewers in Montenegro. Inappropriate management of wastewater and general lack of strategic development in karst areas are not the only issues affecting these fragile ecosystems. Changing of hydrographic conditions, the epigean or the subterranean (hypogean) ones, can also have a negative impact. As an illustrative example, nature of Cetinjsko polje was altered by construction of a reservoir after the extreme floods in 1986. After these, precipitation accumulation in the polje was changed (Radulović 2012), affecting the interconnection between the upper and lower catchment areas. So far, there has been no report on changes in the water regime in Obodska pećina, but we cannot completely exclude its possible effects.

Despite numerous visits, the remarkable size of the subspecies and occasional findings of other crustacean species, no less than 60 years were needed to rediscover the *Sphaeromides* population in Obodska pećina. This and similar findings throughout the Dinaric karst demonstrate that the high frequency of cave visits is an important, yet not sufficient prerequisite for the (successful) exploration of subterranean biodiversity. Additionally, usage of diverse sampling techniques, together with taking into account local climate and effects of altered land use, can bring new insights into the world's hottest subterranean point – the Dinaric karst.

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