First sighting of 'white' and 'black' olms (*Proteus anguinus*) in the same spring

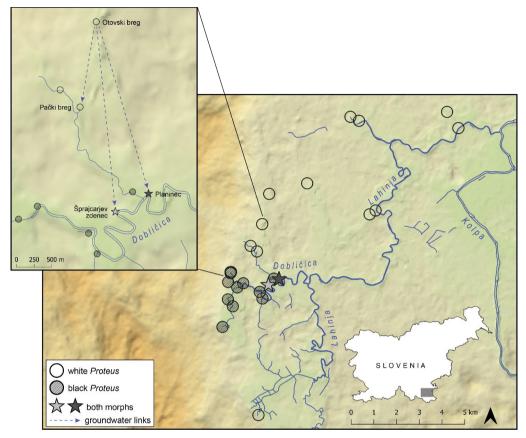
Prvo opažanje belega in črnega močerila (*Proteus anguinus*) v istem izviru

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The olm (Proteus anguinus) is the largest inhabitant of caves and subterranean waters along the Dinaric Karst (Sket 1997). Across its range, distinct evolutionary lineages are found (Gorički & Tronteli 2006, Tronteli et al. 2009), with most of them exhibiting highly troglomorphic features, including the absence of pigmentation and eyes, an elongated snout and limbs (referred to as 'white olms'). However, a single lineage (P. anguinus parkeli) in south-eastern Slovenia has presumably retained ancestral features, including a dark colouration, visible eyes, a shorter snout and limbs, named the 'black olm' (Arntzen & Sket 1997, Bizjak Mali & Sket 2019, Ivanović et al. 2019). Most of the lineages are allopatric, with non-overlapping distributional ranges. In the region of Bela krajina, however, the white olm lineage of southeastern Slovenia comes into contact with the black olm (Gorički et al. 2017). While the southeastern Slovenian lineage is distributed across a large part of Dolenjska and has been reported from over a hundred locations, the black lineage has a very limited distribution and is only known from 14 locations (Fig. 1; Hudoklin & Aljančič 2017, SubBioDB 2021). Direct contact between these two distinct lineages had not been observed before, but a study based on environmental DNA (eDNA) found that a sample from a spring close to the river Dobličica (Šprajcarjev zdenec) contained the DNA of both lineages (Gorički et al. 2017). The distance between the closest locations, where the black and white olms have been visually verified, is around 1.5 km in linear distance (Gorički et al. 2017, Hudoklin & Aljančič 2017, Năpăruș-Aljančič et al. 2017). However, linear distances are not necessarily representative or even meaningful for hydrological connections. A study on hydrological connections in the Dobličica catchment showed that the water from Otovski breg reappears in springs near the Dobličica (Habič et al. 1990). Therefore, while both eDNA data and hydrological connections suggest that black and white olms could have come into contact, this has not been confirmed by field observations as yet. Genetic and morphological data did not reveal any hybrids between black and white olms, although there are too few samples available from the potential contact zone to entirely dismiss sporadic hybridization (Gorički et al. 2017).

We report herewith on the first field observations of black and white olms at the same location, the Planinec spring. During the survey of springs potentially inhabited by black olms (Gorički et al. 2017), on 3.6.2021, we observed a young medium-sized black olm (~15 cm) in Planinec spring at 1:00 in the night. Together with the previous sighting (Gorički et al. 2017), this is the second visual confirmation of the black olm occurring in this spring. On the second survey of this spring on 12.10.2021, a white olm was observed at 22:40. In the late-night hours, at 00:40, presumably the same white olm was observed again. After waiting and checking the spring again at 1:15, the olm was not observed anymore. During the third survey on 25.3.2022, a white olm was observed and caught for closer inspection (Fig. 2). After measuring its length and body mass it was released back into the spring. On the fourth survey on 11.5.2022, three black olms were observed. During all surveys, olms reacted to our white lights and immediately retreated to the subterranean part of the spring. The only exception was the white olm that was caught and inspected on 25.3.2022. The spring was overgrown with plants at the time, and the olm swam into these plants on the shore of the spring.

Based on their eDNA study, Gorički et al. (2017) found that the spring Šprajcarjev zdenec (600 m upstream) contained DNA of both black and white olms, although neither were visually confirmed. However, it was suspected that additional sampling may likely result in further springs nearby with cooccurring olms, potentially by passive dispersal of eDNA. Since Planinec is downstream of Šprajcarjev zdenec, passive dispersal of eDNA to Šprajcarjev zdenec is not likely. Therefore, it can be assumed that black and white olms come into contact in at least these two springs, and probably also in the springs in between.



- Figure 1. Distribution of the white olm (*P. anguinus*) and the black olm (*P. anguinus parkelj*) in Bela krajina, Slovenia. Data retrieved from SubBio Database (SubBioDB 2021) and Gorički et al. (2017). Unreliable and false data according to Hudoklin and Aljančič (2017) excluded. Map produced with QGIS version 3.10.12, using Copernicus data and information funded by the European Union - EU-DEM (European Union 2016) and rivers (Geodetska uprava Republike Slovenije 2010) as base layers. In the top left corner, a magnification of the proposed contact zone between black and white olms is shown, including known groundwater connections between Otovski breg and springs near the river Dobličica (Habič et al. 1990).
- Slika 1. Razširjenost belega (P. anguinus) in črnega močerila (P. anguinus parkelj) v Beli krajini, Slovenija. Podatke smo pridobili iz Baze o podzemnem živalstvu (SubBioDB 2021) in prispevka Gorički in sod. (2017). Podatke, ki so bili glede na Hudoklin in Aljančič (2017) nezanesljivi ali napačni, smo izključili. Zemljevid je izdelan s programom QGIS verzija 3.10.12. Kot osnovna sloja smo uporabili podatke Opernicus in informacije, ki jih financira Evropska unija EU-DEM (European Union 2016) in sloj rek (Geodetska uprava Republike Slovenije 2010). V zgornjem levem kotu je povečano območje kontaktne cone belega in črnega močerila, vključno z znanimi podzemnimi vodnimi povezavami med Otovskim bregom in izviri ob reki Dobličici (Habič in sod. 1990).

Our observations are an important confirmation of the existence of a contact zone between black and white olms in the northern part of the Dobličica catchment. Based on previous knowledge of hydrogeological connections and eDNA, combined with our new observations, the contact zone probably extends at least between the two mentioned springs near the Dobličica and Otovski breg, covering approximately 0.7 km² (Fig. 1). Although extensive research has been carried out in the area following the discovery of the black olm in 1986 (Sket & Arntzen 1994), there is still not much data on the occurrence of olms in springs located within the presumed contact zone. Thus, future research may show the extent of the contact zone and overlap between the two lineages, and whether they are potentially able to hybridize.



- **Figure 2.** The spring Planinec, where black and white olms were observed. The arrow marks the hole to which the olms retreated after we enlightened the spring. In the top right corner, the photograph of a white olm observed on 25 March 2022 in the spring is shown. Photo: Ester Premate.
- Slika 2. Izvir Planinec, kjer smo opazili belega in črnega močerila. Ko smo izvir osvetlili, sta se v obeh primerih umaknila v podzemlje skozi luknjo, označeno s puščico. V zgornjem desnem kotu je fotografija belega močerila, opaženega 25. marca 2022. Foto: Ester Premate.

References

- Arntzen J.W., Sket B. (1997): Morphometric analysis of black and white European cave salamanders, *Proteus anguinus*. J. Zool. 241: 699-707.
- Bizjak Mali L., Sket B. (2019): History and biology of the »black proteus«(*Proteus anguinus parkelj* Sket & Arntzen 1994; Amphibia: Proteidae): a review. Folia Biol. Geol. 60: 5.
- European Union (2016): EU-DEM v1.1. Copernicus Land Monitoring Service. https://land.copernicus.eu/imagery-in-situ/eudem/eu-dem-v1.1 [accessed on 1. 12. 2021]
- Geodetska uprava Republike Slovenije (2010): Zvezna vektorska kartografska zbirka podatkov. https://egp.gu.gov.si/egp/ [accessed on 1. 12. 2021]

- Gorički Š., Stanković D., Snoj A., Kuntner M., Jeffery W.R., Trontelj P., Pavic M., Grizelj Z., Năpăruş-Aljančič M., Aljančič G. (2017): Environmental DNA in subterranean biology: Range extension and taxonomic implications for *Proteus*. Sci. Rep. 7: 1-11.
- Gorički Š., Trontelj P. (2006): Structure and evolution of the mitochondrial control region and flanking sequences in the European cave salamander *Proteus anguinus*. Gene 378: 31-41.
- Habič P., Kogovšek J., Bricelj M., Zupan M. (1990): Izviri Dobličice in njihovo širše kraško zaledje. Acta Carsol. 19: 5-100.
- Hudoklin A., Aljančič G. (2017): Pregled razširjenosti človeške ribice ter njenega odkrivanja na nizkem dolenjskem krasu. Dolenj. kras 7: 212-227.
- Ivanović A., Aljančič G., Arntzen J.W. (2019): Skull shape differentiation of black and white olms (*Proteus anguinus anguinus* and *Proteus a. parkelj*): an exploratory analysis with micro-CT scanning. Contrib. Zool. 82: 107-114.
- Năpăruș-Aljančič M., Gorički Š., Stanković D., Kuntner M., Aljančič G. (2017): GIS analysis to assess the groundwater habitat pollution of black proteus. Nat. Slov. 19: 47-49.
- Sket B., Arntzen J.W. (1994): A black, nontroglomorphic amphibian from the karst of Slovenia: *Proteus anguinus parkelj* n. ssp. (Urodela: Proteidae). Bijdr. tot Dierkd. 64: 33-53.
- Sket B. (1997): Distribution of *Proteus* (Amphibia: Urodela: Proteidae) and its possible explanation. J. Biogeogr. 24: 263-280.
- SubBioDB (2021): Subterranean Fauna Database. Subterranean Biology Lab, Department of Biology, Biotechnical Faculty, University of Ljubljana. https://db.subbio.net/ [accessed on 1. 12. 2021]
- Trontelj P., Douady C.J., Fišer C., Gibert J., Gorički Š., Lefébure T., Sket B., Zakšek V. (2009): A molecular test for cryptic diversity in ground water: How large are the ranges of macrostygobionts? Freshw. Biol. 54: 727-744.