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First review of recent records of sturgeons and paddlefishes (Acipenseriformes) in the Danube River basin in Slovenia

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Abstract. We present recent records of sturgeons and paddlefishes from the rivers in Danube basin in Slovenia after 2000. Strictly, only confirmed and unambiguous records (specimen, picture) were taken into account. The sterlet and the Siberian sturgeon have been occasionally found in rivers and Russian sturgeons in gravel pits, while sterlets, Siberian sturgeons, Russian sturgeons and the paddlefish are still farmed in some ponds. The Siberian sturgeons were released in the Mura and Sava River in 2016, but the species is as »exotic pet fish« present in more water bodies. The presence of sturgeons in gravel pits is unknown. The Siberian sturgeon can be relatively easily misidentified with the sterlet, so the catches in the Mura and Sora Rivers in 2009 were misidentified as sterlet, while the Siberian sturgeon was actually captured. The last sterlet in Slovenian rivers was captured in the Drava River in 2001, and even this individual had probably been released in Austria. The occurrence of sturgeons in Slovenian rivers in the last eighteen years does not seem to be connected with migration and revival of natural populations in the lower part of the Drava and Sava Rivers.

Key words: sterlet, nonindigenous sturgeons, Sava River, Drava River, Mura River, Slovenia

Izvleček. Prvi pregled recentnih podatkov o jesetrovkah (Acipenseriformes) iz donavskega porečja Slovenije – V prispevku predstavljamo najdbe jesetrov v donavskem porečju Slovenije po letu 2000. Rezultati temeljijo izključno na dokaznih fotografijah ali primerkih. V rekah so bili ujeti kečiga in sibirski jeseter, v gramoznicah ruski jeseter, v ribnikih pa so gojili oziroma še gojijo kečige, sibirske jesetre, ruske jesetre in ameriškega veslokljuna. Sibirski jesetri so bili spuščeni v reko Muro in Savo leta 2016, kot okrasne ribe pa jih imajo v precej več vodah. Pojavljanje jesetrovk v gramoznicah je velika neznanka. Sibirskega jesetra zlahka zamenjamo s kečigo, tako da so bili ulovi v Muri in Sori v letu 2009 napačno pripisani kečigi, medtem ko je bil dejansko ujet sibirski jeseter. Zadnjo kečigo so v slovenskih rekah ujeli leta 2001, in sicer v reki Dravi, pa še ta je bila verjetno izpuščena v Avstriji. Pojavljanje jesetrovk v slovenskih rekah po letu 2000 se ne zdi povezano z migracijo in izboljšanjem stanja populacij v spodnjem toku reke Drave ali Save.

Ključne besede: kečiga, tujerodne jesetrovke, Sava, Drava, Mura, Slovenija

Introduction

Six species of sturgeons (Acipenseriformes) are indigenous to the Danube River Basin: beluga (*Huso huso* Linnaeus, 1758) (Slovenian: beluga), Russian sturgeon or Danube sturgeon (*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833) (Slo: kašikar), ship sturgeon (*A. nudiventris* Lovetsky, 1828) (Slo: sim, gladki jeseter), stellate sturgeon or starry sturgeon (*A. stellatus* Pallas, 1771) (Slo: pastruga), European sturgeon or common sturgeon (*A. sturio* Linnaeus, 1758) (Slo: atlantski jeseter) and sterlet (*A. ruthenus* Linnaeus, 1758) (Slo: kečiga) (Holčík 1989, Hensel & Holčík 1997). European sturgeon has always been the rarest species in the Danube River Basin and restricted to the Lower Danube, whereas others have been more common (Hensel & Holčík 1997). Three anadromous species (beluga, Russian and stellate sturgeon) migrated to the Middle and sometimes to the Upper Danube River and to some of its larger tributaries including Drava and Sava, while two are potamodromous species (ship sturgeon, sterlet) (Hensel & Holčík 1997). Sturgeon overharvesting and habitat destruction have caused dramatic population declines worldwide (Ludwig et al. 2009). The Danube River sturgeon populations began to decline in the 16th century, i.e. a long time before severe pollution and changes in the habitat affected the stocks (Debus 1997, Hensel & Holčík 1997). Destruction of spawning grounds, obstructions to migration, deterioration of the water quality followed in the next few centuries (Debus 1997). During the 18th century, the fishing of migratory sturgeons in the Upper and Middle Danube River started to collapse (Reinartz 2002). Remnants of anadromous sturgeon populations in the Danube have vanished due to the blocking of their spawning migratory routes since the construction of 'Iron Gate I' (1970) and 'Iron Gate II' (1984) hydroelectric dams (Hensel & Holčík 1997). Today, only four species (Russian and stellate sturgeons, sterlet, beluga) still reproduce in the Lower Danube and stocks have been drastically decreasing, while only sterlet still persists in the Upper and Middle Danube. The European sturgeon is extinct, while the ship sturgeon is considered functionally extinct in the Danube River Basin (Reinartz 2002).

In Slovenian books, all six sturgeon species are usually listed (*H. huso*, *A. gueldenstaedti*, *A. ruthenus*, *A. stellatus*, *A. nudiventris*, *A. sturio*) that have been historically present in at least one of the Danube's tributaries (the Sava, Drava, Mura Rivers) in Slovenia (Povž & Sket 1990, Veenvliet & Kus Veenvliet 2006, Povž et al. 2015). This information is based mostly on old papers (Freyer 1842, Heckel & Kner 1858, Franke 1892, Glowacki 1885, 1896) without critical judgment. However, in line with other recent assessments of historical records in the Danube catchment (Schmall & Friedrich 2014a, b) as well as with the known range of European sturgeon in the Danube (Holčík 1989), the occurrence of European sturgeon in Slovenia at least is probably either based on misidentification or identification not going down to the species level. Unambiguous are records for the Sava River, while information for the Drava and Mura Rivers is based also on caught specimens in Austria or Croatia. Hensel & Holčík (1997) mentioned only the Russian sturgeon and sterlet for the Slovenian section of the Drava, Mura and Sava Rivers, while some other sturgeon species were caught in the vicinity (beluga in the Sava River close to Zagreb in Croatia). Last review for the Drava and Mura Rivers even shows that there is no other evidence of the historic appearance of other sturgeon species than sterlet in the Austrian Drava and Mura Rivers (Schmall & Friedrich 2014b). However, there is very little exact historic information, and a review of literature in Slovenia has never been done. Especially mediaeval documents from the Slovenian territory should implicitly be reviewed. Historic distribution or even presence of various sturgeon species in the Drava, Sava and Mura Rivers in Slovenia still needs to be clarified.

In the eighties and nineties of the 20th century, sterlets were caught in the Sava, Drava, Mura and Kolpa Rivers (Povž 1984, Jaš 1996, Jeremko 1998, Povž et al. 1998, Povž & Sket 1990). Consequently, sterlet was the only sturgeon species listed in Slovenian freshwater fish species list (Povž & Sket 1990). Two years later, sterlet was assessed as »extinct« in the national Red list of freshwater fish (Povž 1992) and fully protected (Ur. l. RS 1993). In 2003, its status was changed to »rare« (Ur. l. RS 2002) and remained protected (Ur. l. RS 2004). Other Danube sturgeons were not assessed in both Red lists and are thus not protected either. The European sturgeon is included in the list of sea fishes as endangered (Ur. l. RS 2002).

With the decreasing natural populations, aquaculture of sturgeons has undergone a dynamic development worldwide (Bronzi et al. 1999). The Siberian sturgeon (*A. baerii* Brandt, 1869), assessed as endangered in its native range (Ruban & Bin Zhu 2010), has become the preferred species in European aquaculture (Bronzi et al. 1999). In the last few decades, the occurrence and spreading of several nonindigenous sturgeon species (Siberian sturgeon, white sturgeon (*A. transmontanus* Richardson, 1836), paddlefish *(Polyodon spathula* Walbaum, 1792), etc.) as well as various hybrids have been observed in the Danube River basin (Friedrich 2009, 2013, Weiperth et al. 2014). The increasing catches of nonindigenous Siberian sturgeon in European rivers correlates strongly with their increasing number represented in hatcheries (Ludwig et al. 2009). Unintentional escape of sturgeons from hatcheries located near rivers is quite common and documented (Ludwig et al. 2009). Nonindigenous sturgeons can escape into natural waters during floods from large gardens, angling ponds or aquaculture facilities in the drainage area during the flood events and can drift into the main river channels (Weiperth et al. 2014). Hybridization poses a serious threat for the survival of sterlet populations. Also, natural reproduction of the Siberian sturgeon has already been observed in Europe (Ludwig et al. 2009). Additionally, intentional stocking is taking place, both illegally (ornamental fish becoming too large, attraction stocking for anglers) as well as legally with unchecked or misidentified stocks.

In Austria between 1982 and the early 1990s, a stocking program of sterlet was carried out in the Drava River, although it was very unlikely that the species ever occurred in this section of the Drava River (Friedrich & Schmall 2014). Until 1995, around one thousand sterlets were released (Honsig-Erlenburg & Friedl 1999), also close to Lavamünd, near the Slovenian border (Friedrih 2009). Between 1988 and 1998, seven sterlets were caught in the Lavamünd area in Austria (Honsig-Erlenburg & Friedl 1999). Regular recoveries showed that the animals have grown well in the river and that some migrated downstream through the turbines (Honsig-Erlenburg & Friedl 1999). Catches of sterlets in the Drava River in Slovenia (Jaš 1996, Jeremko 1998) probably originate from the stocking program in Austria. Catches of eels (*Anguilla anguilla*) and whitefishes (*Coregonus* spp.) in the Drava River in Slovenia (Povž et al. 2015) originate from Austrian stocking programs as well. Release of sterlets into the Mura River in Austria has been conducted sporadically. In 2001, sturgeons were released into the Mura River close to Graz. Later on, however, it was established that nonindigenous Siberian sturgeons had most probably been released (Friedrich 2013). In 2005, one Siberian sturgeon was found dead in the upper section of the Mura River, and additionally two Siberian sturgeons were caught in 2010 close to Spielfeld (Šentilj) (Friedrich 2013). There are no proven records of sterlets being caught in the Austrian section of the Mura River (Friedrich 2013).

Slovenian hatcheries breed at least three sturgeon species: indigenous sterlet, nonindigenous Siberian sturgeon and paddlefish (Zabric et al. 2006, Vogrin 2007, Urbas 2011, Povž & Gregori 2014, Povž et al. 2015, Omerzu 2017). In Slovenia, at least two »sterlets« have been caught in the 21st century, particularly in the Sora (ZZRS 2009) and Mura Rivers (Povž 2016). In 2016, »sterlets« were released into the Mura (Pojbič 2016) and Sava Rivers (Mavsar 2016a, b). Soon it was established, however, that a Siberian sturgeon was actually released. On web forums, information on more »sterlets« caught in Slovenia can be found, not only from rivers but gravel pits as well. We decided to investigate this information and present the first review of sturgeon records in Slovenian rivers of the Danube drainage area after 2000.

Materials and methods

In this paper we gathered records of sturgeons of the Danube river basin in Slovenia after 2000. Web forums, photo galleries and other local newspapers were surveyed for information. Additionally, information from anglers was gathered. Fish were identified using combination of different characters that are preserved on stuffed fish or visible on pictures (Holčík 1989, CITES 2001).

Results

Despite several »anecdotal« pieces of information on caught sturgeons, it is very hard to find primary sources of information. Only proved and unambiguous record (specimen, picture) after 2000 were therefore strictly taken into account.

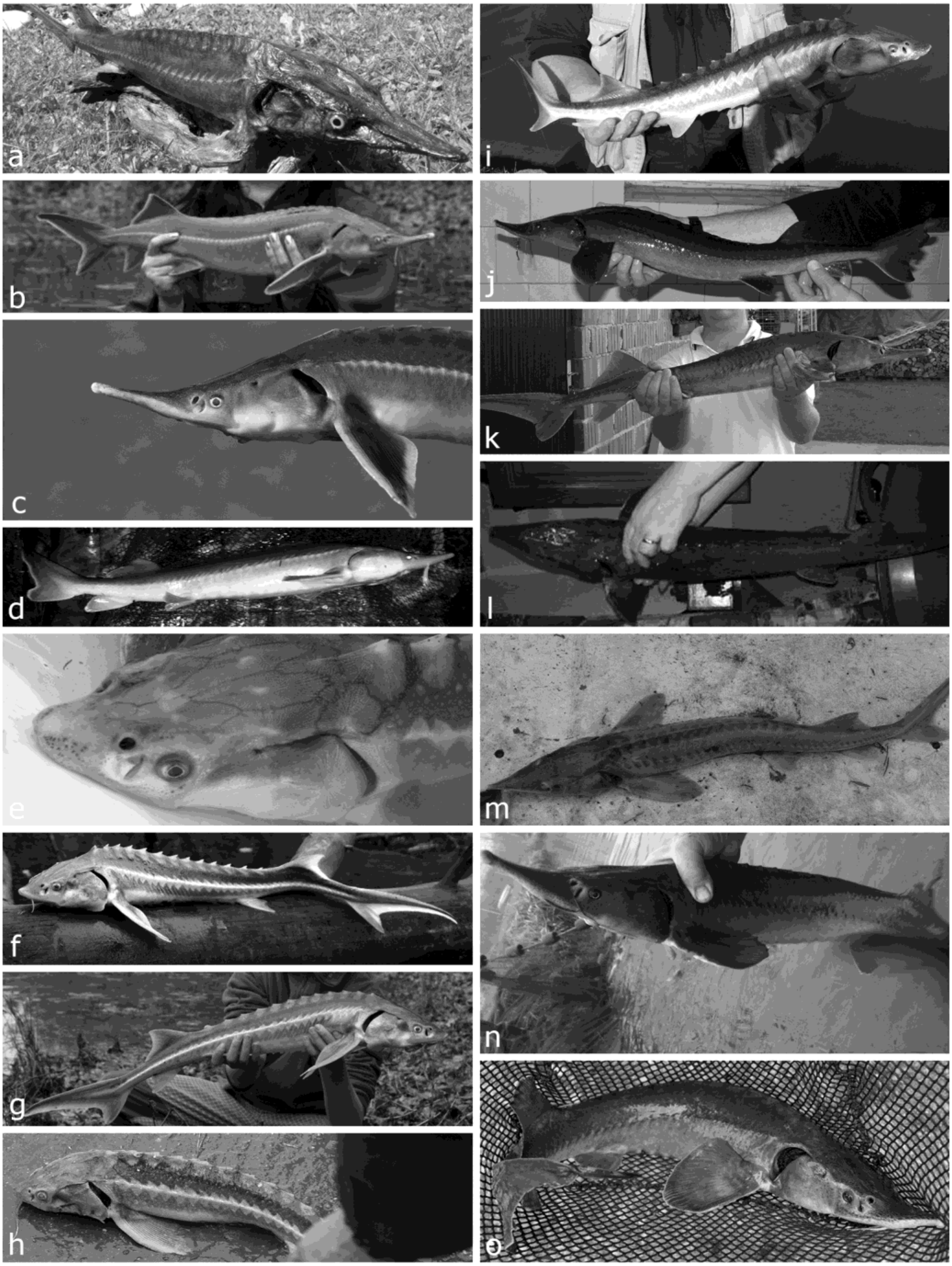

Figure 1. Sturgeons from Danube River basin in Slovenia after year 2000. For detailed information see Tab. 1 (letters correspond to ID letters in Tab. 1).

Slika 1. Jesetri iz iz donavskega porečja Slovenije po letu 2000.

Table 1. Data on Sturgeons from the Danube River basin in Slovenia after 2000.
Tabela 1. Podatki o jesetrih iz donavskega porečja Slovenije po letu 2000.

ID letter/ ID oznaka	Species/ Vrsta	Locality/ Lokacija	Date/ Datum	Photo/ Foto
a	*A. ruthenus*	Drava River (Trbonje)	17.7.2001	Anonymus
b	*A. ruthenus*	Požeg reservoir	2005	Stane Omerzu
c	*A. ruthenus*	Turnovi ribniki	2008	Milan Vogrin
d	*A. ruthenus*? (*A. ruthenus* × *Huso huso*?)	Tržec gravel pit	2014	Uroš Lovrec
e	*A. gueldenstaedti*	Požeg reservoir	8.11.2003	Stane Omerzu
f	*A. gueldenstaedti*	Požeg reservoir	6.11.2004	Stane Omerzu
g	*A. gueldenstaedti*	Požeg reservoir	5.11.2005	Stane Omerzu
h	*A. gueldenstaedti*	Požeg reservoir	7.11.2009	ZZRS archive
i	*A. gueldenstaedti*	Rače	2015	Silvo Koren
j	*A. baeri*	Sora River	23.2.2009	ZZRS archive
k	*A. baeri*	Mura River (Veržej)	summer 2009	Anonymus
l	*A. baeri*	Drava River (Vuzenica)	June 2018	Anonymus
m	*A. baeri*	Požeg reservoir	7.11.2009	ZZRS archive
n	*A. baeri*	Požeg reservoir	25.11.2016	Slavko Prijatelj
o	*A. baeri*	Mura River (Ceršak)	1.6.2016	Marjian Gaber

Sterlet *Acipenser ruthenus* Linnaeus, 1758 (Slo: kečiga)

Single proved unambiguous catch in this century dates to 2001. The sterlet (80 cm total length; Fig. 1a) was caught on 17. 7. 2001 in the Drava River close to Trbonje between Dravograd and Vuzenica hydropower plant, 11 km downstream from Austria. The sterlet was probably released within the reintroduction program in Austria. It coincides with the catch of the sterlets every few years in the Drava River in Slovenia (Jaš 1996, Jeremko 1998) and Austria (Honsig-Erlenburg & Friedl 1999). We could not confirm any other rumours from the Drava River in Slovenia, although it is possible that more sterlets were caught there. Honsig-Erlenburg & Friedl (1999) concluded that sterlets migrated downstream through turbines, but it should not be neglected that during high discharges spill gates at hydropower plants are open. No other record of sturgeon from Slovenian rivers after year 2000 can be identified as sterlet. Records from the Sora (ZZRS 2009) and Mura Rivers (Povž 2016) have to be discarded due to misidentification.

Sterlets have been reared in Požeg water reservoir (Zabric et al. 2006, Urbas 2011). Urbas (2011) also published a picture of a sterlet, but one from 2005 was also found by us (Fig. 1b). Sterlets have been reared also in Turnovi ribniki (Turn ponds) in the area of Rače (Vogrin 2007; Fig. 1c).

In a gravel pit close to Tržec near Ptuj one sturgeon was caught in 2015, which, however, cannot be identified with total certainty as sterlet due to the poor quality of the pictures. It is possible that it was a hybrid between sterlet and beluga (Fig. 1d).

Paddlefish *Polyodon spathula* (Walbaum, 1792) (Slo: ameriški veslokljun)

This species has an unusual external appearance, so its misidentification is not expected. Paddlefish were cultivated in a facility close to Rogaška Slatina (Povž 2012, Povž & Gregori 2014, Povž et al. 2015). There is no information that paddlefish is present or was caught in lakes or rivers in Slovenia.

Russian sturgeon *Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833 (Slo: kašikar)

On web forums we found pictures of specimens with unambiguous morphological characters that can be recognised as those of the Russian sturgeon. In 2003, 2004, 2005 and 2009 at least, the Russian sturgeon was reared in Požeg water reservoir (Fig 1. e, f, g, h). In 2005, it was also caught in a gravel pit close to Rače (Fig. 1i). There is no information that Russian sturgeon is present or was caught in Slovenian rivers after 2000.

Siberian sturgeon *Acipenser baerii* Brandt, 1869 (Slo: sibirski jeseter)

Siberian Sturgeon is cultivated in an aquaculture facility at Dvor in the Dolenjska region along the Krka River (Omerzu 2017). These fish can be bought at fish markets in large supermarkets.

Without pictures of caught specimens we would be still convinced that the Siberian sturgeon is present in Slovenia only at fish farm Dvor. On 23. 2. 2009, Siberian sturgeon was caught in the Sora River (Fig. 1j). It was misidentified as sterlet (ZZRS 2009), and therefore released, as protected species, back into the river. In 2009, nobody paid enough attention to species determination and the sturgeons were simply attributed to the sterlets. The Sora River is not a suitable habitat for sturgeons and neither was it expected that sterlet could occur there. As no »sterlet« was introduced by the Angling Society that manages the area, the only logical conclusion was that it was released by some amateur. Due to the habitat type, which is typical of the pre-alpine Sora River, sturgeons cannot survive there for a long period of time.

In summer 2009, one Siberian sturgeon was caught and taken from the Mura River close to Veržej, 43 km downstream from the Spielfeld hydropower plant in Austria (Govedič & Miličič 2018). At first it was misidentified as sterlet (Povž 2016) but photographs show that it was actually a Siberian sturgeon (Fig. 1k). The catch coincides with two Siberian sturgeons in 2010 close to Spielfeld (Šentilj) in Austria (Friedrich 2013). Additionally, one Siberian sturgeon was caught in the Drava River below Vuzenica hydropower plant in June 2018 (Fig. 1l).

Pictures from the Požeg water reservoir during fish harvesting in 2009 (Fig. 1m) and 2016 (Fig. 1n) prove that Siberian sturgeon has been reared there as well. Even more photos featuring Siberian sturgeons can be found on Facebook. So it seems that Siberian sturgeon is kept as exotic species in some private ponds (Benda 2018) and small fish farms (Anonymus 2014).

In 2016, the Slovenian state authority issued permission for additional stocking of the Mura and Sava River with sterlets without any previous survey on the presence of indigenous sturgeons. 15 fish were released in the Mura River close to Ceršak (3 km downstream from Spielfeld hydropower) on 1. 6. 2016 and 93 of them in the dammed Sava River close to Brestanica between Blanca and Krško hydropower plants on 5. 6. 2016 (Mavsar 2016a, b, Pojbič 2016). Due to good media cover of these events, pictures circulated on web and soon after it was discovered that the stocked fish were in fact nonindigenous Siberian sturgeons (Fig. 1o).

Due to the release of nonindigenous sturgeons into the Mura and Sava Rivers, the government changed regulations on supplemental stocking (Ur. l. RS 2016). As sterlet was not officially extinct in Slovenia (Ur. l. RS 2002), permission for supplemental stocking was issued. For extinct species, permission for resettlement should be issued. Today, authorities have to follow the same procedure for supplemental stocking and resettlement of protected species, and their genetic origin has to be checked as well. But the authorities face a new challenge; what are anglers supposed to do when they catch sturgeons? It is very hard to differentiate between sterlet and Siberian sturgeon, especially for anglers, since they often lack special knowledge. The first species should be immediately released as a protected species, while the nonindigenous one should be taken from the site. In the last century, sturgeons usually considered sterlets were caught, but this simplified method is not possible any more due to 10 species and various hybrids being traded in Europe. All caught fish should be verified directly with the specimen or photograph in order for experts to identify the species. In Austria, a hotline was established where anglers can send their photos via WhatsApp and immediately they get feedback on the species identification.

Sturgeons are very popular for sport fishing. Following the example in other European countries, there are probably more and more gravel pits in Slovenia stocked with sturgeons from aquaculture. And further intensification of sturgeon aquaculture will increase this trend. In Slovenia, the trade and origin of sturgeons are not sufficiently controlled. Due to intentional and unintentional stocking of sturgeons, more catches of sturgeons are expected in Slovenia in near future.

The occurrence of sturgeons in Slovenian rivers in the last eighteen years seems not to be connected with migration and revival of natural populations in the lower part of the Drava and Sava Rivers, but rather with human activities, illegal and misidentified stockings as well as escapees from commercial fish farms. This sturgeon review is not complete. Information on caught sturgeons is still circulating around and waiting to be confirmed. We hope that this paper will encourage anglers to disclose their catch.

Povzetek

V povodju Donave je domorodnih šest vrst jesetrovk (Acipenseriformes): beluga (*Huso huso*), kašikar (*Acipenser gueldenstaedtii*), sim (*A. nudiventris*), pastruga (*A. stellatus*), atlantski jeseter (*A. sturio*) in kečiga (*A. ruthenus*), ki so pogosto tudi navedene v splošnih slovenskih knjigah o ribah. Navedbe temeljijo na starih virih, ki niso bili kritično presojani. Ta pregled je treba še narediti in razjasniti, katere vrste jesetrovk so zgodovinsko živele v naših rekah.

V letih 1980–2000 je bilo v Sloveniji ujetih nekaj kečig v Savi, Dravi, Muri in Kolpi. Po letu 2000 je bilo v naših rekah ujetih nekaj jesetrov, ki so bili sprva napačno prepoznani kot kečige. Rezultati, ki temeljijo izključno na dokaznih fotografijah ali primerkih, kažejo, da so zadnjo kečigo v slovenskih rekah ujeli leta 2001 v reki Dravi, pa še ta je bila verjetno izpuščena v Avstriji. Informacije o kečigah iz Sore in Mure po letu 2000 je treba zavreči zaradi napačne prepoznave. Leta 2009 je bil v Muri pri Veržeju ujet sibirski jeseter (*A. baerii)*, leto kasneje pa dva pri Šentilju v Avstriji. Leta 2009 je bil v Sori ujet in nazaj v reko izpuščen sibirski jeseter. Junija 2018 je bil ujet sibirski jeseter v Dravi pod HE Vuzenica. V letu 2016 so sibirske jesetre zaradi napake izpustili v reko Muro in Savo. Podatkov o ulovu drugih domorodnih ali tujerodnih vrst jesetrovk iz naših rek po letu 2000 ni.

Gojenje jesetrovk je razvito po celem svetu. V Evropi največ gojijo tujerodnega sibirskega jesetra*.* Tudi v slovenskih ribogojnicah gojijo ali pa so gojili domorodno kečigo in ruskega jesetra ter tujerodnega sibirskega jesetra in ameriškega veslokljuna (*Polyodon spathula)*. Sibirskega jesetra gojijo v Dvoru na Dolenjskem. Leta 2007 so kečige gojili v Turnovih ribnikih pri Račah. V zadrževalniku Požeg so gojili kečige leta 2005 in 2010, v letih 2003–2005 in 2009 tudi ruskega jesetra, sibirskega pa 2009 in 2016.

Jesetri so v Evropi priljubljene ribe za športni ribolov. Tako so bili jesetri verjetno tudi v Sloveniji izpuščeni v več gramoznic in privatnih ribnikov, kot smo zbrali podatkov. V gramoznici Tržec je bil leta 2015 ujet jeseter, ki ni nujno kečiga, morebiti je bil križanec med kečigo in belugo. Načrtno gojenje križancev jesetrov je namreč pogosto. V gramoznici pri Račah je bil 2015 ujet ruski jeseter. Kot okrasne ribe jih imajo verjetno v precej več vodah.

Zaradi napak pri izpustitvi jesetrov v Muro in Savo je bila spremenjena Uredba o zavarovanih prosto živečih živalskih vrstah. Sedaj bo treba tudi za doselitev upoštevati strožja pravila, podobno kot za ponovno naselitev. Pravnih praznin na področju jesetrovk ne manjka. Formalno je od živečih zavarovana le kečiga, ruski jeseter pa sploh ni zavarovan. Nobenega izmed jesetrov v rekah ni dovoljeno upleniti, hkrati pa tujerodnih vrst ali križancev ne bi smeli vračati v naravo. Ločevanje jesetrov, še posebej sibirskega in kečige, za neizkušene ni preprosto. V prejšnjem stoletju so bili ulovi jesetrov preprosto pripisani kečigi, danes pa to ni več mogoče. Za zanesljivo potrditev je nujna fotografija.

Prisotnost jesetrovk v slovenskih rekah po letu 2000 ni povezana z migracijo in izboljšanjem stanja populacij v spodnjem toku reke Drave ali Save, temveč s človekovo dejavnostjo. Ribe so bile izpuščene ali pa so pobegnile iz ribogojnic.

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References

Anonymus (2014): Facebook profil: Apartmaji in ribogojstvo Povh, Objava 29. 6. 2014. https://www.facebook.com/714522805272563/photos/a.728056253919218/735809473143896 [accessed on 19. 8. 2018]

Benda B. (2018): Facebook profil: Ribiška družina Mozirje, Objava 31. 3. 2018. https://www.facebook.com/groups/33030740877/permalink/10155446077970878/ [accessed on 19. 8. 2018]

Bronzi P., Rosenthal H., Arlati G., Williot P. (1999): A brief overview on the status and prospects of sturgeon farming in Western and Central Europe. J. Appl. Ichthyol. 15: 224-227.

CITES (2001): CITES Identification guide - sturgeons and paddlefish: Guide to the identification of sturgeon and paddlefish species controlled under the Convention on international trade in endangered species of wild fauna and flora. Minister of supply and services Canada, 180 pp.

Debus L. (1997): Sturgeons in Europe and causes of their decline. In: Birstein V.J., Bauer A., Kaiser-Pohlmann A. (Eds.), Sturgeon stocks and caviar trade workshop. Occasional paper of the IUCN Species Survival Commission No. 17, IUCN - The World Conservation Union, pp. 55-67.

Franke J. (1892): Die Gewässer in Krain und ihre nutzbare Fauna. Jahresbericht der k. k. Staats-Oberrealschule in Laibach für das Schuljahr 1891/1892, Laibach, pp. 1-24.

Freyer H. (1842): Fauna der in Krain bekannten Säugethiere, Vögel, Reptilien und Fische. Gedr. In D. Egerschen Buchdruckerey, Laibach, 90 pp.

Friedrich T. (2009): Störartige in Österreich – Chancen und Perspektiven im Freigewässer. Österreichs Fischerei 62: 250-258.

Friedrich T. (2013): Sturgeons in Austrian rivers: historic distribution, current status and potential for their restoration. World sturgeon conservation sciety, Special publication n°5, Norderstedt, 75 pp.

Friedrich T., Schmall B. (2014): Die Störarten der Donau. Teil 3: Sterlet, »Stierl« (*Acipenser ruthenus*) und aktuelle. Österreichs Fischerei 67: 167-183.

Glowacki J. (1885): Die Fische der Drau und ihres Gebietes. XVI. Jahresberichte des Steiermärk. Landsch. Untergymnasiums zu Pettau, Pettau, 18 pp.

Glowacki J. (1896): Die Fischfauna der Save und des Isonzo. I. Jahresbericht des K.K. Staats Untergymnasium in Cili, Cili, pp. 1-37.

Govedič M., Miličič I. (2018): Ujemite naravo!: zbranih 10.000 fotografij. Ribič 77(1-2): 6-9.

Heckel J., Kner R. (1858): Die Süsswasserfische der Östreichischen monarchie mit Rücksicht auf die Angränzenden Länder. Verlag von Wilhelm Engelmann, Leipzig, 388 pp.

Hensel K., Holčik J. (1997): Past and current status of sturgeons in the upper and middle Danube River. Environ. Biol. Fishes 48: 185-200.

Holčík J. (Ed.) (1989): The freshwater fishes of Europe. Vol. 1, Part II: General introduction to fishes/Acipenseriformes. AULA-Verlag, Wiesbaden, 469 pp.

Honsig-Erlenburg W., Friedl M. (1999): Zum Vorkommen des Sterlets (A. ruthenus L.) in Kärnten. Österreichs Fischerei 52: 129-133.

Jaš S. (1996): Takšne ribe pa še ne. Ribič 55(1-2): 35.

Jeremko B. (1998): Kečiga iz kanala Drave. Ribič 57(5): 120.

Ludwig A., Lippold S., Debus L., Reinartz R. (2009): First evidence of hybridization between endangered sterlets (*Acipenser ruthenus*) and exotic Siberian sturgeons (*Acipenser baerii*) in the Danube River. Biol. Invasions 11: 753-760.

Mavsar B. (2016a): V Savo ponovno naselili kečigo. Posavski obzornik 20(12): 21.

Mavsar B. (2016b): V Savi ponovno domuje kečiga. Posavski obzornik 20(13): 29.

Omerzu S. (2017): Tržno poročilo za dobre in trajnostne dobre obete za proizvode iz akvakulture. Racoon d.o.o., Center za promocijo in pospeševanje športnega ribolova in ribiškega turizma, Ljubljana, 120 pp.

Pojbič J. (2016): Kečiga postaja ambasadorka prosto tekoče Mure. Delo 58(127): 20.

Povž M. (1984): Ihtiofavna reke Mure I. Biol. vestn. 32(1): 87-92.

Povž M. (1992): Rdeči seznam ogroženih sladkovodnih rib (Pisces) in piškurjev (Cyclostomata) v Sloveniji. Varst. narave 17: 51-59.

Povž M. (2012): Ameriški veslokljun. Ribič 71(1-2): 16-17.

Povž M. (2016): Ribe in piškurji v porečju Mure v Sloveniji. Proteus 78(6-8): 339-345.

Povž M., Gregori A. (2014): Tujerodne sladkovodne ribe v Sloveniji. Zavod Umbra, Ljubljana, 31 pp.

Povž M., Sket B. (1990): Naše sladkovodne ribe. Mladinska knjiga, Ljubljana, 369 pp.

Povž M., Šumer S., Budihna N. (1998): Ribe in raki Pokolpja (razširjenost, biologija in ekologija sladkovodnih rib in rakov ter naravovarstvene smernice). Ljubljana, 95 pp.

Povž M., Gregori A., Gregori M. (2015): Sladkovodne ribe in piškurji v Sloveniji. Zavod Umbra, Ljubljana, 293 pp.

Reinartz R. (2002): Sturgeons in the Danube River. Literature study on behalf of IAD. Landesfischereiverband Bayern e.V. und Bezirk Oberpfalz, 150 pp.

Ruban G., Bin Zhu (2010): *Acipenser baerii*. The IUCN Red List of Threatened Species 2010: e.T244A13046607. http://dx.doi.org/10.2305/IUCN.UK.2010-1.RLTS.T244A13046607.en [accessed on 19.8.2018].

Schmall B., Friedrich T. (2014a): Die Störarten der Donau Teil 1: Hausen (*Huso huso*), Europäischer Stör (*Acipenser sturio*) & allochthone Störarten. Österreichs Fischerei 67: 95-109.

Schmall B., Friedrich T. (2014b): Die Störarten der Donau. Teil 2: Waxdick (*Acipenser gueldenstaedtii*), Glattdick (*Acipenser nudiventris*), Sternhausen (*Acipenser stellatus*) und historische Störnachweise zweifelhafter Identität. Österreichs Fischerei 67: 129-143.

Ur. l. RS (1993): Uredba o zavarovanju ogroženih živalskih vrst. Uradni list RS 3(57): 2851-2854.

Ur. l. RS (2002): Pravilnik o uvrstitvi ogroženih rastlinskih in živalskih vrst v rdeči seznam. Uradni list RS 12(82): 8893-8975.

Ur. l. RS (2004): Uredba o zavarovanih prosto živečih živalskih vrstah. Uradni list RS 14(46): 5963-6016.

Ur. l. RS (2016): Uredba o spremembah in dopolnitvah Uredbe o zavarovanih prosto živečih živalskih vrstah. Uradni list RS 26(64): 9013-9014.

Urbas T. (2011): Bogata žetev še ne pomeni bogastva. Ribič 70(1-2): 24-25.

Veenvliet P., Kus Veenvliet J. (2006): Ribe slovenskih celinskih voda, priročnik za določanje. Zavod Symbiosis, Grahovo, 168 pp.

Vogrin M. (2007): Nepozabna riba se vrača. Novice občine Rače–Fram 37-4: 51-52.

Weiperth A., Csányi B., György Á.I., Szekeres J., Friedrich T., Szalóky Z. (2014): Observation of the sturgeon hybrid (*Acipenser naccarii* × *Acipenser baerii*) in the Hungarian section of River Danube. Pisces Hung. 8: 111-112.

Zabric D., Podgornik S., Kosi G., Brancelj A. (2006): Ciljni raziskovalni program (CRP) »Konkurenčnost Slovenije 2001-2006« Vpliv gojenja rib v toplovodnih ribogojnicah in gramoznicah na vodni ekosistem (zaključno poročilo). Zavod za ribištvo Slovenije, Ljubljana, 114 pp.

ZZRS (2009): Kečiga v Sori. Zavod za ribištvo Slovenije, novice 10. 3. 2009, https://www.zzrs.si/blog/keciga-v-sori/ [accessed on 6. 7. 2018]

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First record of the invasive Asian clam *Corbicula fluminea* (O.F. Müller, 1774) (Bivalvia: Corbiculidae) in Slovenia

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Abstract. The Asian clam (*Corbicula fluminea*) is considered one of the most invasive freshwater bivalves in the world. It has been introduced to several European countries. During the field surveys conducted in August 2018, a total of 61 specimens of the Asian clam were found along the Drava River between Ormož and Središče ob Dravi in Northeast Slovenia. These are the first records of this invasive species' occurrence in Slovenia.

Key words: invasive species, *Corbicula fluminea*, Slovenia, Drava River

Izvleček. Prva najdba invazivne vrste azijske školjke *Corbicula fluminea* (O.F. Müller, 1774) (Bivalvia: Corbiculidae) v Sloveniji – *Corbicula fluminea* je ena najbolj invazivnih školjk celinskih voda. Zanešena je bila v številne evropske države. Med terenskim popisom avgusta 2018 smo na odseku reke Drave med Ormožem in Središčem ob Dravi skupno našli 61 primerkov te školjke. To so hkrati tudi prvi podatki o pojavljanju te invazivne vrste v Sloveniji.

Ključne besede: invazivne vrste, *Corbicula fluminea*, Slovenija, Drava

Introduction

The Asian clam (*Corbicula fluminea*) is a small (usually up to 30 mm long) freshwater bivalve inhabiting rivers, canals or lake sediments. It can tolerate low salt concentrations (Franco et al. 2012), thus it can be also found in brackish waters. It is native to Southeast Asia, but at the beginning of 20th century it started to spread to other continents (Sousa et al. 2008, Basen et al. 2017). In Europe, it is hard to track the defined invasion pathways. The first records of the species' introduction were received from a number of different locations (Crespo et al. 2015). The species was first found in the Tagus Estuary in Portugal and the Garrone Estuary in France in 1980, followed by the River Rhine, near Rotterdam in 1985 (Crespo et al. 2015). It disperses through passive transport of juveniles by fluvial navigation or tidal currents (Sousa et al. 2008). It can actively secrete long mucous threads that enable its flotation (Prezant & Chalermwat 1984), which germinates its dispersion in lakes. Unaided upstream movement may be an important dispersal mechanism in free running rivers (Voelz et al. 1998). Rapid dispersion is caused mostly due to different human activities such as: ballast water transport, use as fish bait or as a food resource, use by aquarium hobbyists and juveniles' byssal attachment to boat hulls (Sousa et al. 2008, Franco et al. 2012, Crespo et al. 2015). Ectozoochorous dispersal by birds or endozoochorous dispersal by birds or fish is also possible, although most likely a rare occurrence (Coughlan et al. 2017). Today it is present in almost all European river basins, from the Iberian Peninsula to Ireland and the UK, and Bulgaria and Romania in the east (Ferreira-Rodriguez et al. 2018). In less than 100 years, it has invaded all continents, except Antarctica. Accordingly, this species is one of the most successful invasive species in aquatic ecosystems (Crespo et al. 2015).

The rapid spread and the invasion success is related to its biological characteristics (rapid growth, early sexual maturity, high fecundity, hermaphroditism, planktonic larvae, and the potential to reach high population density levels), extensive dispersal capacities, its physiological tolerance and its association to human activity. The Asian clam reaches sexual maturity within the first 3 to 6 months (Sousa et al. 2008). It reproduces twice per year (Crespo et al. 2015) – the majority of population is hermaphrodite, capable of androgenic self-fertilization (Pigneur et al. 2012). It releases pediveligers with reduced mobility to the water column, which rapidly settle into sediment. All this may result in an annual fecundity rate as many as 68,000 juveniles per individual (Denton et al. 2012). A single individual in optimal environmental conditions has the potential to start a new population (Crespo et al. 2015). It can also tolerate temperatures up to 36 °C (Basen et al. 2017). The Asian clam grows rapidly due to its high filtration and assimilation rates (Sousa et al. 2008). The major part of its energy is allocated to growth and reproduction and only a small proportion is devoted to respiration (Sousa et al. 2008). Where habitat conditions are favourable (fine sediment), the Asian clam can build up massive stocks and dominate the local community comprising up to 90% of total benthic biomass (Basen et al. 2017). Franco et al. (2012) report on densities up to 11,142 individuals m^{-2}. Specimens of the Asian clam spend much of their life burrowed into substrate at depths up to tens of centimetres. Juvenile and adult individuals migrate to the substrate surface when exposed to environmental stressors such as low dissolved oxygen levels (Forrest et al. 2017). The species has a short life span ranging from 1 to 5 years (Sousa et al. 2008).

Materials and methods

The survey was conducted between 15.8.2018 and 16.8.2018 after coincidental finding of one half of an empty shell of the Asian clam on 1.7.2018 on gravel bar of the Drava River near Središče ob Dravi in Northeast Slovenia. The sampling method used was the hand netting of live animals in the river sediments in the shallow water. Due to their small size and because they are totally anchored into the river sediment, it was harder to spot them with bathyscopes compared to the success this survey technique delivers for indigenous unionid species. All the potential locations along the short section of the Drava River in Slovenia between Ormož and Središče ob Dravi were surveyed. Only shallow sections (up to depth of about 1 m) of the river were sampled. Additionally, gravel bars in this section of Drava River were surveyed for empty shells. All animals and empty shells were taken from the site and later on individual shell length and height were measured with an ESD Safe Dial Caliper (0.1 mm reading).

The species was identified by its external morphological characteristics such as pattern of shell sculpture, shape and concentric ridges. *Corbicula fluminea* can be easily misidentified with *C. fluminalis*. Collected specimens of *C. fluminea* had coarser ridges with a whitish inner surface compared to *C. fluminalis*, the shells of which develop finer ridges, with a violet inner surface (Ciutti & Cappelletti 2009). The height/length (<1) ratio was in favour of *C. fluminea* as well.

Results and discussion

A total of 52 (min-max length: 7.2–23.1 mm) live specimens of the Asian clam and 9 empty shells (min-max length: 11.7–20.8 mm) were collected at four locations along the Drava River between Obrež and Središče ob Dravi (Fig. 1). The clams were found only in fine sediment in the shallow water next to the river bank. Various other studies report on the presence of Asian clams in fine sediment (Paunović et al. 2007). This fact corresponds to some references (Crespo et al. 2015, Gama et al. 2015), which state that the species is restricted only to well-oxygenated water. Empty shells were additionally found on the surface of fine sediment at the downstream end of gravel bars together with empty shells of the painter's mussel (*Unio pictorum*), thick shelled river mussel (*Unio crassus*), duck mussel (*Anodonta anatina*) and zebra mussel (*Dreissena polymorpha*). From the length frequency distribution (Fig. 2), all captured clams were considered sexually mature, which corresponds to Sousa et al. (2008). The largest shells suggest that the first generation of the Asian clam occurred in the Drava River at least 2 years earlier.

Due to the presence of Asian clams in the neighbouring countries, i.e. Italy (Ciutti & Cappelletti 2009, Kamburska et al. 2013), Croatia (Lajtner 2015, Lajtner et al. 2016) and Hungary (Csanyi 1999), its occurrence in Slovenia has been expected. In the Drava River the species was found as early as in 2016 in Ormož Lake on Croatian side and downstream in Lake Varaždin in 2018 (Lajtner J., personal information).

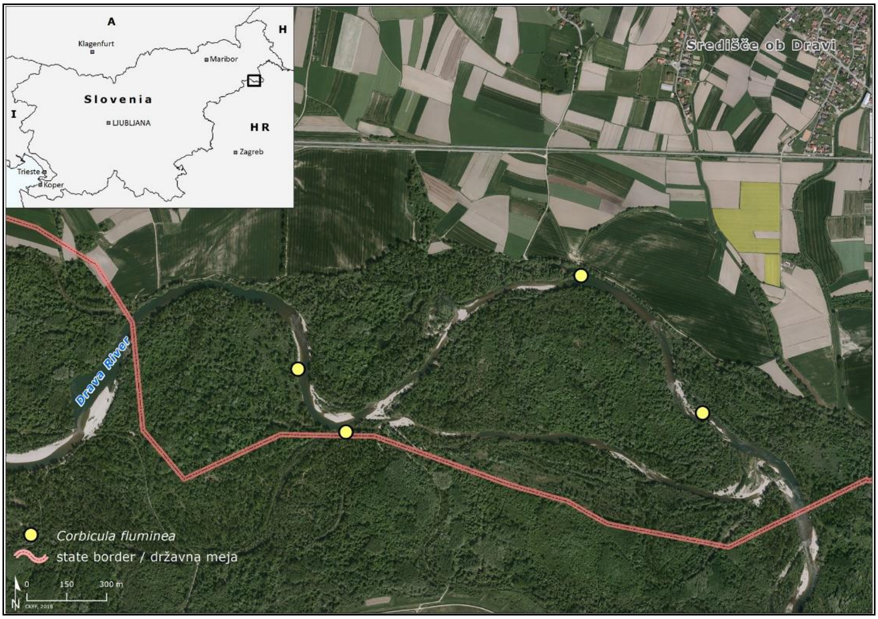

Figure 1. Records of the live Asian clam *Corbicula fluminea*.
Slika 1. Mesta najdbe živih školjk vrste *Corbicula fluminea*.

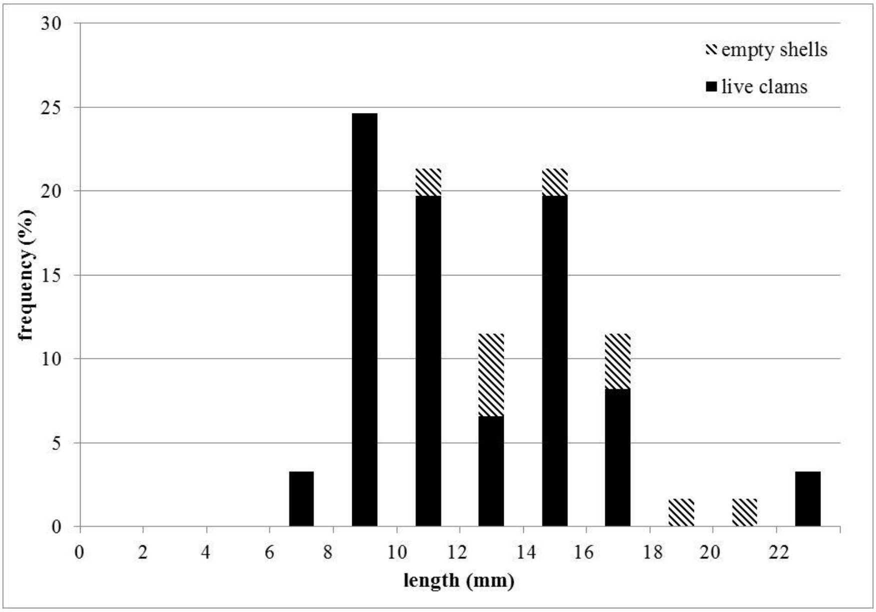

Figure 2. Length frequency distribution of Asian clam *Corbicula fluminea* in the Drava River between Obrež and Središče ob Dravi.
Slika 2. Frekvenčna distribucija dolžine lupin vrste *Corbicula fluminea* iz reke Drave od Obreža do Središča ob Dravi.

Invasive bivalves cause serious ecological and economic impact due to their effects on natural ecosystems and the damaging impacts on man-made structures (Rosa et al. 2011). The Asian clam can have a detrimental impact on native bivalves due to its burrowing and bioturbation activity, high abundances, displacing and/or reducing available habitats for juvenile unionids (Unionidae) and sphaeriids (Sphaeriidae). Dense populations of the Asian clam may also ingest large numbers of unionids sperm, glochidia and newly metamorphosed juveniles (Sousa et al. 2008). It may also compete for food resources with sphaeriids and unionids and consequently has the potential to limit planktonic food available to native bivalves (Pigneur et al. 2014, Ferreira-Rodriguez et al. 2018). Contrary to Asian clams, unionids are slow-growing species with a long lifespan and life-cycles that are dependent on fish hosts (Lopes-Lima et al. 2014). Additionally, this invasive species can be a vector for the introduction of new parasites and diseases to the biotic components of invaded ecosystems (Sousa et al. 2008).

In lowland parts of Slovenia and Croatia, relatively large and shallow reservoirs were built, whereas hydroelectric power stations have been constructed on side channels. This completely altered the hydrological and ecological regimes of the Drava River. In the stretch of the Drava River where the Asian clam was found, the hydrological regime is controlled. The discharge is set at 8 m^3 s^{-1} during the summer and winter. There are no water level oscillations after regular rainy days. Most of the sediment is deposited in the accumulation lakes. The river substrate contains mainly gravel with only a small amount of deposited fine sediment. During high discharges, the greater part of fine sediment is deposited in a flood zone outside the main river channel. Only small patches of fine sediment are deposited at the inside of the meander bends, where the water velocity locally drops. Thus fine sediment may be found after meander bends as well as at the bottom of the deeper sections. Since we sampled only shallow and accessible sections of the Drava River, we do not know whether the Asian clam lives in the deeper sections as well. The amount of empty shells on gravel bars will reveal this in the future. The accumulation lakes where large amounts of fine sediment occur have big potential for the Asian clam to develop big populations. The Asian clam is so far the third (together with Chinese pond mussel and zebra mussel) invasive bivalve species discovered in the Drava River in Slovenia. Time will reveal if all three invasive bivalve species will remain here and whether they will affect the three indigenous bivalve species (painter's mussel, thick shelled river mussel, duck mussel). Due to the Asian clam's biology we assume that the competition for habitat (fine sediment) and food between the Asian clam, Chinese pond mussel and all three indigenous species will be the main driver.

As the species is spreading fast upstream the Drava River, it is expected to occur also in Ptuj Lake. There it may establish a large population, since the Asian clam can reach high density in similar habitats; up to 3,206 individuals m^{-2} in rivers, up to 1,278 individuals m^{-2} in lakes and up to 796 individuals m^{-2} in reservoirs (Lucy et al. 2012). Preventive measures have to be taken immediately to stop the species from spreading out of the Drava River. Due to the popular use of Ptuj Lake for water sports it may spread fast around Slovenia.

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References

Basen T., Fleckenstein K., Rinke K., Rothhaupt K.O., Martin-Creuzburg D. (2016): Impact of temperature and nutrient dynamics on growth and survival of *Corbicula fluminea*: A field study in oligotrophic Lake Constance. Int. Rev. Hydrobiol. 102: 15-28.

Ciutti F., Cappelletti C. (2009): First record of *Corbicula fluminalis* (Müller, 1774) in Lake Garda (Italy), living in sympatry with *Corbicula fluminea* (Müller, 1774). J. Limnol. 68(1): 162-165.

Coughlan N.E., Stevens A.L., Kelly T.C., Dick J.T.A., Jansen M.A.K. (2017): Zoochorous dispersal of freshwater bivalves: an overlooked vector in biological invasions? Knowl. Manag. Aquat. Ecosyst. 2017(418): 42.

Crespo D., Dolbeth M., Leston S., Sousa R., Pardal M.A. (2015): Distribution of *Corbicula fluminea* (Müller, 1774) in the invaded range: a geographic approach with notes on species traits variability. Biol. Invasions 17: 2087-2101.

Csanyi B. (1999). Spreading invaders along the Danubian highway: first record of *Corbicula fluminea* (O.F. Müller, 1774) and *C. fluminalis* (O.F. Müller, 1774) in Hungary (Mollusca: Bivalvia). Folia Hist. Nat. Musei Matraensis 23(1998-1999): 343-345.

Denton M.E., Chandra S., Wittmann M.E., Reuter J., Baguley J.G. (2012): Reproduction and population structure of *Corbicula fluminea* in an oligotrophic subalpine lake. J. Shellfish Res. 31(1): 145-152.

Ferreira-Rodriguez N., Sousa R., Pardo I. (2018): Negative effects of *Corbicula fluminea* over native freshwater mussels. Hydrobiologia 810: 85-95.

Forrest A.L., Andradottir H.O., Mathis T.J., Wittmann M.E., Reuter J.E., Schladow S.G. (2017): Passive transport of a benthic bivalve (*Corbicula fluminea*) in large lakes: implications for deepwater establishment of invasive species. Hydrobiologia 797: 87-102.

Franco J.N., Ceia F.R., Patricio J., Thompson J., Marques J.C., Neto J.M. (2012): Population dynamics of *Corbicula fluminea* (Müller, 1774) in mesohaline and oligohaline habitats: Invasion success in a Southern Europe Estuary. Estuar. Coast. Shelf S. 112: 31-39.

Gama M., Crespo D., Dolbeth M., Anastacio P. (2015): Predicting global habitat suitability for *Corbicula fluminea* using distribution models: The importance of different environmental databases. Ecol. Model. 319: 162-169.

Kamburska L., Lauceri R., Beltrami M. (2013): Establishment of *Corbicula fluminea* (O.F. Müller, 1774) in Lake Maggiore: a spatial approach to trace the invasion dynamics. BioInvasions Rec. 2: 105-117.

Lajtner J. (2015): Invazivne vrste školjkaša u slatkovodnim ekusustavima Hrvatske. Prvi znanstveni simpozij o biologiji slatkih voda, Hrvatsko udruženje slatkovodnih ekologa, Biološki odsjek Prirodoslovno-matematičkog fakulteta, Zagreb, 20. 2. 2015. http://www.husek.hr/wp-content/uploads/2014/10/Lajtner.pdf [accesed on 2.9.2018].

Lajtner J., Crnčan P., Ćuk R., Dekić S., Gottstein S., Hudina S., Kovačević S., Lucić A., Paunović M., Simić V., Tomović J., Žganec K. (2016): Distribution of the invasive alien bivalve *Corbicula fluminea* (Müller, 1774) in Croatia. In: Jelaska S.D. (Ed), Book of Abstracts, 2nd Croatian Symposium on Invasive Species, Croatian Ecological Society, Zagreb, p. 57.

Lopes-Lima M., Teixeira A., Froufre E., Lopes A., Varandas S., Sousa R. (2014): Biology and conservation of freshwater bivalves: past, present and future perspectives. Hydrobiologia 735: 1-13.

Lucy F.E., Karatayev A.Y., Lyubov E.B. (2012): Predictions for the spread, population density, and impacts of *Corbicula fluminea* in Ireland. Aquat. Invasions 7: 465-474.

Paunović M., Csanyi B., Knežević S., Simić V., Nenadić D., Jakovčev-Todorovič D., Stojanović B., Cakić P. (2007): Distribution of Asian clams *Corbicula fluminea* (Müller, 1774) and *C. fluminalis* (Müller, 1774) in Serbia. Aquat. Invasions 2(2): 99-106.

Pigneur L.M., Fallise E., Roland K., Everbecq E., Deliege J.F., Smitz J.S., Doninck K., Descy J.P. (2014): Impact of invasive Asian clams, *Corbicula* spp., on a large river ecosystem. Freshw. Biol. 59: 573-583.

Pigneur L.M., Hedtke S.M., Etoundi E., Donick K. (2012): Androgenesis: a review through the study of the freshwater shellfish *Corbicula* spp. Heredity 108: 581-591.

Prezant R.S., Chalermwat K. (1984): Flotation of the bivalve *Corbicula fluminea* as a means of dispersal. Science 225: 1491-1492.

Rosa I.C., Pereira J.L., Gomes J., Saraiva P.M., Gonçalves F., Costa R. (2011): The Asian clam *Corbicula fluminea* in the European freshwater-dependent industry: A latent threat or a friendly enemy? Ecol. Econom. 70: 1805-1813.

Sousa R., Antunes C., Guilhermino L. (2008): Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. Int. J. Lim. 44(2): 85-94.

Voelz N.J., McArthur J.V., Rader R.B. (1998): Upstream mobility of the Asiatic clam *Corbicula fluminea*: Identifying Potential Dispersal Agents. J. Fresh. Ecol. 13: 39-45.

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SHORT COMMUNICATION

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A twist of nature: a left-handed *Bythinella schmidti* (Küster, 1852) (Caenogastropoda: Bythinellidae)

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Abstract. As most extant snails, *Bythinella schmidti* is characterised by dextral (right-handed) coiling of the shell. Nevertheless, a small sinistral (left-handed) individual from the spring on a mountain pasture was sampled, together with its larger dextral conspecifics. In our report on this first case of sinistrality within the superfamily Truncatelloidea, we discuss its shell abnormalities and provide a review on chirality in snails.

Key words: *Bythinella*, sinistrality, shell abnormalities, spring, Slovenia

Izvleček. Zasuk narave: levosučna *Bythinella schmidti* (Küster, 1852) (Caenogastropoda: Bythinellidae) – Kot za večino obstoječih vrst je tudi za vrsto *Bythinella schmidti* značilna desnosučnost, torej zavitost hišice v smeri urinega kazalca. A v izviru na planinskem pašniku smo med večjimi desnosučnimi sovrstniki našli tudi majhnega levosučneža te vrste. V poročilu o prvem primeru levosučnosti znotraj naddružine Truncatelloidea obravnavamo opažene nepravilnostih v zgradbi hišice in podajamo pregled objav o sučnosti polžev.

Ključne besede: *Bythinella*, levosučnost, nepravilnosti hišice, izvir, Slovenija

Introduction

Handedness is the phenomenon relating to the ability to classify chiral objects into right- and left-handed. Most snail species in nature show either uniform right- or left-handedness. Sinistral species are rare, especially in the marine realm, where such taxa have independently originated only 19 times among Cenozoic gastropod clades (Vermeij 2002). Some 90–99% of snail species exhibit dextral shell coiling (Asami 1993, van Batenburg & Gittenberger 1996); i.e., when oriented with the shell apex pointing upwards and the shell's aperture opening facing the viewer, the opening is on the right-hand side. However, a few cases of the consistent mirror-image shell coiling within the same snail species exist as well. The earliest discovery of a single-gene mutation that can cause a complete left-to-right and right-to-left inversion of the body axis was made on pond snails *Lymnaea stagnalis* (Linnaeus, 1758) and

Radix peregra (O. F. Müller, 1774), which is nowadays synonymized with *R. baltica* (Linnaeus, 1758). Moreover, this was the first maternal effect gene mutation discovered (Freeman & Lundelius 1982, Gurdon 2005). Both snails have been used to study asymmetry for more than 120 years (references listed in Liu et al. 2013) and the sinistral individuals are reported to represent up to 2% of their populations (Wandelt & Nagy 2004; while one author of this notice (AF) did not find a single sinistral individual among about 3,000 examined *R. peregra* from Poland). On the other hand, in the south-east-Asian tree snail *Amphidromus (Amphidromus) inversus* O. F. Müller, 1774, and some 30 other species from this subgenus, dextrals and sinistrals appear at about even rates (Craze et al. 2006, Sutcharit et al. 2007, Schilthuizen & Haase 2010). Their balanced chiral dimorphism is one of the extremely rare cases of genetic antisymmetry. In most other snail species, such reversals of asymmetry are rather exceptional. Among thousands of right-handed shells there may be one or the other unique sinistral specimen, which are informally referred to as snail kings. These »*conchylia sinistralia*« can be found as special addition to different shell collections from the Renaissance era onwards. Such findings have been reported already from the Cambrian (Parkhaev 2007) to the Cenozoic (Pierce 1996).

Within the uniformly dextral superfamily Truncatelloidea, the genus *Bythinella* Moquin-Tandon, 1856 comprises tiny (2–4 mm shell height) dioecious freshwater snails belonging to the family Bythinellidae. It has been demonstrated that it is impossible to identify and separate out *Bythinella* species without molecular data (Falniowski & Szarowska 2011), although the morphology must be considered in identification as well (Bichain et al. 2007, Haase et al. 2007). More than 120 recognised species and subspecies occur in springs, caves and groundwater (Giusti & Pezzoli 1977, Falniowski 1987, Yıldırım et al. 2006) from northern Africa and NE Spain across central Europe to W Turkey, with at least two richness centres in France and the Balkans (Glöer & Pešić 2014). Many taxa are liable to become endangered due to their limited range and vulnerable habitat.

While checking for the presence of *Belgrandiella* A.J. Wagner, 1927 in the Karavanke Mountains (Slovenia), several specimens of *Bythinella* were found in a spring. It was only when the individuals were examined in the laboratory under the stereo microscope that it turned out that among dextral *Bythinella* cf. *schmidti* (Küster, 1852) much smaller snail king of the species was present as well. Here, we present this first case of sinistrality within the superfamily Truncatelloidea.

Materials and methods

Several live miniature snails were collected from the stones in a small spring bubbling up just beside a marked trail to Kofce mountain chalet (N Slovenia) by the first author of this article (SP). Forceps were used to remove individuals from the stones and put into the plastic vial filled with spring water. The spring is located on a pasture grazed by livestock some 50 m above the Matizovec farm, north-east of Podljubelj village (N of the town Tržič, 1100 m a.s.l., coordinates; 46°24'55.56"N 14°18'30.0"E) (Fig. 1).

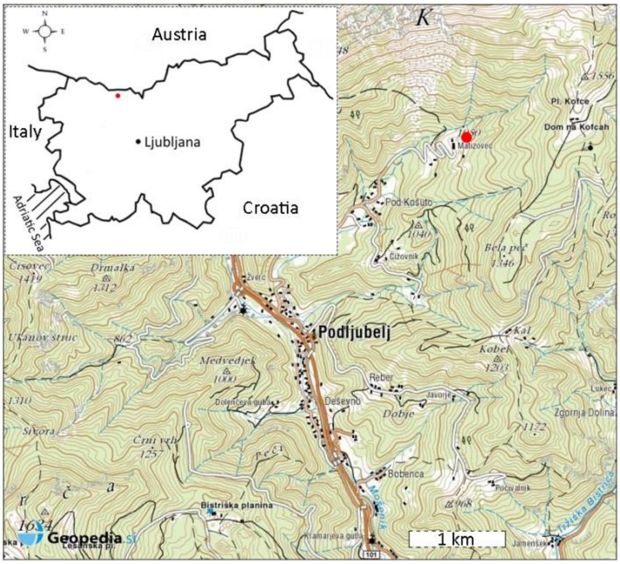

Figure 1. Map showing locality inhabited by left-handed individual of *Bythinella* cf. *schmidti*. The base map was taken from the portal geopedia.si.

Slika 1. Lokacija izvira, kjer je bil najden levosučni osebek vrste Bythinella cf. schmidti. Osnovna karta je bila vzeta s portala geopedia.si.

Results and discussion

The sinistral specimen (Figs. 2A–B) is not a mirror image of its dextral conspecifics (Figs. 2C–E): there are fewer whorls present, the suture is deeper (Fig. 2A), the shell shows scalarity (tendency to open coiling) (Fig. 2B) and the umbilicus is abnormally broad (Fig. 2B). In addition, it is about twice smaller than the dextral ones. Despite its smallness, the specimen seems to be adult or nearly adult since the shell aperture is surrounded by abnormally broad and prominent lip (Fig. 2A).

Coiling direction in snails, studied mainly in pulmonates, is known as determined by a single Mendelian locus. Either the »dextral« or »sinistral« allele is dominant (Schilthuizen & Davison 2005, Schilthuizen & Haase 2010), even though Utsuno & Asami (2010) discovered also a chirality randomizing gene in *Bradybaena*. The long history of various, not necessarily correct theories about the genetic background of the chirality (the so-called maternal inheritance) (e.g. Boycott & Diver 1923, Sturtevant 1923, Diver et al., Boycott & Garstang 1925) was caused by the fact, that phenotypical expression of this maternal effect gene is delayed by a generation. Since the opposite direction of the shell coiling results in the mirror organization of the ventral sac and pallial organs, such conspecifics can't orient their bodies for copulation, which leads to interchiral reproductive isolation.

Figure 2. Shells of *Bythinella* cf. *schmidti* from the spring above the Matizovec farm (Podljubelj): A–B - sinistral specimen, C–E - "normal", dextral specimens; a bar equals 0.5 mm (photo: A. Falniowski).
Slika 2. Polžki vrste *Bythinella* cf. *schmidti* iz izvira nad kmetijo Matizovec (Podljubelj): A–B – levosučni osebek, C–E - "normalni" desnosučni osebki; merilce meri 0.5 mm (foto: A. Falniowski).

Nevertheless, the latter is usually due to behavioral rather than purely physical constraints, thus possibly promoting saltational, single-gene and sympatric speciation (Asami et al. 1998, Gittenberger 1988, Schilthuizen & Davison 2005, Davison et al. 2009). This and other hypotheses about evolution and persistence of sinistral lineages have been tested using computer simulations (e.g. Johnson et al. 1990, Orr 1991, van Batenburg & Gittenberger 1996, Stone & Björklund 2002) and through study of populations (e.g. Asami 1993, Asami et al. 1998, Ueshima & Asami 2003, Davison et al. 2005, Schilthuizen et al. 2005, Schilthuizen et al. 2007, Sutcharit et al. 2007). Importantly, Davison et al. (2005, p. 1569) noted that, precisely due to the maternal effect gene, the reproductive isolation is unstable. While some researchers suggested the possibility of one-gene saltational sympatric speciation (e.g. Ueshima & Asami 2003), some other remained unconvinced (e.g. Davison et al. 2005, Schilthuizen & Davison 2005) due to individual cases that show almost balanced intra-population coil dimorphism (i.e. previously mentioned species in the subgenus *Amphidromus*). Such dimorphism is probably maintained by sexual selection favouring mates of the opposite chirality (Sutcharit et al. 2007, Schilthuizen et al. 2007, Schilthuizen & Looijestijn 2009). However, in hermaphroditic pulmonates, a population of new sinistral species could also be established through self-fertilization of the sole sinistral individual, despite their effective behavioural restrictions against interchiral copulation. In dioecious caenogastropods, like *Bythinella*, this is not possible. Vermeij (2002) has demonstrated that among living dioecious sinistral neogastropods, all have a nonpelagic (intracapsular) development. This confirms that a new (sub)population of sinistral snails within such population of dextrals may evolve only if the offspring of a parent is not scattered across larger area.

Shell abnormalities are not a typical feature of snail kings, although anomalies associated with sinistrality have been reported in *Lymnaea stagnalis* (Asami 2007), *Achatinella* (Asami 2001), *Conus adversarius* (Hendricks 2009) and *Bradybaena* (Utsuno & Asami 2007). Also, Shibazaki et al. (2004) have demonstrated that the oppositely coiling embryos of *L. stagnalis* are not the perfect mirror images of one another. The observed shell abnormality of our *Bythinella*, i.e. its slight scalarity, deeper suture, broader umbilicus and smallness, can be due to pleiotropic effects resulting from incompatibility of the reversed chirality and the rest of the genomic and developmental environment, causing a reduction of fitness. Similar irregularities have been reported in *Cerion* (Gould et al. 1985) and *Partula suturalis*, in which the sinistral shells are somewhat lower and squatter (Gould et al. 1985, Johnson 1987, Johnson et al. 1993, Davison et al. 2009). Our snail king's pronounced dwarfism is most likely not reflecting possible damage inflicted in an early stage of life, but rather another generation, whose development took place in harsh conditions (e.g., during winter). This is not a rare phenomenon among hydrobioid members (Wilke & Falniowski 2001, Falniowski et al. 2012).

References

Asami T. (1993): Genetic variation and evolution of coiling chirality in snails. Forma 8: 263–276.

Asami T. (2001): Evolution of left-right asymmetry: why isn't the snail mirror flat? In: Salvini-Plawen L., Voltzow J., Sattmann H., Steiner G. (Eds.), Proceedings of the world congress of malacology 2001. Unitas Malacologica, Vienna, p. 13.

Asami T. (2007): Developmental constraint and stabilizing selection against left-right reversal in snails. In: Jordaens K., Van Houtte N., Van Goethem J., Backeljau T. (Eds.), Proceedings of the world congress of malacology 2007. Unitas Malacologica Antwerp, p. 13.

Asami T., Cowie R.H., Ohbayashi K. (1998): Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. Am. Nat. 152: 225-236.

van Batenburg F.H.D., Gittenberger E. (1996): Ease of fixation of a change in coiling: computer experiments on chirality in snails. Heredity 76: 278-286.

Bichain J.M., Boisselier-Dubayle M.C., Bouchet P., Samadi S. (2007): Species delimitation in the genus *Bythinella* (Mollusca: Caenogastropoda: Rissooidea): a first attempt combining molecular and morphometrical data. Malacologia 49: 293-311.

Boycott A.E., Diver C. (1923): On the inheritance of sinistrality in *Limnaea peregra*. P. Roy. Soc. B–Biol. Sci. 95: 207-213.

Craze P.G., Elahan B., Schilthuizen M. (2006): Opposite shell-coiling morphs of the tropical land snail *Amphidromus martensi* show no spatial-scale effects. Ecography 29: 477-486.

Davison A., Chiba S., Barton N.H., Clarke B. (2005): Speciation and gene flow between snails of opposite chirality. PLoS Biol. 3: 1559-1571.

Davison A., Constant N., Tanna H., Murray J., Clarke B. (2009): Coil and shape in *Partula suturalis*: the rules of form revisited. Heredity 103: 268-278.

Diver C., Boycott A.E., Garstang S.L. (1925): The inheritance of inverse symmetry in *Lymnaea peregra*. J. Genet. 15: 113-200.

Falniowski A. (1987): Hydrobioidea of Poland (Prosobranchia: Gastropoda). Folia Malacol. 1: 1-122.

Falniowski A., Szarowska M. (2011): Radiation and phylogeography in a spring snail *Bythinella* (Mollusca: Gastropoda: Rissooidea) in continental Greece. Ann. Zool. Fennici 48: 67-90.

Falniowski A., Szarowska M., Glöer P., Pešić V. (2012): Molecules *vs* morphology in the taxonomy of the *Radomaniola/Grossuana* group of Balkan Rissooidea (Mollusca: Caenogastropoda). J. Conchol. 41: 19-36.

Freeman G., Lundelius J.W. (1982): The developmental genetics of dextrality and sinistrality in the gastropod *Lymnaea peregra.* Wilhelm Roux's Archives 191: 69-83.

Gittenberger E. (1988): Sympatric speciation in snails; a largely neglected model. Evolution 42: 826-828.

Giusti F., Pezzoli E. (1977): Primo contributo alla revisione del genre *Bythinella* in Italia. Natura Bresciana, Annurario del Museo Civico di Storia Naturale di Brescia 14: 3-80.

Glöer P., Pešić V. (2014): Two new species of the genus *Bythinella* Moquin-Tandon, 1856 (Mollusca: Gastropoda: Hydrobiidae) from the Western Balkan Peninsula. Ecol. Mont. 1(4): 249-255.

Gould S.J., Young N.D., Kasson B. (1985): The consequences of being different: sinistral coiling in *Cerion*. Evolution 39: 1364-1379.

Gurdon J.B. (2005): Sinistral snails and gentlemen scientists. Cell 123(5): 751-753.

Haase M., Wilke T., Mildner P. (2007): Identifying species of *Bythinella* (Caenogastropoda: Rissooidea): A plea for an integrative approach. Zootaxa 1563: 1-16.

Hendricks J.R. (2009): Sinistral snail shells in the sea: developmental causes and consequences. Lethaia 42: 55-66.

Johnson M.S. (1987): Adaptation and rules of form: chirality and shape in *Partula suturalis*. Evolution 41: 672-675.

Johnson M.S., Clarke B., Murray E. (1990): The coiling polymorphism in *Partula suturalis* does not favor sympatric speciation. Evolution 44: 459-464.

Johnson M.S., Murray J., Clarke B. (1993): The ecological genetics and adaptive radiation of *Partula* on Moorea. Oxf. Surv. Evol. Biol. 9: 167-238.

Liu M.M., Davey J.W., Banerjee R., Han J., Yang F., Aboobaker A., Blaxter M.L., Davison A. (2013): Fine Mapping of the Pond Snail Left-Right Asymmetry (Chirality) Locus Using RAD-Seq and Fibre-FISH. PLoS ONE 8(8): e71067.

Orr H.A. (1991): Is single-gene speciation possible? Evolution 45: 764-769.

Parkhaev P.Y. (2007): Shell chirality in Cambrian gastropods and sinistral members of the genus *Aldanella* Vostokova, 1962. Paleontol. J. 41: 233-240.

Pierce H.G. (1996): On sinistral coiling among fossil North American Lymnaeidae. The Veliger 39: 220-225.

Schilthuizen M., Davison A. (2005): The convoluted evolution of snail chirality. Naturwissenschaften 92: 504-515.

Schilthuizen M., Haase M. (2010): Disentangling true shape differences and experimenter bias: are dextral and sinistral snail shells exact mirror images? J. Zool. 282: 191-200.

Schilthuizen M., Looijestijn S. (2009): The sexology of the chirally dimorphic snail species *Amphidromus inversus*. Malacologia 51: 379-387.

Schilthuizen M., Scott B.J., Cabanban A.S., Craze P.G. (2005): Population structure and coil dimorphism in a tropical land snail. Heredity 95: 216-220.

Schilthuizen M., Craze P.G., Cabanban A.S., Davison A., Gittenberger E., Stone J., Scott B.J. (2007): Sexual selection maintains whole-body chiral dimorphism. J. Evol. Biol. 20: 1941-1949.

Shibazaki Y., Shimizu M., Kuroda R. (2004): Body handedness is directed by genetically determined cytoskeletal dynamics in the early embryo. Curr. Biol. 14: 1462-1467.

Stone J., Björklund M. (2002): Delayed prezygotic isolating mechanisms: evolution with a twist. P. Roy. Soc. B–Biol. Sci. 269: 861-865.

Sturtevant A.H. (1923): Inheritance of direction of coiling in *Lymnaea*. Science 58: 269-270.

Sutcharit C., Asami T., Panha S. (2007): Evolution of wholebody enantiomorphy in the tree snail genus *Amphidromus*. J. Evol. Biol. 20: 661-672.

Ueshima R., Asami T. (2003): Single-gene speciation by left-right reversal. Nature 425: 679-679.

Utsuno H., Asami T. (2007): Genetic basis of adaptive evolution of left-right reversal under developmental constraint. In: Jordaens K., Van Houtte N., Van Goethem J., Backeljau T. (Eds.), Proceedings of the world congress of malacology 2007, Unitas Malacologica Antwerp, pp. 227-228.

Utsuno H., Asami T. (2010): Maternal inheritance of racemism in *Bradybaena similaris*. J. Hered. 101: 11-19.

Vermeij G.J. (2002): The geography of evolutionary opportunity: hypothesis and two cases in gastropods. Int. Comp. Biol. 42: 935-940.

Wandelt J., Nagy L.M. (2004): Left-right asymmetry: more than one way to coil a shell. Curr. Biol. 14(16): 654-656.

Wilke T., Falniowski A. (2001): The genus *Adriohydrobia* (Hydrobiidae: Gastropoda): polytypic species or polymorphic populations? J. Zoo. Syst. Evol. Research 39: 227-234.

Yıldırım M.Z., Bahadır Koca S., Kebapçı Ü. (2006): Supplement to the Prosobranchia (Mollusca: Gastropoda) Fauna of Fresh and Brackish Waters of Turkey. Turk. J. Zool. 30: 197-204.

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FIELD NOTE

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An interesting new record of Egyptian locust *Anacridium aegyptium* (Linnaeus, 1764) (Orthoptera: Acrididae) for Slovenian inland

Zanimiva nova najdba egipčanske kobilice *Anacridium aegyptium* (Linnaeus, 1764) (Orthoptera: Acrididae) v notranjosti Slovenije

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The Egyptian locust (also known as the Egyptian bird grasshopper; *Anacridium aegyptium* (Linnaeus, 1764)) is one of the largest grasshoppers of Europe (Harz 1975) and Slovenia (Gomboc 2003). It reaches the adult stage in late summer, with adult animals surviving colder months and winter till next May (Bellman 2009). It is known to occur throughout the European Mediterranean (Gomboc 2003, Bellman 2009, Hochkirch et al. 2016) as well as in North Africa and the Middle East (see Hochkirch et al. 2016). Reports from Austria also show that the species is capable of reaching inland areas by different means of human assistance, e.g. with train transport (Zuna-Kratky 2017). Considered rare in the country (Gomboc 2003), in the last three decades, the species has only been recorded in Slovenian Littoral, with the easternmost data being noted in the Vipava valley (Gomboc 2013). Only a single old inland record of *A. aegyptium* has been known from Slovenia so far. The species was reported by Us (1971, 1992) from Grintovec near Kočevje, where the author recorded 2 female larvae on 28. 7. 1969.

On 23. 4. 2018, bat mist-netting (Kunz & Kurta 1988) was carried out as a part of the »Netopirji - skrivnostni Ljubljančani 3« project (SDPVN 2018). The nets were placed in an almost straight line of 33 metres in length and about 4 meters in height across several shallow ponds in the area of Jarše gravel pit, located in the north-eastern part of Ljubljana (46.076463° N, 14.544973° E; 287 m a. s. l.) The field work was conducted between 20:00 and approximately 22:30 hrs. The weather on that day was dry with an occasional breeze. Apart from two bat species (*Pipistrellus pygmaeus* and *P. kuhlii*), we also captured one individual of *A. aegyptium*. The locust was entangled in the net sometime after 22:00 and was immediately rescued. Photographs (Fig. 1) were taken and the animal was released unharmed. The species identity was suggested by initially consulting Bellman's manual (2009) and afterwards verified by consulting three Slovenian orthopterists (D. Galjot, P. Trontelj & S. Gomboc).

Figure 1. The caught individual of *A. aegyptium*, Jarše gravel pits, NE Ljubljana, 23. 4. 2018 (photo: Nejc Poljanec).
Slika 1. Ujeti osebek *A. aegyptium*, betonarna Jarše, SV Ljubljana, 23. 4. 2018 (foto: Nejc Poljanec).

As confirmed by expert consultancy (D. Galjot, S. Gomboc & M. Bedjanič, pers. comm.) and browsing through available literature (e.g. Us 1971, 1992, Gomboc 2013), this is the first recent record of the species in Slovenian inland after almost 50 years. The Egyptian locust is a keen flyer (Gomboc 2003) and the aerial distance of about 50 km from the nearest parts of the Littoral makes the find not so unusual. The caught individual, however, probably did not overwinter in the nearby area due to quite severe winter conditions between late February and early March 2018 (ARSO 2018). Another set of possible explanations for our find is related to human activity. It is possible that the caught individual was brought in by accidental transport, either by car or train, since Ljubljana is an important junction for main Slovenian highways and railway tracks. For example, at least a few findings of *A. aegyptium* from neighbouring Austria can be attributed to trains (Zuna-Kratky 2017). This possibility is further supported by a single observation of *Decticus albifrons,* another Mediterranean species, found in Ljubljana in close proximity to the railway line (Trontelj 2004). Zuna-Kratky (2017), however, also comments that the majority of Austrian observations of *A. aegyptium* were probably due to accidental import with Mediterranean plant material, which is also an option in our case, considering the urban location of our find. Regardless of the real explanation for our find, it is perhaps reasonable to expect further individuals of the species in continental Slovenia as a result of human-mediated introduction in the future.

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References

ARSO (2018): Mraz, sneg in veter od 18. februarja do 6. marca 2018. Urad za meteorologijo in hidrologijo, Agencija Republike Slovenije za okolje, Ljubljana, 29 pp. http://www.meteo.si/uploads/probase/www/climate/text/sl/weather_events/mraz-sneg-veter_18feb-6mar2018.pdf [Accesed in May 2018]

Bellman H. (2009): Naše in srednjeevropske žuželke. Založba Narava, Olševek, 445 pp.

Gomboc S. (2003): Kobilice – Orthoptera (Saltatoria). In: Sket B., Gogala M., Kuštor V. (Eds.), Živalstvo Slovenije, Tehniška založba Slovenije, Ljubljana, pp. 308-318.

Gomboc S. (2013): Kobilice/Orthoptera. In: Pavšič J. (Ed.), Vipavska dolina. Slovenska matica, Ljubljana, pp. 136-145.

Harz, K. (1975): Die Orthopteren Europas II. Series Entomologica 11, Junk, The Hague.

Hochkirch A., Kristin A., Szovenyi G., Presa J.J., Rutschmann F., Chobanov D. P., Kleukers R., Willemse L. P. M. (2016): *Anacridium aegyptium*. The IUCN Red List of Threathened Species. International Union for Conservation of Nature and Natural Resources. http://www.iucnredlist.org/details/16084533/1 [accesed in May 2018]

Kunz T. H., Kurta A. (1988): Capture methods and holding devices. In: Kunz T. H. (ed.), Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington D.C., London, pp. 1-29.

Trontelj P. (2004): Unexpected record of the white-faced bush-cricket *Decticus albifrons* (Fabricius, 1775) (Orthoptera: Tettigoniidae) in Ljubljana, central Slovenia. Nat. Slov.6(2): 57.

Us P. A. (1971): Beitrag zur Kenntnis der Orthopteren-Fauna (Saltatoria) von Slowenien. Beitr. Entomol. 21(1/2): 5-31.

Us P.A. (1992): Favna ortopteroidnih insektov Slovenije. SAZU, Raz. za prir. vede 32(12), 314 pp.

SDPVN (2018): Netopirji – skrivnostni Ljubljančani 3. http://www.sdpvn-drustvo.si/netopirji-skrivnostni-ljubljancani-3.html [accesed in December 2018]

Zuna-Kratky T. (2017): Eingeschleppte, nicht dauerhaft etablierte Arten. In: Zuna-Kratky T., Landmann A., Illich I., Zechner L., Essl F., Lechner K., Ortner A., Weißmair W., Wöss G. (Eds.), Die Heuschrecken Österreichs. Denisia 39, pp. 816-818.

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»SOS *Proteus*« – EDITORIAL

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Third International meeting SOS *Proteus*: »Conservation of proteus and its habitat – 250 years after its scientific description« / Tretji mednarodni posvet SOS *Proteus*: »Varstvo človeške ribice in njenega habitata – 250 let po znanstvenem opisu«

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Upon the 250th anniversary of the first published scientific description of proteus, the Tular Cave Laboratory (Society for Cave Biology) organised, in partnership with Škocjan Caves Park, Slovenia, the 3rd International meeting SOS *Proteus*: »Conservation of *Proteus* and its habitat – 250 years after its scientific description«. It was held on 14. 4. 2018 at the Promotion and congress centre of Škocjanske jame Caves Park, in Matavun in Slovenia, under the honorary patronage of His Excellency the President of the Republic of Slovenia Mr. Borut Pahor.

The meeting gathered over 90 researchers and experts on proteus, speleobiology, karstology, herpetology, conservation and public outreach from Slovenia, Italy, Croatia, Hungary, Bosnia and Herzegovina, Germany, Portugal, Romania, USA, UK, China and Denmark. The meeting started with two keynote lectures: »Short history of Slovenian Karst«, held by Academician Andrej Kranjc, and »250th anniversary of the taxonomic description of *Proteus anguinus*« given by Academician Boris Sket. The program of 21 presentations was divided into six sessions: Conservation/Habitat, Conservation/Management and Health, Research Highlights and Perspectives, Education, Poster session, closing the meeting with the final session Discussions, Conclusions and Next steps. The lectures included the latest findings in the field of research and conservation of proteus and pollution of karst groundwater. The presentation part of the meeting was concluded by a short documentary showing the importance of film in promotion of awareness on vulnerability of proteus, and an educational computer game, visually exploring ecology of proteus against threats of groundwater pollution (Tular 2018).

In the closing session, participants were invited to participate in a debate related to current practices, legislation or protective measures needed or applied for the conservation of proteus along the Dinaric karst. The discussion was centred on the international cooperation among all countries hosting or researching proteus, in order to establish a better communication channel with European decision makers in research, conservation and public awareness, towards a practical protection of this endangered cave animal and its habitat.

The 3rd »SOS *Proteus*« meeting also launched its new logo, showing the black and white proteus coupled in a drop of (drinking) water (Fig. 1).

Figure 1. Participants of the 3rd International meeting SOS *Proteus*: »Conservation of proteus and its habitat – 250 years after its scientific description«, Škocjan Caves Park, Slovenia, 14. 4. 2018 (photo: Matej Blatnik).
Slika 1. Udeleženci 3. Mednarodnega posveta SOS *Proteus*: »Varstvo človeške ribice in njenega habitata – 250 let po znanstvenem opisu«, Park Škocjanske jame, Slovenija, 14. 4. 2018 (foto: Matej Blatnik).

The assembly of the meeting honoured Professor David C. Culver, American University, USA, for his outstanding contributions to the study and conservation of subterranean biodiversity, and Academician Andrej Kranjc, Slovenian Academy of Sciences and Arts, Slovenia, for his outstanding studies of Karst research history.

The submitted extended abstracts of the lectures are presented on the following pages of this issue of *Natura Sloveniae*.

Acknowledgements

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References

Tular (2018): Program of the 3rd International Meeting SOS *Proteus*: »Conservation of proteus and its habitat – 250 years after its scientific description«. Tular Cave Laboratory, Society for Cave Biology, Kranj, 4 pp. http://www.tular.si/images/Tular_docs/SOS_Proteus_2018_Program.pdf [accessed on 18.12. 2018]

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The threshold concentration for nitrate in groundwater as a habitat of *Proteus anguinus*

Mejne koncentracijske vrednost za nitrat v podzemni vodi kot okolju močerila

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The aim of the study was to assess the risk that nitrate might pose to the groundwater ecosystem in the LIFE Kočevsko project area (http://life-kocevsko.eu). We identified most relevant sources of nitrate in groundwater of the project area as well as in the entire karst region where the proteus (*Proteus anguinus*) populations are present. Based on toxicity data on amphibians we calculated the threshold concentration for nitrate in groundwater as a habitat of proteus. The main sources of nitrate emissions in groundwater were identified as wastewater treatment plant effluents that immediately sink into karst underground, as well as emissions from livestock farming and the potentially inappropriate use of manure. The calculated threshold concentration of nitrate for proteus of 9.2 $mgNO_3^-/L$ comprises the predicted no-effect concentration (PNEC), the natural background concentration and the expected variation of the natural background concentration. Based on results obtained, we proposed possible risk mitigation measures to reduce the impact of nitrate on groundwater as the proteus' habitat.

The groundwater directive provides the groundwater quality standard (GQS) for nitrate of 50 $mgNO_3^-/L$ (2006/118/EC 2006). This value is based on epidemiological evidence for methaemoglobinaemia in infants, which results from short-term exposure to nitrate. The nitrate GQS is protective for bottle-fed infants and, consequently, other population groups (World Health Organization 2011). It is obvious that the goal of nitrate GQS is to protect groundwater as a source of drinking water and not as an ecosystem. However, several scientific publications provide information that this value might not be safe for the aquatic ecosystems. It seems that amphibians are more sensitive in their developmental stages than humans (Marco et al. 1999, Rouse et al. 1999). The presented survey was focused on the LIFE Kočevsko project area in southern Slovenia, mainly in the Municipality of Kočevje. However, a broader view on the potential emission of nitrate in the proteus' habitats in the karst region of Slovenia was also presented. Two main sources of nitrate emissions in groundwater were identified:

- Wastewater treatment plants (WWTP) effluents that immediately sink into the karst underground.
- Emissions from livestock farming and the potentially inappropriate use of manure.

Wastewater treatment effluents in the karst region commonly sink directly into the underground and groundwater. The effluents from the tertiary WWTP are, in most cases, of a good quality (regarding the organic pollution, nitrates and phosphorous). However, the secondary treatment, as the most common method of treatment in smaller WWTP, does not provide the adequate effluent quality. Such an example is the WWTP on the border of the project area near Ribnica na Dolenjskem, which releases effluents into the sinker and groundwater.

Intensive pig and cattle production estates are located within the project area. In addition, spreading of manure from the intensive poultry production over the grassland might exert a strong influence on the groundwater habitats of the proteus populations.

The threshold concentration of nitrate for proteus comprises the predicted no-effect concentration (PNEC), the natural background concentration and the expected variation of the natural background concentration. The PNEC is extracted from selected long-term toxicity data (expressed as NOAEL – no observed adverse effect level or NOEC – no observed effect concentration) of $NaNO_3$ and KNO_3 on amphibians available in scientific literature (e.g. Schuytema & Nebeker 1999a, Laposata & Dunson 1998, Camargo et al. 2005).

The predicted no-effect concentration (PNEC) calculation was performed following the method using SSD (Species Sensitivity Distribution) approach and the ETX 2.1. software (van Vlaardingen et al. 2014): the PNEC was calculated based on the 5th percentile (HC5) of toxicity data (Tabs. 1, 2; Fig. 1) by applying the assessment factor 1.

Table 1. Overview of endpoints of toxicity expressed with NOAEL or NOEC, for different amphibians, taken from published references. Markings refer to: * – embryo, ** – tadpole.
Tabela 1. Vrednosti parametrov toksičnosti NOAEL ali NOEC za različne dvoživke, vzete iz literature. Oznake se nanašajo na: * – zarodek, ** – paglavec.

Number	NOAEL/NOEC [mg/L]	Tested species	Reference
1	9.0	*Ambystoma maculatum**	Laposata & Dunson 1998
2	9.0	*Ambystoma jeffersonianum**	Laposata & Dunson 1998
3	9.0	*Rana sylvatica***	Laposata & Dunson 1998
4	66.0	*Xenopus laevis***	Schuytema & Nebeker 1999b, Sullivan & Spence 2003
5	56.7	*Pseudacris regilla**	Schuytema & Nebeker 1999a
6	78.2	*Pseudacris regilla*** – average values of 30.1^A and 126.3^B	A – Schuytema & Nebeker 1999b, B – Camargo et al. 2005
7	29.0	*Rana aurora**	Schuytema & Nebeker 1999c
8	24.8	*Xenopus laevis**	Schuytema & Nebeker 1999a
9	5	*Rana temporaria***	Johansson et al. 2001
10	9	*Bufo bufo***	Baker & Waights 1993

Table 2. Values of the SSD calculation (software ETX 2.1., van Vlaardingen et al. 2014) is expressed as the 5th percentile (HC5) of the long-term effect concentrations of $NaNO_3$ on embryonal and/or larval stage of amphibians.
Tabela 2. Izračun SSD (programsko orodje ETX 2.1., van Vlaardingen et al. 2014) temelji na vrednosti 5 percentile (HC5) koncentracij dolgodobnih učinkov $NaNO_3$ na embrionalne in/ali larvalne stadije dvoživk.

Name	Value	Log10 (value)	Description
LL HC5	1.04	0.019	lower estimate of the HC5
HC5	**3.50**	**0.544**	**median estimate of the HC5**
UL HC5	6.95	0.842	upper estimate of the HC5
sprHC5	6.65	0.823	spread of the HC5 estimate

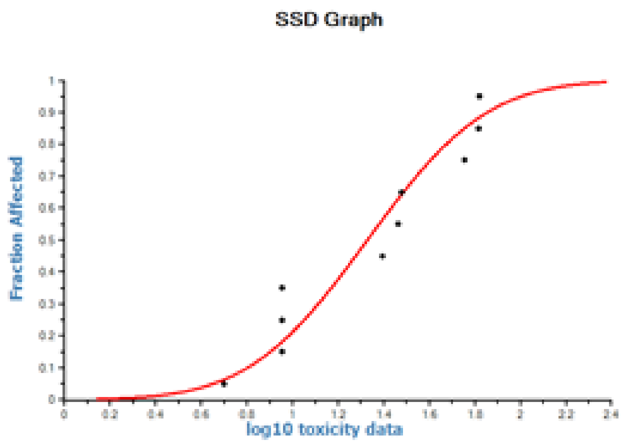

Figure 1. Graphic display of the species sensitivity distribution (SSD) (software ETX 2.1., van Vlaardingen et al. 2014).
Slika 1. Grafični prikaz porazdelitve občutljivosti vrst (SSD – species sensitivity distribution) (programsko orodje ETX 2.1., van Vlaardingen et al. 2014).

The natural background concentration for nitrate in Slovenia is 3.8 $mgNO_3^-/L$ (Mezga 2014). This concentration is estimated for all the areas with identified proteus populations. Therefore, the calculated threshold concertation for nitrate in groundwater can be applied to all these sites. The deviation of the natural background concentration of nitrate was estimated to be 50% (1.9 $mgNO_3^-/L$).

Calculation of PNEC:
$PNEC_{SSD}$= HC5/AF
$PNEC_{SSD}$=3.5 mg NO_3^-/L
The expected background concentration of nitrate: 3.8 $mgNO_3^-/L$
50% of expected deviation of the natural background concentration: 1.9 $mgNO^-_3/L$
The proposed threshold concentration for proteus: 9.2 $mgNO_3^-/L$

The proteus is one of the most remarkable representatives of stygofauna in Slovenia and in Europe. Emissions from agriculture and wastewater effluents can pose a threat to existing populations of this neotenic amphibian. To reduce the risk of nitrate to the proteus, we propose several risk mitigation measures that the risk manager should apply in the LIFE Kočevsko project area as well as at other exposed locations in the karst regions. The measures are as follows:

- To implement the threshold value of 9.2 $mgNO3^-/L$ in groundwater as an environmental quality standard for good chemical status for the proteus habitats.
- To implement appropriate measures within subvention policy to enhance good agricultural practice of manure use and penalize the pollution of environmental compartments with manure.
- To introduce a strict recording of manure application on farms in the karst region.
- Surveillance over the adequacy of dung pits, dung collection sites and possible leaks of slurry into the environment.
- A network of stakeholders, NGOs and public bodies that might have an interest should be established and invited to identify and record all possible sources of nitrates in groundwater.
- Implementation of legal terms that would prevent release of untreated or insufficiently treated wastewater to sink directly into the karst underground and groundwater.

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References

Baker J., Waights V. (1993): The effect of sodium nitrate on the growth and survival of toad tadpoles (*Bufo bufo*) in the laboratory. Herpetol. J. 3: 147-148.

Camargo J.A., Alonso A., Salamanca A. (2005): Nitrate toxicity to aquatic animals: A review with new data for freshwater invertebrates. Chemosphere 58: 1255-1267.

LIFE13 NAT/SI/000314 [WWW Document]. http://life-kocevsko.eu

Johansson M., Räsänen K., Merilä J. (2001): Comparison of nitrate tolerance between different populations of the common frog, *Rana temporaria*. Aquat. Toxicol. 54(1-2): 1-14.

Laposata M.M., Dunson W.A. (1998): Effects of Boron and Nitrate on Hatching Success of Amphibian Eggs. Arch. Environ. Contam. Toxicol. 35: 615-619.

Marco A., Quilchano C., Blaustein A.R. (1999): Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. Environ. Toxicol. Chem. 18(12): 2836-2839.

Mezga K. (2014): Natural Hydrochemical Background and Dynamics of Groundwater in Slovenia: dissertation. University of Nova Gorica Graduate School, Nova Gorica.

Rouse J.D., Bishop C.A., Struger J. (1999): Nitrogen pollution: An assessment of its threat to amphibian survival. Environ. Health Persp. 107(10): 799-803.

Schuytema G.S., Nebeker A.V. (1999a): Comparative effects of ammonium and nitrate compounds on Pacific treefrog and African clawed frog embryos. Arch. Environ. Contam. Toxicol. 36: 200-206.

Schuytema G.S., Nebeker A.V. (1999b): Comparative toxicity of ammonium and nitrate compounds to Pacific treefrog and African clawed frog tadpoles. Bull. Environ. Contam. Toxicol. 18(10): 2251-2257.

Schuytema G.S., Nebeker A.V. (1999c): Effects of Ammonium Nitrate, Sodium Nitrate, and Urea on Red-Legged Frogs, Pacific Treefrogs, and African Clawed Frogs. Bull. Environ. Contam. Toxicol. 63(3): 357-364.

Sullivan K.B., Spence K. (2003): Effects of sublethal concentrations of atrazine and nitrate on metamorphosis of the african clawed frog. Environ. Toxicol. Chem. 22: 627-635.

The European Parliament and the Council of the European Union (2006): DIRECTIVE 2006/118/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 December 2006 on the protection of groundwater against pollution and deterioration. Official Journal of the European Communities L 372: 1-18.

van Vlaardingen P.L.A., Traas T.P., Wintersen A.M., Aldenberg T. (2014): ETX 2.1. Normal Distribution based Hazardous Concentration and Fraction Affected. National Institute of Public Health and environment, Bilthoven.

World Health Organization (2011): Nitrate and nitrite in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality 37(4): 227-231.

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Analysis of the skull of *Proteus anguinus anguinus* by high-resolution X-ray computed microtomography

Analiza lobanje *Proteus anguinus anguinus* z uporabo visokoločljivostne rentgenske računalniške mikrotomografije

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Proteus anguinus (Laurenti, 1768) is a cave amphibian of the order Caudata. Nearly all proteus' populations have very similar morphology with reduced pigmentation and vestigial eyes. This stygobiont amphibian lives in underground waters of the karst along the East Adriatic coast, from the Isonzo (Soča) river in the Northeast Italy to the Trebišnjica river in the Southeast Bosnia and Herzegovina (Sket 1997).

The skull morphology of proteus and its cartilaginous parts are interesting to study because they could be related to life in darkness. The first detailed description of the proteus skull was performed by Dolivo-Dobrovolsky (1926) and later by Sket & Arntzen (1994) through a comparative analysis of skeleton of both white and black proteus subspecies. A few years later, Ivanović et al. (2013) conducted a detailed analysis of the skull by using X-ray computed microtomography (micro-CT) technique. This approach allows to investigate the three-dimensional (3D) microstructure of a biological sample and to visualize regions with different density and/or chemical composition, using virtual slicing or volume rendering procedures.

In the Erwin Pichl Speleovivarium (Società Adriatica di Speleologia) of Trieste (Italy), operating under the supervision of the Natural History Museum of Trieste, the proteus individuals have been investigated applying micro-CT techniques. We aim to obtain high spatial and contrast resolution images, which allow high accuracy in measuring the anatomical details of the skull. Here we present results of the skull measurements of one adult proteus individual. The individual was collected in Postojnska jama (Slovenia) in 1989, and kept in in Speleovivarium laboratory until 1999 when preserved in alcohol. The first micro-CT measurements were performed in 2012 at the TomoLab station of Elettra Sincrotrone Trieste facility (situated in Basovizza near Trieste in Italy; https://www.elettra.trieste.it/). The TomoLab station was conceived as a micro-CT instrument complementary to the SYRMEP beamline (Tromba et al. 2010) and devoted to synchrotron-based micro-CT (Zandomeneghi et al. 2010). At TomoLab, it is possible to achieve a spatial resolution close to the minimum focal spot size (~5 µm) working in a voltage range of 40-130 kV and with a cone-beam geometry. Moreover, a micro-CT system based on a hard X-ray microfocus source shows limited (but clearly detectable) phase-contrast effects (Wilkins et al. 1996) also if with a limited potential with respect to a synchrotron X-ray micro-CT set up. This property could be particularly beneficial for the visualization and subsequent image segmentation of the soft tissues in biological samples.

The examined proteus had a total body length of 276 mm. The individual was placed in an empty plastic cylindrical tube sealed with Parafilm® for the micro-CT scan. The use of a non-destructive method allowed us to visualize bones, cartilages and soft tissues in their original position, without damaging the individual. Volume rendering (obtained with the commercial software VGStudio Max 2.0®, Volume Graphics) and morphological measurements were based on an X-ray microtomographic (micro-CT) scan. The micro-CT scan was performed with the following experimental parameters: Voltage: 65 kV, current: 123 µA, filter = 0.75 mm Al, 2400 projections recorded over a scan angle of 360°, exposure time/projection: 5.5 s, source-to-sample distance: 220 mm; source-to-detector distance: 320 mm, isotropic voxel size: 17.2 µm. A 3D image segmentation, based on a manual thresholding, was performed to visualize only the hard tissues of the animal.

The morphological analysis of the skull was performed using the same landmarks as in Ivanović et al. (2013) (Tab. 1, Fig. 1). Our results (Tab. 1) are in agreement with the range of values reported by Ivanović et al. (2013). We observed 6 teeth on the right premaxilla, with two missing teeth and 7 teeth on the left premaxilla and only one missing tooth. We counted 27 teeth on the right vomer and 26 teeth on the left one (with a probably missing tooth). In this individual, we did not detect teeth on palatopterigoide bones.

Table 1. The list of measurements done on proteus' skull, with distances measured between the landmarks as presented in Fig. 1.
Tabela 1. Seznam meritev, opravljenih na lobanji močerila, z razdaljami med merskimi točkami, ki so označene na Sl. 1.

Distance measured	**Landmarks, as shown in Fig. 1**	**Size (μm)**
Premaxilla (Pmax)	1 ↔ 2	937
	5 ↔ 6	3389
	3 ↔ 4	1311
Vomer (Vom)	7 ↔ 8	4759
Palato-pterigoid (Pal-pt)	9 ↔ 10	4295
	11 ↔ 12	6310
Quadrate (Quad)	13 ↔ 14	10349
	15 ↔ 16	10389
Cranium width	15 ↔ 16	10389
Parietal (Par)	17 ↔ 18	4667
Exoccipital (Exoc)	19 ↔ 20	6484
Cranium length	1,2 ↔ 21	22163
	1,2 ↔ 22	19550
	1,2 ↔ 19 or 20	25784

Using phase-contrast X-ray micro-CT, we were able to visualize not only the hard tissues composing the proteus' skeleton but also some of the well-preserved soft tissues (Fig. 2). This revealed that the skeleton and the gill arches were embedded in the muscles constituting a robust and elastic structure characterized by a rather ample cartilaginous joint. In the highlighted detail the joint between the dentary, quadrate and prearticular or gonial bones was visible; the front and posterior depressors of the mandible were inserted in the gonial bone, similarly to what was described for *Necturus maculosus* (Bauer 1997). The muscular structures and their functional organization, together with the shape of the skull, are probably related to predatory activities, the type of prey and other the ecological variables (Herrel et al. 2001).

Investigation techniques are continuously improving and offering new important opportunities for research. The morphological characterization of the proteus skull will allow a 3D reconstruction and modelling of parts of the cranial bones. Furthermore, in 2016, the analyses with synchrotron micro-CT were performed on various sexually mature proteus individuals from different localities at the SYRMEP beamline (Elettra Sincrotrone Trieste). The analyses were performed in collaboration with several national and international institutions, and present the work in progress.

References

Bauer W.J. (1997): A contribution to the morphology of visceral jaw-opening muscles of urodeles (Amphibia: Caudata) J. Morphol. 233: 77-97.

Dolivo-Dobrovolsky V. (1926): Lobanja čoveške ribice (*Proteus anguinus* Laurenti). Rad Jugoslavenske akademije znanosti i umjetnosti 232: 190-209.

Herrell A., Van Damme R., Vanhooydonck B., De Vree F. (2001): The implications of bite performance to diet in two species of lacertid lizards. Can. J. Zool. 79: 662-670.

Ivanović A., Aljančič G., Arntzen J.W. (2013): Skull shape differentiation of black and white olms (*Proteus anguinus anguinus* and *Proteus a. parkelj*): an exploratory analysis with micro-CT scanning. Contributions Zool. 82(2): 107-113.

Sket B. (1997). Distribution of *Proteus* (Amphibia: Urodela: Proteidae) and its possible explanation. J. Biogeogr. 24: 263-280.

Sket B., Arntzen J.W. (1994): A black, non-troglomorphic amphibian from the karst of Slovenia: *Proteus anguinus parkelj* n. s sp. (Urodela: Proteidae). Bijdr. Dierkd. 64: 33-53.

Tromba G., Longo R., Abrami A., Arfelli F., Astolfo A., Bregant P., Brun F., Casarin K., Chenda V., Dreossi D., Hola M., Kaiser J., Mancini L., Menk R.H., Quai E., Quaia E., Rigon L., Rokvic T., Sodini N., Sanabor D., Schultke E., Tonutti M., Vascotto A., Zanconati F., Cova M., Castelli E. (2010): The SYRMEP Beamline of Elettra: Clinical Mammography and Bio-medical Applications. In:

AIP publishing, AIP Conference Proceedings, 1266 (1): 18-23.

Wilkins S., Gureyev T.E., Gao D., Pogany A., Stevenson A. (1996): Phase-contrast imaging using polychromatic hard x-rays. Nature 384(6007): 335-338.

Zandomeneghi D., Voltolini M., Mancini L., Brun F., Dreossi D., Polacci M. (2010): Quantitative analysis of X-ray microtomography images of geomaterials: Application to volcanic rocks. Geosphere 6(6): 793-804.

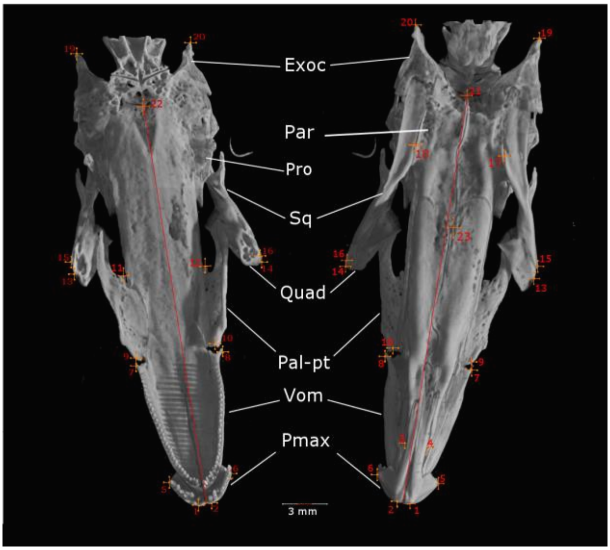

Figure 1. The images show the ventral (on the left) and dorsal (on the right) views of the upper bones of the skull of *Proteus anguinus anguinus*. The parameters of micro CT scans are given in the text. The distances, measured with landmarks 1 to 23, are explained in Tab. 1. Abbreviations on the photo relate to: Exoc – exoccipital, Par – parietal, Pro – prootic, Sq – squamosal, Quad – quadrate, Pal-pt – palato-pterigoid, Vom – vomer, Pmax – premaxilla.

Slika 1. Prikaz ventralnega (levo) in dorzalnega (desno) pogleda na zgornje kosti lobanje *Proteus anguinus anguinus*. Parametri mikro CT skenov so podani v tekstu. Razdalje, ki so bile merjene z oznakami od 1 do 23, so prikazane v Tab. 1. Oznake na fotografiji pomenijo: Exoc – eksokcipitalna kost, Par – parietalna kost, Pro – prootična kost, Sq – skvamozna kost, Quad – kvadratum, Pal-pt – palato-pterigoid, Vom – vomer, Pmax – premaksila.

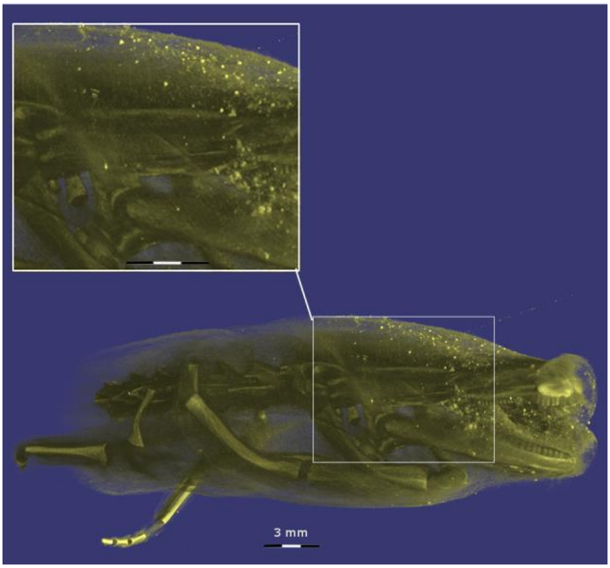

Figure 2. Volume rendering based on micro CT scans acquired with the parameters as given in the text. This picture shows both the hard and soft tissues; in the enlargement (on the left top) the joint between the dentary, quadrate and the prearticular (or gonial) can be observed. The selected level of transparency of the soft tissues allows seeing the hard tissues behind as well.

Slika 2. Volumska predstavitev, ki izhaja iz micro CT skenov s parametri, kot navedeno v tekstu. Ta slika prikazuje tako trda kot mehka tkiva; v povečavi (levo zgoraj) je prikazan sklep med dentarno, kvadratno in preartikularno (ali gonialno) kostjo. Nastavitev prozornosti prikaza mehkih tkiv omogoča tudi prikaz trdih tkiv zadaj.

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Development of eDNA methods for monitoring two stygobiotic species of the Dinaric Karst, *Proteus anguinus* and *Congeria jalzici*, using digital PCR

Razvoj metod eDNA za monitoring dveh stigobiontov Dinarskega krasa, človeške ribice *(Proteus anguinus)* in Jalžićeve jamske školjke (*Congeria jalzici*), z uporabo digitalne PCR

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The welfare of species and ecosystems are assessed by biological field surveys. Without knowing about the existence of particular species, we cannot protect them. Environmental DNA (eDNA) methods detect species at very low densities. When carefully validated and appropriately interpreted (see e.g. Cristescu & Herbert 2018), eDNA-based inventories improve biological surveys of ecosystems and are used to guide conservation efforts (e.g. Zaiko et al. 2018). Apart from establishing the presence of endangered or rare species, eDNA methods are also applied for monitoring the spread of alien invasive species and infectious agents such as fungal or bacterial spores, viruses and parasites. Analyses of total eDNA in a sample (i.e. environmental metagenome) are utilized to obtain information on the species community structure, the relative abundance of different trophic levels, and their interactions. However, a number of properties of eDNA molecules, such as differences in their release rates by different species during different life-history stages, their persistence under different environmental conditions and their variable detection rates using different techniques, are still insufficiently understood. Consequently, while eDNA methods are becoming wildly popular for establishing the presence of species in diverse environments, few studies to date have attempted to use eDNA quantification for determining population sizes and trends in these species (e.g. Doi et al. 2015a, 2017, Buxton et al. 2017, Chambert et al. 2018 and references therein).

Recent records of the olm *Proteus anguinus* outside its historically known range, discovered through detection of its DNA dissolved in groundwater, introduced the eDNA methodology to the study and conservation in the cryptic subterranean environment (Gorički et al. 2017). Since then, we have been able to detect the presence of *P. anguinus* in karst groundwater by analyzing samples of water collected on the surface – at springs or in wells. An upgraded technology, droplet digital PCR (ddPCR), which is reportedly more sensitive, accurate and resistant to PCR inhibitors than classical qPCR (Doi et al. 2015b, Taylor et al. 2017, Baker et al. 2018), is being tested for direct quantification of eDNA molecules in groundwater. We are using the large, accessible and relatively well-characterized natural *P. anguinus* population inhabiting the Planinska jama Cave (southwestern Slovenia) as a model. The size of this population was recently estimated using a genetic mark-recapture method, although only the number of animals actually caught was reported (Zakšek & Trontelj 2017). In succession of the named survey, we sampled water at several sites along the subterranean Pivka River and recorded representative stream profiles and flow velocities at or near the sampling sites (Fig. 1). Thereupon we estimated the number of eDNA molecules specific to *P. anguinus* in three 100 m long sections of the stream (B, F and I of Zakšek & Trontelj 2017). Based on the data reported by Zakšek & Trontelj (2017), we also estimated the number of eDNA molecules per individual animal in the selected stream sections.

Until now we have tested several sampling and filtration strategies and found that their success greatly depends on the amount of suspended particles in the water, which in turn reflects wet weather conditions during a yet undetermined period of time before sampling. On the other hand, during more stable, i.e. better hydrological conditions for the eDNA method, we were able to obtain comparable molecule counts in two consecutive years in all three river sections that were sampled twice. The relative number of eDNA copies per individual animal was the lowest in section F, where the highest number of animals had been observed, but where the slowest water flow was measured. This may indicate a higher rate of eDNA sedimentation (sinking), although we failed to detect more eDNA copies closer to the stream bottom than on the water surface. Alternatively, our result might reflect differences in eDNA release rates between animals from different sections. This explanation is even more plausible when we compare the other two sections with greater hydrologic similarity, yet we consistently detected more eDNA copies in section B than in I – both in absolute and relative terms – which apparently cannot be explained by transfer from the upstream sections alone. Our results indicate the need to investigate and characterize the Planinska jama Cave and its resident *P. anguinus* population in greater detail, as well as to measure the many variables in the eDNA model in a more controlled, experimental setting.

Figure 1. Stream profiling of the Pivka channel in the Planina Cave (photo: C. Mayaud).
Slika 1. Meritev pretoka na Pivškem rokavu Planinske jame (foto: C. Mayaud).

In another line of eDNA research, the utility of ddPCR is being explored for improved detection of the much smaller and rare stygobiont, the cave clam *Congeria jalzici*. The clam is known from only one site in Slovenia and three sites in Croatia (Hudoklin & Ilenič 2012, Bilandžija et al. 2013). The spring of the Krupa River in southeastern Slovenia (Fig. 2) is another presumed site of *C. jalzici*, although only shells, but no living animals were found in it (Sket 1971 in Hudoklin & Ilenič 2012). This spring is the site of one of the greatest ecological disasters in the Dinaric Karst. Between 1962 and 1984, dozens of tons of pure polychlorinated biphenyls (PCBs) were dumped at waste disposal sites and in nearby dolines by a local condenser factory. The undegradable carcinogenic chemicals are still gradually released from the sediment into the groundwater and were detected in the tissues of *P. anguinus* residing in the aquifer (Pezdirc et al. 2011). We detected eDNA of *C. jalzici* in the Krupa spring water and thereby confirmed the presence of a living population there. In the future, we will survey additional sites in the area potentially inhabited by the mollusk to assess the presence and vulnerability of any newly discovered populations, and to monitor the spread of the alien invasive zebra mussel *Dreissena polymorpha*, which may soon become a new major threat to the Slovenian populations of *C. jalzici*.

In conclusion, eDNA holds great potential for monitoring and conservation of fauna in inaccessible subterranean habitats. In the future, the eDNA methodology might be applied in the estimation of *P. anguinus* population sizes without having to see, mark or otherwise disturb the animals themselves. In parallel to eDNA assay development for various stygobiotic species of the Dinaric Karst, a groundwater-sample library is being created. This collection of samples will be available for future analysis of their species composition, once a reference sequence database for known Dinaric Karst species is created. It will serve as a source of information on any changes in species distribution over time, or for some species, as a record of their existence before they are lost forever.

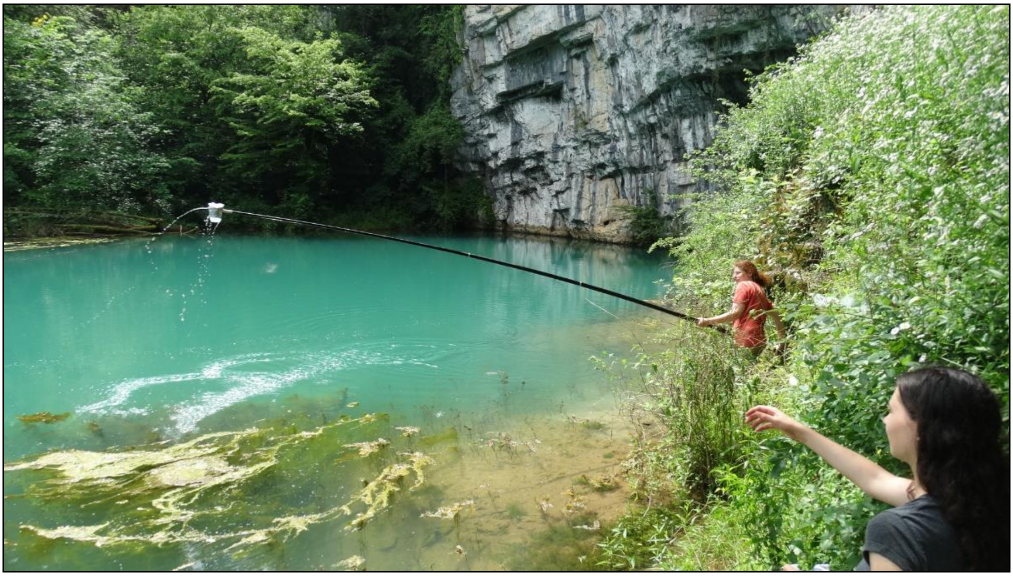

Figure 2. Water sampling at the Krupa spring (photo: P. Presetnik).
Slika 2. Vzorčenje vode na izviru Krupe (foto: P. Presetnik).

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References

Baker C.S., Steel D., Nieukirk S., Klinck H. (2018): Environmental DNA (eDNA) from the wake of the whales: droplet digital PCR for detection and species identification. Front. Mar. Sci. 5: 133.

Bilandžija H., Morton B., Podnar M., Ćetković H. (2013): Evolutionary history of relict *Congeria* (Bivalvia: Dreissenidae): unearthing the subterranean biodiversity of the Dinaric Karst. Front. Zool. 10: 5.

Buxton A.S., Groombridge J.J., Zakaria N.B., Griffiths R.A. (2017): Seasonal variation in environmental DNA in relation to population size and environmental factors. Sci. Rep. 7: 46294.

Chambert T., Pilliod, D.S., Goldberg C.S., Doi H., Takahara T. (2018): An analytical framework for estimating aquatic species density from environmental DNA. Ecol. Evol. 8: 3468-3477.

Cristescu M.E., Hebert P.D.N. (2018): Uses and misuses of environmental DNA in biodiversity science and conservation. Annu. Rev. Ecol. Evol. Syst. 49: 209-230.

Doi H., Uchii K., Takahara T., Matsuhashi S., Yamanaka H., Minamoto T. (2015a): Use of droplet digital PCR for estimation of fish abundance and biomass in environmental DNA surveys. PLoS ONE 10(3): e0122763.

Doi H., Takahara T., Minamoto T., Matsuhashi S., Uchii K., Yamanaka H. (2015b): Droplet digital polymerase chain reaction (PCR) outperforms real-time PCR in the detection of environmental DNA from an invasive fish species. Environ. Sci. Technol. 49(9): 5601-5608.

Doi H., Inui R., Akamatsu Y., Kanno K., Yamanaka H., Takahara T., Minamoto T. (2017): Environmental DNA analysis for estimating the abundance and biomass of stream fish. Freshw. Biol. 62: 30-39.

Gorički Š., Stanković D., Snoj A., Kuntner M., Jeffery W.R., Trontelj P., Pavićević M., Grizelj Z., Năpăruş-Aljančič M., Aljančič G. (2017): Environmental DNA in subterranean biology: range extension and taxonomic implications for *Proteus*. Sci. Rep. 7: 45054.

Hudoklin A., Ilenič, T. (2012): Izvir jamske školjke. Dolenjski kras 6: 139-142.

Pezdirc M., Heath E., Bizjak Mali L., Bulog B. (2011): PCB accumulation and tissue distribution in cave salamander (*Proteus anguinus anguinus*, Amphibia, Urodela) in the polluted karstic hinterland of the Krupa River, Slovenia. Chemosphere 84(7): 987-993.

Taylor S.C., Laperriere G., Germain H. (2017): Droplet digital PCR versus qPCR for gene expression analysis with low abundant targets: from variable nonsense to publication quality data. Sci. Rep. 7: 2409.

Zaiko A., Pochon X., Garcia-Vasquez E., Olenin S., Wood S.A. (2018): Advantages and limitations of environmental DNA/RNA tools for marine biosecurity: management and surveillance of non-indigenous species. Front. Mar. Sci. 5: 322.

Zakšek V., Trontelj P. (2017): Conservation genetics of proteus in the Postojna-Planina Cave System. Nat. Slo. 19(1): 33-34.

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Finds of washed-out proteus from the Pivka intermittent lakes and the Pivka river

Najdbe naplavljenih proteusov s Pivških presihajočih jezer in reke Pivke

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The surface of the Upper Pivka area (SW Slovenia) can be divided into levelled bottom along the Pivka river and higher rocky terrace along the Javorniki mountains. The basins of the Pivka intermittent lakes are deepened into the terrace (Mulec et al. 2005). The area is characterized by close connection between its underground and surface waters. In the karst aquifer, water flows mostly underground, but after periods of more intensive or long-lasting precipitation the water table rises (Petrič & Kogovšek 2005). The underground waters from the shallow karst aquifer of the Upper Pivka pour over the surface and fill the Pivka riverbed and several small karst basins that change into intermittent lakes. The Pivka intermittent lakes constitute a unique hydrological system of 17 intermittent lakes, of which nine occur more frequently, while eight occur less frequently (Kovačič & Habič 2005). The majority of them (11) are located in Landscape Park of the Pivka Intermittent Lakes (also a Natura 2000 site) (Fig. 1).

The Pivka intermittent lakes are a complex hydrological system with different occurrence and duration of lakes, as well as a variety of karst inflows and outflows. The size of the majority of lakes is small (only two lakes larger than 50 ha) and their karst inflows/outflows are also smaller. The more frequently formed intermittent lakes (except Radohovsko jezero) have karst inflows or outflows in the form of springs, estavelles, ponors or swallow holes.

During several years of field observation (from December 2006 to December 2011) of almost all Pivka intermittent lakes (except lakes in Krajnikov dol and Jeredovce) and four selected Pivka springs we explored their geomorphological characteristics and dynamics of lake formation in relation to hydrological conditions of the Pivka river and precipitation in the observation period (Kirn 2016).

According to Sket (1997), some localities of proteus are recorded in the Upper Pivka area, such as Zagorje (the Podlaznica stream and residual pools), Pivka (factories water supply), Palčje with Matijeva jama, Jerinov mlin (near Želje) and Prestranek (Pivka bed). The occurrence of proteus in Matijeva jama at the edge of Palško jezero and at the springs of the Pivka river (Kljunov ribnik) near Kalc Castle and the mill near Želje has been confirmed (Polak 2005). Specifically, proteus are found not just in Matijeva jama, but also in the main Pivka spring in the Pivšce and also in the upper part of the Pivka riverbed when high karst waters eject them (Mulec et al. 2005). According to the Biology Department of the Notranjska Museum Postojna (Polak 2018), additional localities of proteus occurrence in the area are known, such as Jama v Mlaki, Petelinjsko jezero, Želje spings, Tišlerjev mlin and Čadežev mlin (near Želje). Some other past finds of washed-out proteus have also been reported by the locals (e.g. Parsko jezero (ponor) and Klenska Pivka near Mišnik (riverbed close to ruins of the mill); Irena Uršič, pers. comm.), but they are poorly documented. Here and there, finds of washed-out proteus have been published in the media (e.g. Pivka riverbed near Zagorje; Primorske novice, 15. 5. 2008).

During our study of the area focused on hydrology of the Pivka intermittent lakes and the Pivka springs, ten washed-out proteus were also found from 2008 to 2011 and in 2014 (Tab. 1). All finds were reported to Gregor Aljančič (Tular Cave Laboratory). He came to see almost all found proteus after our notice. Researchers at the Tular Cave Laboratory have documented nearly thirty cases of washed-out proteus in Slovenia and Bosnia and Herzegovina since 2008. All animals were found by chance after being reported by the locals (Aljančič et al. 2016).

We recorded proteus in the basins of three intermittent lakes that are formed more frequently (Kljunov ribnik, Kalsko jezero and Petelinjsko jezero), the main Pivka spring and two additional springs (springs near and behind Kljunov ribnik) and one Pivka tributary (Klenska Pivka near Mišnik) (Fig. 1). Most of these localities of washed-out proteus are situated in the Natura 2000 site: SAC Javorniki – Snežnik (except the Pivka spring and Kalsko jezero), as well as in Landscape Park of the Pivka Intermittent Lakes (except Kalsko jezero). Kalsko jezero is a new locality compared to the previously known proteus localities.

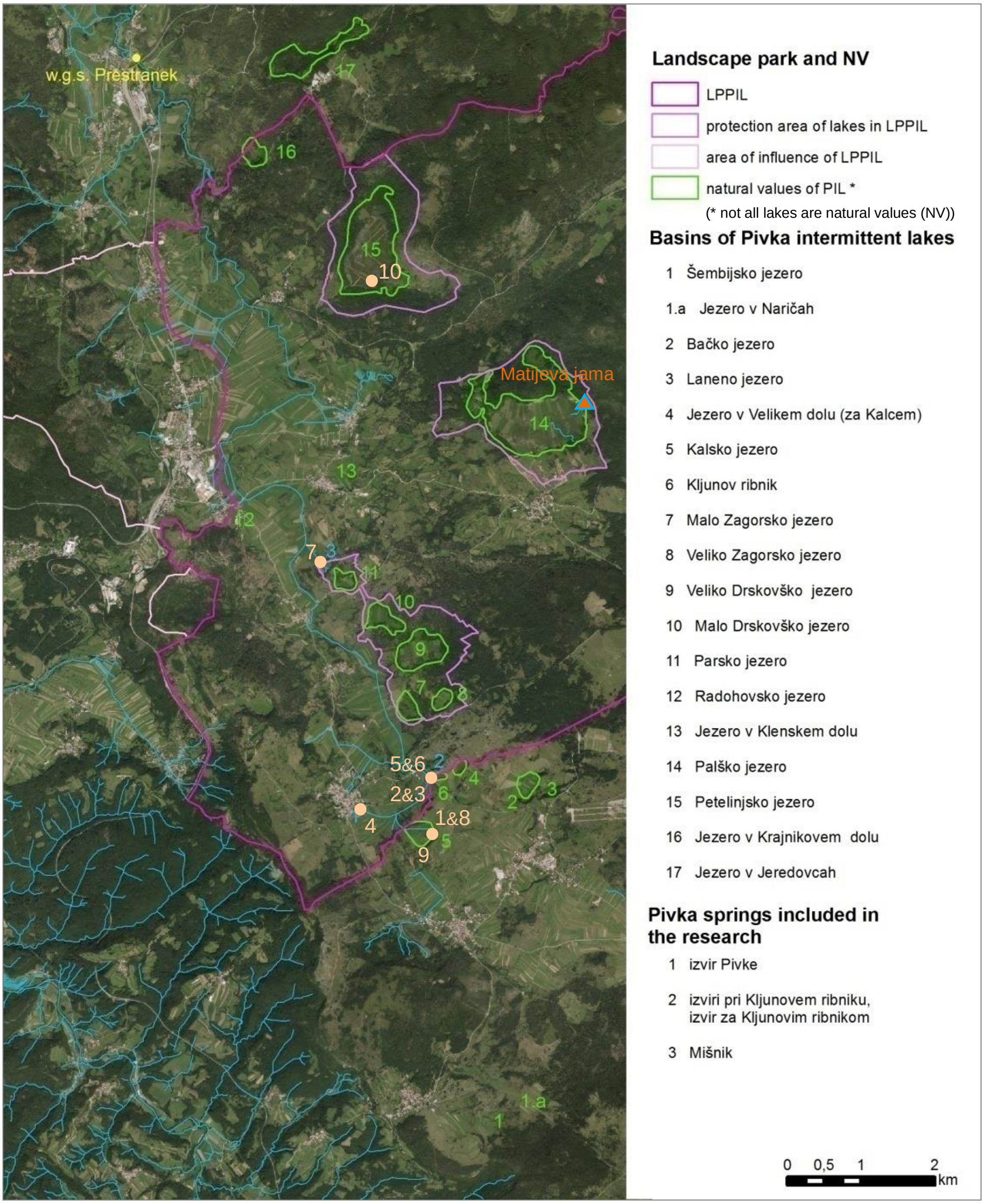

Figure 1. The area of the Pivka intermittent lakes and the Pivka river in Landscape Park of the Pivka Intermittent Lakes (LPPIL) and its surroundings with marked finds of washed-out proteus by localities (lake or Pivka spring/tributary). The finds are numerated as in Tab. 1. Matijeva jama as the release point for washed-out proteus is also presented. Data sources: GURS, ARSO.

Slika 1. Območje Pivških presihajočih jezer in reke Pivke v Krajinskem parku Pivška presihajoča jezera (KPPPJ) in njegovi okolici z označenimi najdbami naplavljenih proteusov po lokalitetah (jezero ali izvir/pritok Pivke). Najdbe so oštevilčene kot v Tab. 1. Prikazana je tudi Matijeva jama kot točka izpusta naplavljenih proteusov. Viri podatkov: GURS, ARSO.

Table 1. Finds of washed-out proteus from the Pivka intermittent lakes and the Pivka river. Coordinates in the Gauss-Krüger coordinate system (GKY and GKX) were read from digital orthophoto maps.
Tabela 1. Najdbe naplavljenih proteusov s Pivških presihajočih jezer in reke Pivke. Koordinate v Gauss-Krügerjevem koordinatnem sistemu (GKY in GKX) so bile odčitane z digitalnih ortofoto načrtov.

No.	Lake/ Pivka	Location	GKY, GKX	Date	State and salvaging of washed-out proteus
1	Kalsko jezero	spring at the northeastern edge of the current lake basin	441115, 54999	23.4.2008	The next day it was taken to the Tular Cave Laboratory where it died due to break of the spine (Aljančič 2009).
2	Kljunov ribnik	western edge part of the lake basin (in shallow water between the spring 4 and the springs near Kljunov ribnik)	441095, 55712	28.12.2008	The next day it was taken to the Tular Cave Laboratory where it died due to being exposed to frost on the back of the body in Kljunov ribnik (Aljančič 2009).
3	Kljunov ribnik	dried up ground along the large borehole (in the morning), while there was still a little water the day before (in the evening) after the water stopped flowing from the borehole on 9. 4. 2009	441119, 55712	11.4.2009	It was released into Matijeva jama on the same day as it was found just when the stream from the lake flowed into the shaft (Aljančič 2009).
4	izvir Pivke	rocks in front of the entrance to the shaft at the spring	440208, 55258	1.1.2010	It was kept one month in Vivarij in Postojnska jama, because Matijeva jama was flooded. It was released into its shaft when the water level was below the surface (Gregor Aljančič, pers. comm.).
5	izvir za Kljunovim ribnikom	dried up spring	441116, 55728	26.5.2010	Dead proteus (dried up)
6	izviri pri Kljunovem ribniku	edge of spring basin	441091, 55712	4.12.2010	It was released into Matijeva jama the next day when the water level was below the surface (in the shaft) (Gregor Aljančič, pers. comm.).
7	Klenska Pivka pri Mišniku	small basin at the bottom of the riverbed where the ditch enters the riverbed through the left embankment	439639, 58608	29.1.2011	It was left in the riverbed and was not noticed the next day.
8	Kalsko jezero	spring at the northeastern edge of the current lake basin	441115, 54999	22.2.2014	It was left at the spring (no photo is available) and was not noticed during the following visits of the lake.
9	Kalsko jezero	northeastern part of the dried up lake bottom	441075, 54977	22.3.2014	Dead proteus with a number of bruises along the body (Aljančič et al. 2015).
10	Petelinjsko jezero	estavelle 6 in the southeastern edge part of the lake basin when the water was only at the bottom of the estavelle	440339, 62461	11.5.2014	It was released into Matijeva jama the same day when the water level was below the surface (in the shaft) (Aljančič et al. 2015).

Figure 2. Photos of washed-out proteus from the area of the Pivka intermittent lakes and the Pivka river. They are numerated as in Tab. 1 (photo: Tina Kirn).
Slika 2. Fotografije naplavljenih proteusov z območja Pivških presihajočih jezer in reke Pivke. Najdbe so oštevilčene kot v Tab. 1 (foto: Tina Kirn).

We found one to three proteus per year (two in 2008, one in 2009, three in 2010, one in 2011, and three in 2014). The state of these animals is shown in Tab. 1 and Fig. 2 (including photos). Eight proteus were found alive, two of which later died due to injuries, one proteus was left in the riverbed and one in the spring, while four were rescued (Aljančič 2009, Aljančič et al. 2015, Aljančič, pers. comm.). Three of them were put into the bucket with water captured at the spring. They waited for the arrival of Gregor Aljančič, who then captured the last proteus by himself after our notice. He released these four proteus into Matijeva jama (an estavelle) in the basin of Palško jezero as the most appropriate release point for washed-out proteus since it is the longest water cave in the area of the Pivka intermittent lakes. The other two proteus were found already dead. Dead proteus are kept in the Study collection of proteus preparations, the Tular Cave Laboratory (Aljančič et al. 2015).

In the basin of Kalsko jezero three animals were found. The first proteus was found in the spring during regular larger lake formation in April 2008. The second proteus was found in the same spring in February 2014, when the extremely large lake formation was decreasing. We saw only a tail of proteus peeping out of the cracks and left it at the spring. After the end of this extremely large lake formation in March 2014 we noticed another proteus (already dead) on the dried up lake bottom.

Most proteus (four) were found in the basin of Kljunov ribnik and springs along it. Therefore, we assume that it is a locality where animals occur quite frequently. The first proteus was in shallow water in the marginal part of the lake basin during the very large lake formation in December 2008. The second proteus (being saved) was on dried up ground along the large borehole when the large lake formation was being discharged in April 2009.

The third find of proteus is from the spring behind Kljunov ribnik (izvir za Kljunovim ribnikom) in the bed of stream from Kljunov ribnik that flows into the Pivka river. Proteus was noticed after the end of regular larger lake formation of Kljunov ribnik in May 2010 when spring dried up. It was caught in the crack among rocks (already dead). The fourth find of proteus is from the springs near Kljunov ribnik (izviri pri Kljunovem ribniku) that flow into the Pivka river. It was during the very large lake formation of Kljunov ribnik in December 2010. The proteus (being saved) lay partially out of the crack at the edge of the spring basin.

One proteus (being saved) was found in the main Pivka spring (izvir Pivke) in January 2010 when the water level was about 30 cm. It was noticed among the rocks in front of the entrance to the shaft. One proteus was found in Klenska Pivka near the Mišnik spring at the end of the very large lake formation of Parsko jezero in January 2011. It was moving in quite deep water in small basin at the bottom of the riverbed. We wanted to capture (save) proteus on the next day, but was not to be seen anymore. The last (tenth) proteus was found in the basin of Petelinjsko jezero during the discharge of the extremely large lake formation in May 2014. It was noticed in almost dried up estavelle 6. The salvaging of this proteus was carried out in cooperation with the Ecomuseum of the Pivka intermittent lakes.

To conclude, the intermittent lakes are the groundwater dependent ecosystems and are therefore also interesting for observing subterranean animals like proteus. Dynamics of lake formation of the Pivka intermittent lakes in a particular year is primarily dependent on precipitation regime and the saturation of the underground (and soil) with water.

The research and monitoring of proteus is highly challenging due to the inaccessibility of its underground habitats. It is sensible to carry out monitoring of springs along the Pivka river and the Pivka intermittent lakes (Fučka et al. 2007). We believe that it is more likely to find proteus in small springs where the water discharge is not so high and proteus can resist the surface water flow after being washed out. With respect to monitoring of the washed-out proteus, special attention should therefore be given to small springs. Animals were found in winter or spring from regular larger to extremely large lake formation of intermittent lakes. We assume that finds of washed-out proteus are more likely when the extent of lake formation is greater since more animals were recorded during very large or extremely large lake formations.

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References

Aljančič G. (2009): Poročilo o najdbah človeške ribice. Jamski laboratorij Tular, Kranj, 4 pp.

Aljančič G., Aljančič M., Golob Z. (2015): Poročilo o reševanju izplavljenih človeških ribic v letu 2014. Zatočišče za človeške ribice (Jamski laboratorij Tular/Društvo za jamsko biologijo), Zatočišče za živali prosto živečih vrst (Golob d. o. o.), Kranj, 5 pp.

Aljančič G., Aljančič M., Golob Z. (2016): Salvaging the washed-out *Proteus*. Nat. Slo. 18(1): 65-67.

Fučka D., Polak S., Habič E., Matijašić D., Vrček D., Danev G. (2007): Podrobnejši načrt upravljanja za projektno območje Snežnik v sklopu akcije A3 projekta LIFE III – Narava: LIFE04NAT/SI/000240 z naslovom Natura 2000 v Sloveniji – Upravljavski modeli in informacijski sistem (NATURA 2000 in Slovenia – Management Models and Information System). Zavod Republike Slovenije za varstvo narave, Območna enota Nova Gorica, Nova Gorica, 195 pp.

Kirn T. (2016): Naravovarstvena izhodišča za varovanje Pivških presihajočih jezer. Magistrsko delo, Univerza v Ljubljani, Biotehniška fakulteta, Ljubljana, 280 pp.

Kovačič G., Habič Š. (2005): Kraška presihajoča jezera Pivke (JZ Slovenija) ob visokih vodah novembra 2000. Acta carsologica 34(3): 619-649.

Mulec J., Mihevc A., Pipan T. (2005): Presihajoča jezera na Pivškem. Acta carsologica 34(3): 543-565.

Petrič M., Kogovšek J. (2005): Hidrogeološke značilnosti območja presihajočih Pivških jezer. Acta carsologica 34(3): 599-618.

Polak S. (2005): Favna kopenskih habitatov Pivških jezer. Acta carsologica 34(3): 660-690.

Polak S. (2018): Izpis podatkov o pojavljanju človeške ribice na Pivškem iz baze podatkov Biološkega oddelka Notranjskega muzeja Postojna z dne 20. 12. 2018. Notranjski muzej Postojna, Postojna.

Sket B. (1997): Distribution of *Proteus* (Amphibia: Urodela: Proteidae) and its possible explanation. J Biogeogr. 24: 263-280.

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Comparative analysis of hematological parameters in wild and captive *Proteus anguinus*

Primerjalna analiza hematoloških parametrov pri močerilu iz narave in v ujetništvu

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Proteus anguinus lives in porous limestone subterranean habitat, which is susceptible to environmental pollution. According to this, and to its specific reproductive biology such as slow and low reproductive rate, longevity and consequently pollutants accumulation as well as its thin non-keratinized skin, proteus is extremely vulnerable to environmental stressors, such as pollution and pathogens, which are of global concern for amphibians. Therefore, assessing health and stress in both wild and captive individuals of proteus is an important and necessary issue in the efforts for protection and conservation of this vulnerable species. Hematological parameters obtained from blood smears, such as white blood cell (WBCs) counts, are an efficient, inexpensive and non-destructive method to assess the health and stress levels of vertebrates, including amphibians (Davis et al. 2008).

In Gredar & Bizjak Mali (2017), we presented the results of a detailed study of blood cell types and their morphology in proteus, which was conducted by Gredar (2016). This study enabled the first preliminary WBCs counts and neutrophils to lymphocytes ratios (N/L ratio) estimations, but the study was carried out on a small number (N=3) of captive animals. Recently, the immunological response of proteus infected with opportunistic black yeast *Exophiala salmonis* was recorded (Bizjak Mali et al. 2018). However, some progression on proteus WBC counts was made up to date (Prša 2018) with efforts to obtain more accurate baseline values of WBCs, especially in wild population of proteus as well as in animals held in captivity. The purpose of this report is to summarize the procedure for safe blood sampling in proteus and to complement previous results on WBC counts and N/L ratio.

Like in other amphibians, the most appropriate site for blood sampling is the heart ventricle. In proteus, the heart is visible through its non-pigmented skin, which makes blood sampling safer (Fig. 1). The blood vessels in the tail, a common site for blood taking in larger urodeles, are too small in proteus. Correct handling in all steps, from anesthesia to recovery, is crucial, including further optimal artificial conditions for rearing these animals in captivity. The common anaesthetic for aquatic vertebrates is tricaine methanesulfonate or MS-222, applied as aqueous solution (Ross & Ross 2008). Both an appropriate concentration of the anaesthetic and the length of anesthesia must be applied and we optimized these two parameters for proteus to be 0.03% and 10–15 min, the latter depending on the animal's weight. Also, the solution must be adjusted to pH 7 with sodium bicarbonate, otherwise it is too acidic and harmful to animal. Before blood sampling, the safe blood volume should be calculated (Heatley & Johnson 2009) and it can be doubled for healthy animals. The safe blood volume is especially important if larger volume of blood is needed, e.g. if blood is needed for several different purposes, such as blood smears, blood culturing or plasma biochemistry. During the procedure, moistening of the skin is necessary and immediately after blood sampling (this takes a few minutes) animals have to be returned to the UV-filtered, aerated and dechlorinated tap water with appropriate temperature for monitored recovering. The awakening from anesthesia is variable but usually takes from few minutes to half an hour. Blood sampling can be repeated in the same animal without any harmful effects on it. However, the recovery time between two consecutive samplings should be long enough. The maximum number of repeated blood samplings in the same animal in our study was 7 times over the course of 3 years. All proteus individuals used for blood sampling have survived. For blood sampling in amphibians, lithium heparin is the recommended anticoagulant because it has the lowest risk for causing artefacts and haemolysis (Wright 2001). Blood smears must be prepared quickly to minimize artefacts and to ensure data quality, and when the blood on the slide is dried, the smears have to be immediately fixed in methanol for 2–3 minutes followed by

Giemsa staining. For optimal differential staining of blood cells, the smears must be stained as soon as possible or at least on the next day.

Figure 1. Blood sampling in proteus (photo: Bizjak Mali L.).
Slika 1. Odvzem krvi močerilu (foto: Bizjak Mali L).

Blood cell counts were made for recently captured individuals of proteus (N=9, Planina Cave, SW Slovenia, body length from 210 to 280 mm), beginning with initial blood sampling within 2–12 days after capture and while they were in captivity over a period of up to 3 years. Counts of WBCs from the initial blood sampling showed extensive variation between animals in every blood cell type. Nevertheless, proteus has a typical urodele pattern of WBCs with the majority of WBCs to be lymphocytes (73.0% ± 12.0) followed by neutrophils (15.9% ± 8.9), monocytes (7.7% ± 4.8) and eosinophils (3.2% ± 2.8), with the exception of basophils that were not found. These WBCs values are similar to the results of previous preliminary research on proteus blood cells from a smaller numerus of animals (Gredar 2016). In all captured animals, the neutrophils to lymphocytes ratio (N/L ratio) was quite variable (between 0.01 and 0.43) but below 1.0 and indicates that animals were not stressed (N/L ratio near 1 is an indicator for stress in amphibians (Davis & Maerz 2008)). In addition, N/L ratios of captured proteuses were within the reference range (between 0.01 and 0.6) that had been reported for other amphibian species (Davis 2009). Surprisingly, in five of the nine individuals amoebas were observed in blood during the initial and subsequent blood sampling (Fig. 2), but this was not reflected in their WBC counts and N/L ratio. We found that WBC counts did not change significantly in animals that had been kept in captivity for varying lengths of time. Although their N/L ratios (ranging from 0.02 to 0.86) generally appear to increase with time in captivity, these differences were not statistically significant ($p = 0.63$). An extremely high N/L ratio (7.8) was found only in one of the nine captive individuals after six months of captivity. However, this animal did not show any obvious symptoms of disease except amoebas in the blood, and is in fact still in good condition at the time of writing this report.

In conclusion, our results showed no statistically significant effect of long-term captivity on the physiological condition of proteuses as revealed by hematological parameters evaluated. The presence of amoebas in proteuses blood is remarkable and further studies are required to clarify the phenomenon of a weak immune response of proteuses to protozoan parasites in their blood.

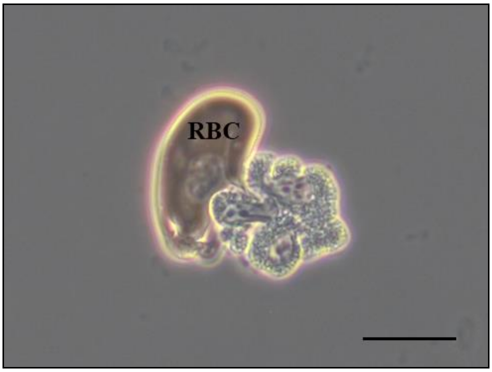

Figure 2. Amoeba in the blood of proteus. RBC – red blood cell, phase contrast, scale bar: 25 µm (photo: P. Prša).
Slika 2. Ameba v krvi močerila. RBC – rdeča krvnička, fazni kontrast, merilo: 25 µm (foto: P. Prša).

All the animals were collected with the approval of the Slovenian Ministry of the Environment and Spatial Planning, permit no. 35601-8/2016-4, and are kept in the Speleobiology Laboratory at the Chair of Zoology, Department of Biology, Biotechnical Faculty, University of Ljubljana, in accordance with the Slovenian animal protection act (Ur.l. RS 37/13).

References

Bizjak Mali L., Zalar P., Turk M., Novak Babič M., Kostanjšek R., Gunde-Cimerman N. (2018): Opportunistic fungal pathogens isolated from a captive individual of the European blind cave salamander *Proteus anguinus*. Dis. Aquat. Org. 129: 15-38.

Davis A.K., Maerz J.C. (2008): Comparison of hematological stress indicators in recently captured and captive paedomorphic mole salamanders, *Ambystoma talpoideum*. Copeia 3: 613-617.

Davis A.K., Maney D.L., Maerz J.C. (2008): The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol. 22: 760-772.

Davis A.K. (2009): The wildlife leukocytes webpage: The ecologist's source for information about leukocytes of wildlife species. http://wildlifehematology.uga.edu [accessed in October 2018]

Gredar T. (2016): Blood cell cultivation of neotenic amphibians and its optimization for cytogenetic analyses. MSc Thesis. University of Ljubljana, Biotechnical faculty, Department of biology, 84 pp.

Gredar T., Bizjak Mali L. (2017): Cultivation and morphology of blood cells of the olm *Proteus anguinus*. Nat. Slo. 19(1): 29-30.

Heatley J.J., Johnson M. (2009): Clinical technique: Amphibian hematology: A practitioner's guide. J. Exot. Pet Med. 18(1): 14-19.

Prša P. (2018): Analysis of the blood count and blood cell proliferation in culture of proteus. MSc Thesis. University of Ljubljana, Biotechnical faculty, Department of biology, 73 pp.

Ross L.G., Ross B. (2008): Anaesthetic and sedative techniques for aquatic animals. 3rd ed. Oxford, Blackwell publishing, 222 pp.

Wright K.M. (2001): Amphibian hematology. In: Wright K.M., Whitaker B.R. (Eds.), Amphibian medicine and captive husbandry. Krieger publishing company, pp. 129-146.

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Identification of cave pollution in the Kras Plateau, Slovenia

Prepoznavanje onesnaženosti jam na planoti Kras, Slovenija

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The biggest issue facing European hydrogeologists is the need to protect the quality and quantity of groundwater resources (Zwahlen 2003). Cave pollution (the term is here used as: caves that are filled with waste) is among the drivers contributing to the pollution and degradation of karst aquifers. Nonetheless, the extent of the problem is neither well described nor systematically monitored at the national level (Prelovšek 2011a, 2011b). In Europe, karst covers around 1.4 million km^2 or 13.8% of the land surface (Chen et al. 2017), and provides an important share of drinking water, e.g. in Austria the share is more than 50%, in Croatia more than 35% and in Belgium more than 30% (COST 1995). In Slovenia, karst landscapes are recognizable and important features at the national level (Habič 1992, Mihevc 1999, Gams 2004, Zupan Hajna 2004, Ribeiro 2017). These landscapes cover approximately 8,800 km^2 or 44% of the country's surface, while the karst springs provide about 43% of drinking water (Lah 1998).

In the past, several attempts were undertaken to evaluate the extent of cave pollution in Slovenia (Prelovšek 2011b). They took place in the Municipality of Novo mesto (Hudoklin 2002), the catchment of the Krka River Spring (Čekada 2011), the karst areas around Celje Plain (Hribernik et al. 2010) and in the Kras Plateau (Prelovšek 2013). In some areas, the pollution can be present in up to 46% of the investigated caves, whereas the estimation of cave pollution for Slovenia is around 20% of the total number of caves (Čekada 2015). Recently, an overview of cave pollution has been made for Bela krajina (Ribeiro & Tičar 2017), where cave pollution affected around 19% of the investigated caves and presents a potential threat to the black olm (*Proteus anguinus parkelj*) (Sket et al. 2003) as well as other subterranean water organisms. At the European level, different remediation projects have been established by speleological associations, caving clubs, municipalities or research institutions, such as the EU project Life Kočevsko in Slovenia (Prelovšek 2015), the project »Clean underground« in Croatia (Novak & Tutiš 2017) and the project »Cleaning up the darkness – Puliamo il Buio« in Italy (Didonna et al. 2018). In order to systematize and prioritize the remediation of karst underground in Slovenia, the evaluation of cave pollution at the national level is essential.

The main objectives of the present study are the following: 1) to identify the level of cave pollution in the Kras Plateau, 2) to compare the results of this study with results of Prelovšek (2013) done in the same geographical area, and 3) to compare the results to the study done in the karst region of Bela krajina (Ribeiro & Tičar 2017).

The Kras Plateau stretches between the Gulf of Trieste, Soča Plain, Vipava Valley and Brkini Hills (Gams 2004) and covers in Slovenia an area of 429 km^2 (Perko 1998). It consists of the Cretaceous and Paleogene Carbonates and has a distinguished flat surface at an elevation of around 300 m a.s.l. with numerous dolines, collapse dolines and caves (Gams 2004). Due to its highly karstified surface, the surface watercourses are absent and the precipitation percolates directly through the karst vadose zone into the underground aquifer. In addition, the underground aquifer is fed by waters of the Reka River which sink in the caves Škocjanske jame. The inlet from the Soča and Vipava Rivers eventually outflows from the Kras Plateau at the springs of the Timavo (Italian name for the Reka River) at Devin, Gulf of Trieste (Doctor 2008). In the first half of the 19th century, exploration of caves in the Kras Plateau gained special attention due to the exploration of caves for water supply of the city of Trieste (Mihevc et al. 2016).

According to the national Cave Registry, 1,077 karst caves have been registered in the Kras Plateau by the end of 2017 (Cave Registry 2018). The cave density is around 2.5 caves/km^2, and the area is considered as one of the hot-spots regarding karst caves concentration in Slovenia (Mihevc et al. 2016, Tičar et al. 2018). Although caves are distributed all over the Kras Plateau, there is a high density of caves between

Škocjanske jame and Sežana and south from Kostanjevica na Krasu. In total, more than 78 km of cave passages have been discovered so far. The average length of the caves is around 73 m and the average depth is 26 m. There are six caves with more than 1 km of passages (the longest being Kačna jama with 15.2 km), and eight caves with 250 m or more vertical drop (the deepest being Jama Sežanske reke with 394 m (Cave Registry 2018). All the deepest caves in the area reach the underground flow of the Reka River.

Data for this study was obtained from the Cave Registry (2018), while data from Prelovšek (2013) were also obtained from field verifications. We gathered the following data: state of the cave, type of waste encountered inside the cave, usage of the cave, as well as other metric data regarding the morphology of the cave. Since 15 registered caves in the Kras Plateau have no information on cave pollution, only 1,062 caves were included in our analyses.

The results show that 817 caves out of 1,062 are without detectable (not seen with the naked eye) pollution (representing 77%) and 245 caves are polluted (representing 23%). Regarding the amount of waste (measured in m^3), 98 caves are considered to be and are labelled as low polluted (0.1–0.9 m^3), 81 are medium polluted (1.0–4.9 m^3) and 66 are highly polluted (more than 5.0 m^3). Considering the physical state of the caves, 198 (19 %) have been damaged. Different types of physical damage can be present in the same cave and refer to artificial widening of passages (135 caves), broken speleothems (26 caves), paintings on walls or on speleothems (26 caves), repositioning of sediments (47 caves), etc.

In most cases, the waste structure was hard to identify from the observation of cave registers. We determined that the greatest part of the waste belongs to the category of organic waste (65%), followed by communal waste (67%) and finally construction waste (25%). It's important to note that 51 polluted caves (21% of polluted caves) contain dangerous waste (e.g. pesticides, dumped motor oils), from which 37 caves (15% of polluted caves) contain explosive remains from WW I or II. Besides, 9 caves are polluted due to a leakage of polluted wastewater and 14 caves contain human remains.

Considering the use of caves, their past uses were quite diverse. 250 caves out of 1,062 were used as landfill sites, 127 as military shelters, 114 as important caves for research purposes, 14 as burial sites, 7 for the acquisition of raw materials, etc.

As part of this study, we also related the size and type of cave entrance to the state of the cave (clean or polluted). The average size of the cave entrance is 21.7 m^2. Results show that clean caves tend to have smaller entrances (18.4 m^2) than polluted caves (32.5 m^2), however, this difference is not statistically significant. The size of the entrances for the low polluted caves is 9.3 m^2, for medium polluted caves 32.5 m^2, and for highly polluted caves 56.3 m^2.

In order to compare the results from this study to the results from Prelovšek (2013), we used 99 caves (the same caves in both studies) and categorized them according to clean, low polluted, medium polluted and high polluted caves (see Tab. 1).

Table 1. Comparison of results on cave pollution in 99 caves from this study and Prelovšek (2013).
Tabela 1. Primerjava rezultatov raziskave z rezultati Prelovšek (2013).

Variable	Category	Prelovšek (2013)	This study
State of the caves [number of caves]	Clean	64	64
	Polluted	25	32
	Destroyed	5	0
	No data	5	3
Level of pollution [number of caves]	Low polluted	7	10
	Medium polluted	7	10
	High polluted	9	12
	Unspecified level of pollution	2	0
Amount of waste in polluted caves [m^3]	Cumulative amount of waste	386.4	727.6
	Average amount of waste per polluted cave	15.4	22.7

The results from the comparison between cave pollution in the Kras Plateau and in Bela krajina (Ribeiro & Tičar 2017) can be seen in Tab. 2.

Table 2. Comparison between cave pollution in the Kras Plateau and in Bela krajina (Ribeiro & Tičar 2017).
Tabela 2. Primerjava rezultatov raziskave onesnaženosti jam na Krasu in v Beli krajini (Ribeiro & Tičar 2017).

Variable	Category	Ribeiro & Tičar (2017)	This study
State of the caves [% of caves]	Clean	81	77
	Polluted	18	23
	Destroyed	1	0
Level of pollution [% of caves]	Low polluted	47	40
	Medium polluted	16	33
	High polluted	37	27
Amount of waste in polluted caves [m^3]	Cumulative amount of waste	974.2	2,385.4
	Average amount of waste per polluted cave	8.3	9.7

To conclude, this study shows the extent of cave pollution in the Kras Plateau and exposes different factors affecting the caves in the area. Due to the past military activities in the study area, explosive remnants of war can be detected in karst caves. Comparison between our results and Prelovšek (2013) showed similar outcomes. The biggest difference between both studies regards the amount of waste within caves. Since our data were based on Cave Registry (2018) without further field observations, the results suggest an overestimation of the amount of waste in caves comparing to Prelovšek (2013). This finding points out the importance of field observations in addition to the data acquired from the archives. The comparison among regions (Kras Plateau and Bela krajina) showed that the share of polluted caves and amount of waste are higher in the Kras Plateau than in Bela krajina (Tab. 2).

Here we highlight one aspect of cave pollution, directly connected with waste disposal. However, there are other critical drivers of groundwater pollution in karst areas, such as wastewater treatment, population density, land use, and transport infrastructure that were not the scope of this study.

References

Cave Registry (2018): Registry of Slovenian Caves. Karst Research Institute ZRC SAZU, Postojna.

Chen Z., Auler A.-S., Bakalowicz M., Drew D., Griger F., Hartmann J., Jiang G., Moosdorf N., Richts A., Stevanović Z, Veni G., Goldscheider N. (2017): The World karst aquifer mapping project: concept, mapping procedure and map of Europe. Hydrogeol. J. 25: 771-785.

COST (1995): Hydrogeological aspects of groundwater protection in karstic areas: Final report. European Commission, Brussels, 446 pp. https://cordis.europa.eu/publication/rcn/199511660_es.html

Doctor D.-H. (2008): Hydrologic connections and dynamics of water movement in the Classical Karst (Kras) aquifer: evidence from frequent chemical and stable isotope sampling. Acta Carsol. 37(1): 101-123.

Čekada M. (2011): Terenski pregled jam v hidrogeološkem zaledju izvira Krke. Jamar 4(2): 32-34.

Čekada M. (2015): Kraljestvo smeti – več kot 2000 onesnaženih jam v Sloveniji. Jamar 7: 53.

Didonna F., Maurano F., Pani D. (2018): Cleaning up the darkness – »Puliamo il buio (PiB)«, over a decade of cave depollution activities in Italy 2005-2017. In: Mattes J., Plan L., Christian E. (Eds.), Proceedings of the 12th EuroSpeleo Forum, Verein für Höhlenkunde Ebensee, Ebensee, p. 36.

Gams I. (2004): Kras v Sloveniji v prostoru in času, 2. edition. Založba ZRC, Ljubljana, 515 pp.

Habič P. (1992): Kras and karst in Slovenia. In: Černe A. (Ed.), Slovenia, geographic aspects of a new independent European nation, The Association of the Geographical Societies of Slovenia, Ljubljana, pp. 31-39.

Hribernik M., Bračič R., Čekada M., Novak T., Ravljen J. (2010): Varstvo kraških jam in virov pitne vode: Velenjsko in Konjiško hribovje, Dobroveljska planota, Ložniško in Hudinjsko gričevje ter Savinjska ravan. Koroško-šaleški jamarski klub Speleos – Siga, Velenje, 65 pp.

Hudoklin A. (2002): Onesnaženost jam v Mestni občini Novo mesto. In: Hudoklin A. (Ed.), Dolenjski kras 4, Jamarski klub Novo mesto, Novo mesto, pp. 69-76.

Lah A. (1998): Voda – vodovje: poglavitni življenjski vir narave in gospodarstva. Svet za varstvo okolja Republike Slovenije, Ljubljana, 63 pp.

Mihevc A. (1999): Morfologija krasa. In: Čuliberg M., Kranjc A. (Eds.), Kras: pokrajina, življenje, ljudje, Založba ZRC, Ljubljana, pp. 41-47.

Mihevc A., Gabrovšek F., Knez M., Kozel P., Mulec J., Otoničar B., Petrič M., Pipan T., Prelovšek M., Slabe T., Šebela S., Zupan Hajna N. (2016): Karst in Slovenia. Boletín Geológico y Minero 127(1): 79-97.

Novak R., Tutiš S. (2017): »Čisto podzemlje«. In: Lučić N., Janjanin Ž., Paar D., Gregov A. (Eds.), Zbornik sažetaka – Skup speleologa Hrvatske, Čilipi 2017, Hrvatsko planinarsko društvo Snježnica, Dubrovnik, p. 47.

Perko D. (1998): The regionalization of Slovenia. Acta Geogr. Slov. 38: 11-57.

Prelovšek M. (2011a): Vulnerability, pressures and protection of karst caves. In: Prelovšek M., Zupan Hajna N. (Eds.), Pressures and protection of the underground karst – cases from Slovenia and Croatia, Karst Research Institute ZRC SAZU, Postojna, pp. 11-17.

Prelovšek M. (2011b): Pollution and cleanup of karst caves in Slovenia. In: Prelovšek M., Zupan Hajna N. (Eds.), Pressures and protection of the underground karst – cases from Slovenia and Croatia, Karst Research Institute ZRC SAZU, Postojna, pp. 101-111.

Prelovšek M. (2013): Projekt 99, popis onesnaženosti jam na Krasu (poročilo). ZRC SAZU in Jamarska zveza Slovenije, Ljubljana, 16 pp.

Prelovšek M. (2015): Zaključno poročilo o popisu onesnaženosti 90 jam na Kočevskem (poročilo). Inštitut za raziskovanje krasa ZRC SAZU in Jamarski klub Novo mesto, Postojna, 20 pp.

Ribeiro D. (2017): Impact of landscape features on land use and regional development in karst areas: a case study of Bela krajina. Doctoral thesis, University of Ljubljana, Ljubljana, 281 pp.

Ribeiro D., Tičar J. (2017): The problematics of cave pollution in Bela krajina. Nat. Slo. 19(1): 43-45.

Sket B., Gogala M., Kuštor V. (2003): Živalstvo Slovenije. Tehniška založba Slovenije, Ljubljana, 664 pp.

Tičar J., Perko D., Volk Bahun M. (2018): Geodediščina in pokrajinska raznolikost Slovenije. In: Ciglič R., Geršič M., Perko D., Zorn M. (Eds.), Pokrajina v visoki ločljivosti, Založba ZRC, Ljubljana, pp. 57-74.

Zupan Hajna N. (2004): Karst in Slovenia. In: Orožen Adamič M. (Ed.), Slovenia: a geographical overview, Založba ZRC, Ljubljana, pp. 39-44.

Zwahlen F. (2003): Vulnerability and risk mapping for the protection of carbonate (karst) aquifers, scope – goals – results. European Commission, COST action 620, Directorate-General Science, Research and Development, Luxembourg, 39 pp. http://www.bgr.bund.de/EN/Themen/Wasser/Projekte/abgeschlossen/F+E/Cost620/cost620_fb_02_pdf.pdf?__blob=publicationFile&v=1

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A short history of »Kras«

Kratka zgodovina »Krasa«

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To clearly distinguish between »Kras« and »karst«, it would be useful to repeat what is essential for the karst in general. To develop a special type of surface and/or underground relief, the following four conditions must usually be fulfilled:

- the bedrock has to be of a soluble rock (carbonate, most frequently limestone);
- rock must be fissured by joint fissures (enabling the water to enter the fissure at one end and leave it at the other end);
- the prevailing process is corrosion (solution of carbonate rock by water);
- the special type of karst (underground) hydrology must exist;

When these conditions are fulfilled – adequate surface and underground features – karst relief develops. Good example is the subterranean connection of the Reka River which sinks into Škocjanske Jame and springs as the Timavo River on the Adriatic coast.

On a global scale, karst covers 15–25% of the Earth's surface (Salomon 2006). On the continental scale, despite the existence of numerous karst terrains, the Dinaric Karst, which stretches along the Eastern Adriatic coast, holds an important position with its 800 km long, 150 km wide and approximately 60,000 km^2 large surface area (Roglić 1965). On its NW tip, the Kras plateau is located. More than by its size, the Dinaric Karst is important for its outstanding karst features, while the Kras plateau is especially important for its history.

Springs of the Timavo River are mentioned in Pseudo-Skylax's Periplous (4th century BC) as an important source of drinking water. They are also referred to in Virgil's Eneide, and Poseidonios of Apamea is the first to mention the underground connection between the Upper Timavo River (the Reka River) and the springs of Timavo (Pfeiffer 1963). One of the first recorded tracing tests was performed by Father Pietro Imperato, living near the Timavo springs, by putting floats into the Reka River in front of Škocjanske jame and trying to collect them in the river's resurgence. This test was erroneously attributed to the well-known Naple's naturalist and mineralogist Ferrante Imperato or his son Francesco (Shaw 1992), but was in fact performed by Pietro Imperato (Tavagnutti 2013). During the 17th and 18th centuries important books and reports, containing descriptions of Kras and Carniola's karst, appeared, their authors being: Valvasor (1689), Nagel (1748), Steinberg (1758), Hacquet (1778), and Gruber (1781) (Fig. 1). Nagel (1748) estimated the age of one of the columns in Vilenica cave, which most probably presents the oldest speleodatation. During the 19th century the most important caves, specifically Labodnica (Abisso Trebiciano in Italian) and Škocjanske jame were explored. At the end of the century, two internationally renowned karstologists F. Kraus (1894) and E.A. Martel (1894) published their works which included fruitful discussions on the topic of Kras (Gams 2004).

Figure 1. On his map of Carniola, B. Hacquet used, inter alia, Slovene names »Na Krassi« (On the Karst) (Hacquet 1778).

Slika 1. B. Hacquet je na svoji karti Kranjske uporabljal slovenska imena, med drugim tudi »Na Krassi« (Na Krasu) (Hacquet 1778).

And how the name of the relatively unknown and unimportant Kras plateau became the international term for limestone terrains and their phenomena and features? In the 2nd century BC, Kras belonged to the kingdom of Histrians, and then fell into Roman hands. The Romans Latinized the name into Carsus in which the original root *kar- (*gar-), meaning a stone, was retained (Snoj 2009). From the accusative form of the Latin name Carsum three actual names derived: Carso (as the so-called »inherited« name) in Italian, Karst in German taken from the Italian name, and Kras in Slovene. According to the Slavic language, the form *Kars(u) changed via liquid metathesis into Kras during the 9th century at the latest (Snoj 2009). Since the only practicable road connecting Central European and Habsburg's lands with the Mediterranean – i.e. with the port of Trieste – passed the karst land between Vrhnika and Trieste and crossed the Kras, several travellers were writing about an unusual karst landscape (Kranjc 1998). As they predominantly wrote in German, they used the German form of the name – Karst. So anywhere else scholars began to compare limestone landscape with that of Kras. Finally, F.H. Hohenwart (Fig. 2) wrote that karst is not only the plateau of Kras, but that it stretches from the surroundings of Udine (Friaul) to the Greek island of Cephalonia (Hohenwart 1830). Thus the toponym Kras became the general term for karst.

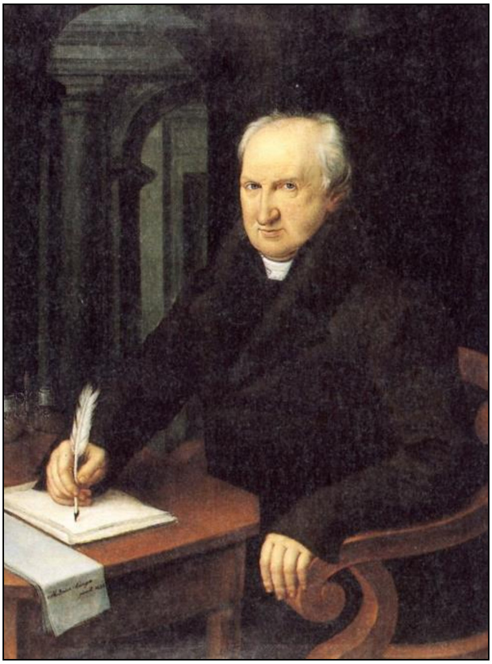

Figure 2. F.H. Hohenwart is the first who clearly asserted that the phenomenon of Karst lies not just on the Kras (Karst) plateau (Hohenwart 1830). The author of the portrait from 1835 is Matevž Langus; the original is held in the National Museum of Slovenia.
Slika 2. F.H. Hohenwart je bil prvi, ki je jasno zapisal, da kraški pojavi niso le na planoti Kras (Hohenwart 1830). Avtor portreta iz 1835 je Matevž Langus; original je v Narodnem muzeju v Ljubljani.

References

Gams I. (2004): Kras v Sloveniji v prostoru in času. Založba ZRC, Ljubljana, 515 pp.

Gruber T. (1781): Briefe hydrographischen und physikalischen Inhalts aus Krain. J.P. Krauss, Wien, 159 pp.

Hacquet B. (1778): Oryctographia Carniolica oder Physikalische Erdbeschreibung des Herzogthums Krain, Istrien, und zum Theil der benachbarten Länder. Erster Theil. G.I. Breitkopf, Leipzig, VII-XVI, pp. 1-162.

Hohenwart F.H. (1830): Wegweiser für die Wanderer in der berühmten Adelsberger und Kronprinz Ferdinands-Grotte bey Adelsberg in Krain. Ignaz Aloys Edlen u. Kleinmayr, Laibach, 16 pp.

Kranjc A. (1998): Kras (The Classical Karst) and the development of karst science. Acta carsologica 27: 151-164.

Kraus F. (1894): Höhlenkunde. Carl Gerold's Sohn, Wien, 308 pp.

Martel E.A. (1894): Les abîmes, les eaux souterraines, les cavernes, les sources. Charles Delagrave, Paris, 580 pp.

Nagel J.A. (1748): Beschreibung deren auf allerhöchsten Befehl Ihro Röm. kaiserlich königlichen Maytt. Francisci I untersuchten, in dem Herzogthume Crain befindlichen Seltenheiten der Natur. Nationalbibliothek, Handschrift Nr. 7854, Wien, ff. 91.

Pfeiffer D. (1963): Die geschichtliche Entwicklung der Anchaunngen über das Karstgrundwasser. Beih. geol. Jb. 57: 1-111.

Roglić J. 1965): The delimitations and morphological types of the Dinaric Karst. Naše jame VII(1-2): 12-20.

Salomon J.-N. (2006): Précis de Karstologie. Presses Universitaires de Bordeaux, Bordeaux, 288 pp.

Shaw T.R. (1992): History of cave science. Sydney Speleological Society, Broadway (NSW, Australia), 338 pp.

Snoj M. (2009): Etimološki slovar slovenskih zemljepisnih imen. Modrijan, Založba ZRC, Ljubljana, 603 pp.

Steinberg F.A. (1758): Gründliche Nachricht von dem in dem Inner-Crain gelegenen Czirchnitzer-See (Reproducirani ponatis izdaje iz 1758), Cankarjeva založba, Ljubljana, 265 pp.

Tavagnutti M. (2013): Giovanni Fortunato Bianchini e le prime ricerche sul Timavo sotterraneo nell'antica Contea di Gorizia. Atti del XXI Congresso Nazionale di Speleologia, EUT Edizioni Università di Trieste, Trieste, pp. 497-505.

Valvasor J.W. (1689): Die Ehre dess Hertzogthums Crain. I. Theil, Wolfgang Moritz Endter, Laybach, 696 pp.

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Capacity building for conservation of the subterranean biodiversity of the Skadar/Shkodra Lake basin (Montenegro and Albania)

Krepitev zmogljivosti za varstvo podzemne biotske raznovrstnosti v bazenu Skadarskega jezera (Črna gora in Albanija)

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Here we briefly describe the capacity building part of the project »Assessment of the endangered subterranean biodiversity of the Skadar/Shkodra Lake Basin (Montenegro and Albania)« conducted in 2016 with the support of the Critical Ecosystem Partnership Fund (CEPF, www.cepf.net), a global nature conservation fund which enables civil society to protect the world's biodiversity hotspots. The partnership included participants from the Tular Cave Laboratory as the leading partner, and four more organisations: the Biospeleological Society of Montenegro (Montenegro), University of Shkodra »Luigj Gurakuqi« (Albania), the Scientific Research Centre of the Slovenian Academy of Sciences and Arts (Slovenia), and the Department of Life Sciences at the University of Trieste (Italy). The project continued the work started in 2013–2014 during Tular's prior CEPF project in Bosnia and Herzegovina and Montenegro (Aljančič et al. 2014, Gorički et al. 2017). The project in 2016 was both research and capacity building orientated, focusing on three main objectives: i) assessment of the endangered subterranean biodiversity; ii) public promotion and academic outreach, and iii) extending the Trans-Balkan conservation alliance and capacity building.

The project has enhanced the Slovenia–Montenegro–Albania–Italy trans-border cooperation on conservation of the endangered subterranean biodiversity and protection of groundwater, through organizing events and meetings, study visits and specialized trainings, connecting local communities as well as non-governmental and governmental organisations.

Particular attention was given to the increasing negative anthropogenic pressure on karst, in particular the pollution of groundwater, which receives virtually no concern in Montenegro, while the situation in Albania is only worse. Inappropriate management of karst and its ecosystem services, lack of practical response and low public awareness result in high pollution of groundwater and serious subterranean habitat destruction. The following main threats to the subterranean habitats of the study area were identified during this project:

- unregulated infrastructure (sewage systems only in larger towns, with ineffective wastewater treatment; public landfills and illegal dumps placed on vulnerable karst locations); one such case is the town of Cetinje, with an underground outlet into Skadar Lake through the cave system of the Obodska pećina;
- agriculture, through massive use of fertilizers, particularly viticulture in the catchment area of karst springs;
- no organized trash collection and recycling in rural areas, rubbish dumped in nature (nearly all caves around settlements serve as illegal dumping sites);
- unregulated construction of tourist facilities, mostly on the coast of the Adriatic Sea and Skadar Lake, with notable pressure on

important trans-border biodiversity areas of Montenegro and Albania, such as Buljarica, Ulcinj Salina, parts of Skadar Lake National Park, etc.;

- uncontrolled growing of built-up areas around Shkodra's karst region (Albania) coupled with exploitation of new quarries and intensive water pumping for drinking water necessities for the local community;
- several type localities of endemic subterranean species have already been destroyed (B. Sket, pers. comm. Apr. 2016).

The above mentioned threats were addressed in four key capacity building activities, which involved young researchers, scientists, conservationists, as well as local communities in the study area:

1. Training for vertical cave explorations: At the start of this project, there was only one trained caver still active in Albania (Enis Shehu, pers. comm. Oct. 2016), meaning that the subterranean bio- and geodiversity hidden behind vertical parts in caves would be almost impossible to access without assistance of foreign experts. To build safe cave exploration capacity, Albanian conservationists were invited to attend a 5-day course to learn basic caving knowledge and skills. The instructor-led training was focused on the safe use of single-rope technique, needed to visit vertical caves. The course was first led indoors on artificial climbing walls in Tirana (Fig. 1a), followed by two-day outdoor practice in four caves in the karst area on the northeast side of Shkodra Lake, Albania. The training was successfully accomplished by ten young Albanian researchers and conservationists, all attending fieldwork training and workshop.

2. Training on fieldwork in caves: Participants continued with practical training on survey and protection of karst was performed during caving practice trips mentioned above. There, sampling techniques to collect cave animals, as well as methods to collect water samples for eDNA analysis for the presence of proteus were also demonstrated during the fieldwork.

3. International workshop on biodiversity of the Southeast Dinaric Karst: In order to raise attention of the Albanian and Montenegrin nature conservation community on the research and conservation of the Southeast Dinaric karst, the workshop »*Conservation of cave biodiversity in Southeast Dinaric Karst*« was organized on 29. 10. 2016 in Shkodër, Albania. The workshop gathered twenty participants from six countries presenting their experiences, methods and solutions, participating in the discussions on protection of the endangered karst biodiversity of Montenegro and Albania. Students from Albania and Montenegro were invited to present their recent work (conservation action within their NGOs or their research projects at universities) through scientific communications. For many of them, this was the first opportunity to present their work in English, to a specialized public (Fig. 1b; Tular 2016).

Figure 1. A: Single-rope technique training for safe cave exploration, conducted at the »Rock Tirana« climbing wall in Tirana, Albania (photo: Magdalena Năpăruș-Aljančič); B: Participants of the international workshop »Conservation of cave biodiversity in Southeast Dinaric Karst«, Shkodër, Albania, 29. 10. 2016 (photo: Gregor Aljančič).

Slika 1. A: Tečaj vrvne tehnike na plezalni steni »Rock Tirana« v Tirani, Albanija (foto: Magdalena Năpăruș-Aljančič); B: Udeleženci mednarodne delavnice »Varstvo jamske biotske raznovrstnosti na jugozahodu Dinarskega krasa«, Skadar, Albanija, 29. 10. 2016 (foto: Gregor Aljančič).

4. International team visits: In addition to visits from Slovenia to Albania and Montenegro, three most perspective young Albanian biologists were invited for a short study visit to Slovenia. The program was adapted according to their field of interest in nature conservation, visiting a wide range of nature conservation and research institutions, meeting their Slovenian colleagues, active in research and conservation of subterranean biodiversity, herpetology, bats and large mammals.

However, an important outcome of the project was the extension of an informal trans-boundary *Proteus* conservation network (Aljančič et al. 2014), connecting five conservation NGOs in Albania (Năpăruș-Aljančič et al. 2016). Capacity building performed during this project included training on the conservation of groundwater, which was explained through cases of pollution and their impact on groundwater biodiversity in Slovenia, showing similar examples in the study area. Particularly valuable was the involvement of partners and local communities in fieldwork (information on caves and karst springs, sources of pollution), as well as outreach activities. Conservationists from Albania and Montenegro were invited to advocate protection of karst biodiversity, and groundwater, their main source of drinking water.

The above described project had several outcomes related both with scientific and capacity building activities, which have enhanced a long needed exchange of information, knowledge and practice between the northwest and southeast Dinaric karst. In the scientific part of the 2016 project we reconfirmed the presence of proteus environmental DNA trace at several sites in Montenegro, pointing at the extension of its range as far as to the NW edge of Skadar Lake. We also sampled the subterranean invertebrate fauna of selected caves in the Skadar/Shkodra Lake Basin (samples in determination at specialists), improving the general knowledge on the subterranean biodiversity of the study area (Năpăruș-Aljančič et al. 2017).

The overall outcomes of the project showed that the activities performed during the capacity building were bringing an added value to the scientific part of the project, building bridges and trustworthy partners for future international conservation actions and research in the Southeastern Dinaric karst.

Further capacity building is needed to support countries such as Albania and Montenegro – in order to establish their research infrastructure, to start more ambitiously the study and conservation of a potentially very rich spot of the subterranean biodiversity.

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References

Aljančič G., Năpăruș-Aljančič M., Stanković D., Pavićević M., Gorički Š., Kuntner M., Merzlyakov L. (2014): A survey of the distribution of *Proteus anguinus* by environmental DNA sampling, CEPF Final Project Completion Report. Society for Cave Biology, Kranj, 30 pp. https://www.cepf.net/sites/default/files/sg60185-technical-report.pdf [accessed on 13.11.2018].

Gorički Š., Stanković D., Snoj A., Kuntner M., Jeffery W., Trontelj T., Pavićević M., Grizelj Z., Năpăruș-Aljančič M., Aljančič G. (2017): Environmental DNA in subterranean biology: range extension and taxonomic implications for *Proteus*. Sci. Rep. 7: 45054.

Năpăruș-Aljančič M., Aljančič G., Stanković D., Merzlyakov L., Pavićević M. (2017): Assessment of the endangered subterranean biodiversity of the Skadar/Shkodra Lake Basin. CEPF Final Project Completion Report. Society for Cave Biology, Kranj, 30 pp. https://www.cepf.net/sites/default/files/sg73473-final-report.pdf [accessed on 13.11.2018].

Tular (2016): Program of the International workshop »*Conservation of cave biodiversity in Southeast Dinaric Karst*«. Tular Cave Laboratory, Society for Cave Biology, Kranj, 4 pp. http://www.tular.si/images/Tular_docs/Subterranean_biodiversity_Shkoder_Program.pdf [accessed on 18.12. 2018]

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The »Trebinje *Proteus* Observatorium and *Proteus* Rescue and Care Facility«, Bosnia and Herzegovina

Opazovalni center in center za reševanje ter oskrbo proteusov v Trebinju, Bosna in Hercegovina

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The Town of Trebinje is situated in Trebinjsko Polje at the upstream end of Popovo Polje in Eastern Hercegovina, Bosnia and Herzegovina. This area is a biodiversity »hotspot« for hypogean fauna, including the enigmatic *Proteus anguinus anguinus* Laurenti, 1768, the focal species for the »*Proteus* Project« in Bosnia and Herzegovina. Forward planning of objectives for the Phase 3 period (2021–2030) of the project included the creation of a multifunctional facility based on the concept of a »*Proteus* Observatorium«. It was intended that such a combined facility would eventually be used as:

(a) a location at which the visitors could actually see one of the most famous cave animals in the world in its natural habitat, without the use of white light and without entering the cave – a »*Proteus* Observatorium«;
(b) a »Rescue & Care Facility« for stray, damaged and undamaged proteus;
(c) a research facility to study and record the behaviours of proteus;
(d) an ecotourist visitor location; and
(e) a demonstration centre for conservation activities and associated scientific research.

It was also realised that such a facility could not be developed quickly, due to the constraints placed upon such a plan by the inherent characteristics required by (b) above. The creation and operation of a »*Proteus* Observatorium« such as we had in mind, would also have to be designed in strict compliance with the »Prime Directive and Ethical Code of Practice« of the »*Proteus* Project«. As such, it must pose absolutely no risk to the health and well-being of any proteus population and must not adversely impact its natural habitat.

A suitable natural location at which to develop the Observatorium was found to be at the main entrance area of the Vrelo »Vruljak 2« cave in the Gorica urban district of Trebinje. This cave is part of the Vrelo »Vruljak« Cave System which contains a large population of adult and juvenile proteus (Lewarne et al. 2010).

After much work, the natural entrance was eventually walled up and the access hole was secured by a gate (Fig. 1). Infrared underwater lights and infrared video cameras were then installed in the underwater passages (Fig. 2) and a 12 V DC electrical supply system was fitted just inside the entrance.

This is the first such »*Proteus* Observatorium« in Bosnia and Herzegovina or indeed anywhere within the natural geographical range of proteus, where a viable population can be observed and studied in its native habitat without disturbance either by the regular presence of human visitors or by the use of white light. In comparison with captive proteus held in other subterranean facilities such as the Experimental Ecology Station of the CNRS at Moulis, France (http://www.ecoex), the Tular Cave Laboratory, Slovenia (Aljančič et al. 2016), Hermann's Cave, Germany (Ipsen & Knolle 2017) or the Speleovivarium »Erwin Pichl« in Trieste, Italy (Papi & Mauri 2016), the proteus in the Trebinje facility are not physically confined to living in concrete or glass aquaria or in other artificial conditions. Consequently, the observations we record are not subject to the effects of any unnatural environmental constraints placed upon the animals. Our ability to observe and record proteus behaviours under natural conditions relies on a specific behaviour of proteus – extreme site fidelity (Gergely et al. 2015, Gergely & Lewarne 2017).

It is not unusual for proteus individuals to become forced or washed-out of their underground habitat onto the surface, especially when the underground flow rates in the karst aquifer respond to prolonged periods of high rainfall (Aljančič et al. 2016). During the process of being washed-out or forced-out, they are liable to incur physical damage, either from tiny superficial scratches or even the more major types of damage to their gills or even loss of a limb. Moreover, in such a way, injured washed-out proteus are highly susceptible to fungal infections.

Figure 1. The completed protected entrance to the »Trebinje *Proteus* Observatorium« at the Vrelo »Vruljak 2« cave (photo: B. Lewarne).
Slika 1. Zaprt vhod v v jamo Vrelo »Vruljak 2« , kjer je postavljen opazovalni center »Trebinje *Proteus* Observatorium« (foto: B. Lewarne).

Figure 2. Installation of an IR camera with integral IR lights (top) and an independent stand-alone IR LED emitter array below it (photo: A. Sári).
Slika 2. Postavitev IR kamere z vgrajenimi IR lučmi (zgoraj) in neodvisno samostoječe IR LED svetilo pod njo (foto: A. Sári).

The »*Proteus* Project« has an inherent »duty of care« to protect proteus and its natural habitats and this responsibility also naturally extends to those proteus individuals that are forced from the underground onto the surface during floods. It has been apparent to us for many years that there is a need for a service to rescue and re-home stray proteus in the Trebišnjica River Basin and even to protect, treat and nurse damaged individuals in a suitable quarantine facility before returning them to a suitable hypogean location.

In 2017, we visited the Tular Cave in Kranj, Slovenia to observe and learn how Gregor Aljančič and his team were dealing with the washed-out proteus problem (Aljančič et al. 2016). As a result of our visit to the Tular Cave, we were sufficiently inspired to design and install the necessary infrastructure to replicate their proteus veterinary treatment model in the Trebinje Observatorium, albeit on a much smaller scale. We also modified their procedures to adapt them to our own circumstances. This unique combined facility is now at a sufficient stage of development whereby it is fully suited to the task and now includes a quarantine or isolation aquarium, specifically designed for this veterinary purpose and which has been installed just inside the entrance of the Observatorium. After our standard chemotherapeutic treatment for fungal infections has been applied to the rescued proteus, they are returned to the natural habitat afforded by the »Trebinje Proteus Observatorium« in the Vrelo

»Vruljak 2« cave. Thus far, we have already successfully treated 5 stray proteus.

During our »*Proteus* Project's« educational outreach programme, the Observatorium is always rigorously advertised among the local people as being available for accepting lost or stray proteus from anywhere in the Trebišnjica river basin. We will collect such individuals from any location in the river basin to safely transport them to the »Trebinje *Proteus* Observatorium« for initial veterinary treatment prior to their return to the natural underground habitat already occupied by a thriving proteus population.

In conclusion, the »Trebinje *Proteus* Observatorium« cannot accommodate rescued proteus from any habitat location in the Trebižat or Una/Sana river basin areas in Bosnia and Hercegovina. This comes from the the »*Proteus* Project's« extensive and on-going hydrological programme which continues to demonstrate that the aquatic chemistry under all hydrological conditions is considerably different to that in the Trebišnjica river basin. Additionally, we do not wish to compromise the gene pool of the proteus populations living in one river basin area by introducing proteus individuals from other river basin areas, where they could be genetically different (Gorički & Trontelj 2006).

References

Aljančič G., Aljančič M., Golob Z. (2016): Salvaging the washed-out *Proteus*. Nat. Slo. 18(1): 65-66.

Gergely B., Lewarne B., Herczeg G. (2015): *In situ* underwater tagging of aquatic organisms: A test using the cave-dwelling olm, *Proteus anguinus*. Ann. Zool. Fennici 52: 160-166.

Gergely B., Lewarne B. (2017): Observations on the olm *Proteus anguinus* population of the Vrelo Vruljak System (Eastern Herzegovina, Bosnia and Herzegovina). Nat. Slo. 19(1): 39-41.

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.

Ipsen A., Knolle F. (2017): The olm of Hermann's Cave, Harz Mountains, Germany – eggs laid after more than 80 years. Nat. Slo. 19(1): 51-52.

Lewarne B., Gergely B., Smith R.P.S. (2010): The Vrelo »Vruljak« (Gorica) hypogean part-ecosystem. Speleobiologica Bosniae et Hercegovinae 1, 80 pp.

Papi F., Mauri E. (2016): *Proteus* and education at the Speleovivarium »Erwin Pichl« in Trieste (Italy). Nat. Slo. 18(1): 63.

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Lubin Y.D., Eberhard W.G., Montgomery G.G. (1978): Webs of Miagrammopes (Araneae: Araneaidae) in the neotropics. Psyche 85: 1-13.
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Edmonds D.T. (1997): The contribution of atmospheric water vapour to the formation of a spider's capture web. In: Heimer S. (Ed.), Proceedings of the 17th European Colloquium of Arachnology. Oxford Press, London, pp. 35-46.

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Lubin Y.D., Eberhard W.G., Montgomery G.G. (1978): Webs of Miagrammopes (Araneae: Araneaidae) in the neotropics. Psyche 85: 1-13.

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