Univerza
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NIB
NACIONALNI INŠTITUT ZA BIOLOGIJO

NATURA SLOVENIAE

Revija za terensko biologijo • Journal of Field Biology

Letnik • Volume 19 Številka • Number 1

Ljubljana
2017

NATURA SLOVENIAE

Revija za terensko biologijo • Journal of Field Biology

Izdajata • Published jointly by

Biotehniška fakulteta, Univerza v Ljubljani
Jamnikarjeva 101, SI-1000 Ljubljana
Tel.: (0)1 320 30 00; Telefax: (0)1 256 57 82
http://www.bf.uni-lj.si

Nacionalni inštitut za biologijo
Večna pot 111, SI-1000 Ljubljana
Tel.: (0)59 232 700; Telefax: (0)1 2412 980
http://www.nib.si

http://www.bf.uni-lj.si/bi/NATURA-SLOVENIAE/index.php

Naslov uredništva • Address of the Editorial Office
NATURA SLOVENIAE, Večna pot 111, SI-1111 Ljubljana, Slovenija

Izvlečki prispevkov so zavedeni v zbirkah **ASFA, AGRIS, Biological Abstracts, Biosis Previews, COBISS** in **Zoological Records**

ISSN: 1580-0814 **UDK: 57/59(051)=863=20**

Lektorji • Language Editors
za angleščino (for English): Henrik Ciglič
za slovenščino (for Slovene): Henrik Ciglič

Oblikovanje naslovnice • Layout
Daša Simčič akad. slikarka, Atelje T

Natisnjeno • Printed in
2017

Tisk • Print
Miha Košenina s.p., Brezovica pri Ljubljani

Naklada • Circulation
300 izvodov/copies

Sofinancira • Cofinanced by
Javna agencija za raziskovalno dejavnost RS/Slovenian Research Agency

Kazalo vsebine

NATURA SLOVENIAE 19(1): 5-21
SCIENTIFIC PAPER

Prejeto / Received: 24.2.2017
Sprejeto / Accepted: 16.6.2017

The biting midge *Forcipomyia paludis* (Macfie, 1936) (Diptera: Ceratopogonidae) in Slovenia, Bosnia and Herzegovina, Croatia and Sweden

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Abstract. Records of the biting midge *Forcipomyia paludis* (Macfie, 1936) from Slovenia and Bosnia and Herzegovina are reported herewith as the first finds of *F. paludis* in both countries, together with new records from Croatia and Sweden. This biting midge is a temporary ectoparasite of dragonfly imagines and the only ceratopogonid species known in Europe to feed specifically on this insect group. *Forcipomyia paludis* is already known in 18 European countries. Prior to this report, *F. paludis* was known to infest 67 dragonfly species in Europe. Thirteen dragonfly imagines from 11 sites in Slovenia, 27 imagines from 13 sites in Bosnia and Herzegovina and six imagines from two sites in Croatia having *F. paludis* on their wings were recorded. Additional data for 50 imagines from 15 sites in Sweden are also presented. In Slovenia, the species is known to occur in the Gorenjska, Goriška Brda, Vipava River Valley, Coastal-Karst region, Central Slovenia, Kočevska region and Bela krajina, while in Bosnia and Herzegovina it is known only from south Herzegovina (Ljubuški, Čapljina, Mostar and Stolac areas). In Croatia, the species is present in several parts of the country, while in Sweden it occurs only in the southern and middle parts of the country (Skåne, Öland, Gotland, Göteborg and Gävle). Six new dragonfly host species and the northernmost occurrence of *F. paludis* are also reported.

Key words: *Forcipomyia paludis*, Diptera, Ceratopogonidae, biting midge, parasite, Odonata, dragonflies, distribution

Izvleček. Kačjepastirska mušata *Forcipomyia paludis* (Macfie, 1936) (Diptera: Ceratopogonidae) v Sloveniji, Bosni in Hercegovini, na Hrvaškem in Švedskem – Za Slovenijo ter Bosno in Hercegovino so prvič predstavljene najdbe kačjepastirske mušate *Forcipomyia paludis* (Macfie, 1936), skupaj z novimi najdbami na Hrvaškem in Švedskem. Kačjepastirska mušata je začasen zunanji zajedavec na odraslih kačjih pastirjih (Odonata) in edina vrsta mušate v Evropi, ki se hrani specifično na tej skupini žuželk. Vrsta je doslej znana iz 18 evropskih držav. V Evropi je znanih že 67 vrst kačjih pastirjev, ki jih kačjepastirska mušata zajeda. Poročamo o najdbah *F. paludis* na 13 odraslih kačjih pastirjih z 11 lokacij v Sloveniji, 27 odraslih s 13 lokacij v Bosni in Hercegovini ter šestih odraslih z dveh lokacij na Hrvaškem. Zabeležene so tudi najdbe kačjepastirske mušate na 50 kačjih pastirjih s 15 lokalitet na Švedskem. V Sloveniji je vrsta znana z Gorenjske, iz Goriških Brd, Vipavske doline, obalno-kraške regije, osrednje Slovenije, Kočevske in Bele krajine. V Bosni in Hercegovini je bila zabeležena le v južni Hercegovini (Ljubuški, Čapljina, Mostar in Stolac), medtem ko je bila na Hrvaškem ugotovljena na več mestih po državi. Na Švedskem je bila zabeležena v južnem in osrednjem delu države (Skåne, Öland, Gotland, Göteborg in Gävle). Poročamo tudi o šestih novih vrstah kačjih pastirjev kot gostiteljev in najbolj severni najdbi kačjepastirske mušate.

Ključne besede: *Forcipomyia paludis*, Diptera, Ceratopogonidae, mušata, zajedavec, Odonata, kačji pastirji, razširjenost

Introduction

Small insects that seemed to be stuck on dragonfly wings were noticed for the first time in 2016 on photos of two dragonfly species taken in Slovenia in previous years. They were subsequently identified as dragonfly-biting midge *Forcipomyia paludis* (Macfie, 1936) (Diptera: Ceratopogonidae), and this encouraged the careful check of numerous other dragonfly photos for presence of these animals. This temporary ectoparasitic small insect species is known to suck haemolymph from the veins of the dragonfly wings (Wildermuth & Martens 2007). As *F. paludis* is not yet known to attack other insects, Martens et al. (2008) concluded that it is a parasite specific to the Odonata. With respect to their choice of host species, *F. paludis* is opportunistic (Wildermuth & Martens 2007). Their females have been reported to be attached to the wings or thorax of many dragonfly species (Martens et al. 2008). They mostly cling to near the wing bases, thus minimizing the centrifugal forces to which they are exposed during wing beats (Wildermuth & Martens 2007). Also, the main veins near the bases are thicker and contain more haemolymph than those in the distal sections, thus yielding a better food supply for the parasite (Wildermuth & Martens 2007). Usually, each dragonfly individual is harbouring a few to a dozen parasites (Manger & van der Heijden 2016), although strikingly, Clastier et al. (1994) reported from France 169 biting midges on a single *Libellula quadrimaculata* individual. Curiously enough, the feeding action of *F. paludis* does not seem to leave visible lesions on the host's integument (Wildermuth & Martens 2007). When catching dragonflies with the insect net, *F. paludis* commonly leaves its host (Martens et al. 2008).

Due to their small size of 1.8 mm (Wildermuth & Martens 2007), *F. paludis* is almost always overlooked during field work, but recognized later on the photographs of dragonflies (Manger & van der Heijden 2016). The midges are quite easily identified on the photographs. When they are attached to the underside of the dragonfly wings, a brown, or sometimes reddish brown, stain is visible. Their head is black and the brown abdomen covered by two colourless wings. The wings are not projected beyond the abdomen tip (Wildermuth 2012). Potential development sites of *F. paludis* larvae are swamp areas with larger water bodies (such as lakes), but also peat bogs (Martens et al. 2012). It is still unclear when *F. paludis* attach themselves to dragonflies, but possibly they prefer freshly hatched teneral and juvenile individuals, whose cuticle has not yet hardened (Wildermuth 2012). Apart from *F. paludis*, other groups of Diptera may also be associated with the dragonfly imagines (Martens et al. 2008).

This paper reports on the first records of *F. paludis* in Slovenia and Bosnia and Herzegovina. Additionally, new data for Croatia and Sweden are presented. New dragonfly species as host and the northernmost occurrence of *F. paludis* are also reported. We propose a Slovene vernacular name.

Materials and methods

This study is based on the inspection of photographs. As no biting midge other than *F. paludis* has so far been reported to be associated with European Odonata (Martens et al. 2008), all records of biting midge presence were included in the analysis as *F. paludis*. In search of both published and new records in Slovenia, the national odonatological bulletin Erjavecia, published by the Slovene Dragonfly Society, and three Slovenian public databases were investigated, together with personal archives of several colleagues. From Foto-narava (2017), 1954 dragonfly photos were investigated; further 1103 photos came from BioPortal (2016) and 326 photos from the Database of Invertebrate Pictures (2017). Also, the Facebook group Metulji in kačji pastirji / Butterflies & Dragonflies / Leptiri i vretenci (2017), where mostly authors from Slovenia and Croatia post their photos, was checked. The authors of photographs recording an apparent presence of *F. paludis* were asked to send their original photo with detailed locality information. None were aware of the presence of *F. paludis* in their photos. Additional photographs were taken by the first author or submitted by other colleagues, while four records are based on personal observations of the first author. From Bosnia and Herzegovina, several thousand photographs of dragonflies from all parts of the country were checked. These photographs were taken mainly by the second author, in the period between 2009 and 2016. For Sweden, besides the individual work of the first three authors, also more than 6000 photos (taken in the periods: June–August in 2013, 2014 and 2015, and all the photos taken in 2016) in the citizens' science Swedish Species Observation System – Artportalen (ArtDatabanken 2017), the Entomological Collections at the Swedish Natural History Museum in Stockholm (Naturarv 2017) and the archives of the Swedish Dragonfly Society were checked.

All material was analysed with respect to date, locality, host species and its sex, as well as number, orientation and position of biting midges on the host's body. The coordinates of the localities were taken from Google maps (2017), and their altitudes from DaftLogic (2017). The following orientations of *F. paludis* were differentiated: towards the host's body, facing the wing tip, parallel with the host's body (facing the costae), and facing the posterior edge of the wing. The position of midges was classified with respect to the fore- and hind wing, upper- or lower side of the wings, basal or distal half of the wings or the nodal part of the wings.

In English, we refer to the species with Latin name only, while in Slovenian, we use also vernacular name. We propose this new name to be »kačjepastirska mušata«, according to the biology of *F. paludis*, being specialized parasite of Odonata.

Results

In total, 95 new records of *F. paludis* attached to Odonata are provided (Tab. 1, 2), with additional two specimens from one site (L.40, Tab. 1) found in a museum collection. We also analysed five previously published records of *F. paludis* from three sites (L.27, L.28, L.38, Tab. 1) (see also below).

Slovene and Swedish public databases proved to be a valuable source of information: six records of *F. paludis*' presence were confirmed from Foto-narava and BioPortal combined, and further 39 records came from Artportalen. At least three records in Artportalen are the result of a blog post by the third author (Billqvist 2014) with the intention to raise awareness of *F. paludis* in Sweden. One additional record from Foto-narava from user AK (2016) with *Orthetrum brunneum* as a host of one *F. paludis* individual was established, but had to be excluded from further study as we were not able to obtain the locality information of the photo – allegedly it was taken in Slovenia. One record in Croatia was discovered on the photo published in the bulletin Erjavecia (Bedjanič 2015).

Five already published records of *F. paludis* from Croatia and Sweden (L.27, L.28, L.38, Tab. 1) lacked the data on the host' species or on the parasite's position/orientation in published sources (Sandhall 2000, Martens et al. 2008, Billqvist 2014) and are therefore included in this study with additional information. Furthermore, *F. paludis* also occurs on the island of Gotland, from where two specimens are stored in the Entomological Collections at the Swedish Natural History Museum in Stockholm (Naturarv 2017). Both individuals were collected at the same site (L.40, Tab. 1) on two separate occasions (12.6.2011, 10.7.2011). We were not able to check this museum collection ourselves and the information whether the individuals were collected alone or attached to the host was not available. Hence, these specimens were not included in further analyses.

On most sites, the biting midges were found on single dragonfly individuals (Tab. 1). Of the sites of *F. paludis* on the Balkan Peninsula, 11 are in Slovenia, 13 in Bosnia and Herzegovina, and two new sites in Croatia – on Pag Island and near the southern border with Bosnia and Herzegovina (Fig. 1). In Sweden, two sites are located in the Skåne province, one near Göteborg, one near Gävle at the northern border of the Uppsala province, nine localities are clustered on Öland Island and two on Gotland Island (Fig. 1). Records from Slovenia are from the end of May to late July, while those from Bosnia and Herzegovina are from early May to early July (Tab. 2). Data from Croatia were collected from the end of June to early September, while data from Sweden are from early June to early August.

Altitudinal distribution varied between 0 and 540 m above sea level. Data of *F. paludis* were recorded in or near 25 lentic and 16 lotic habitats, while other sites were not at water bodies. Habitats were diverse, as given in Tab. 1.

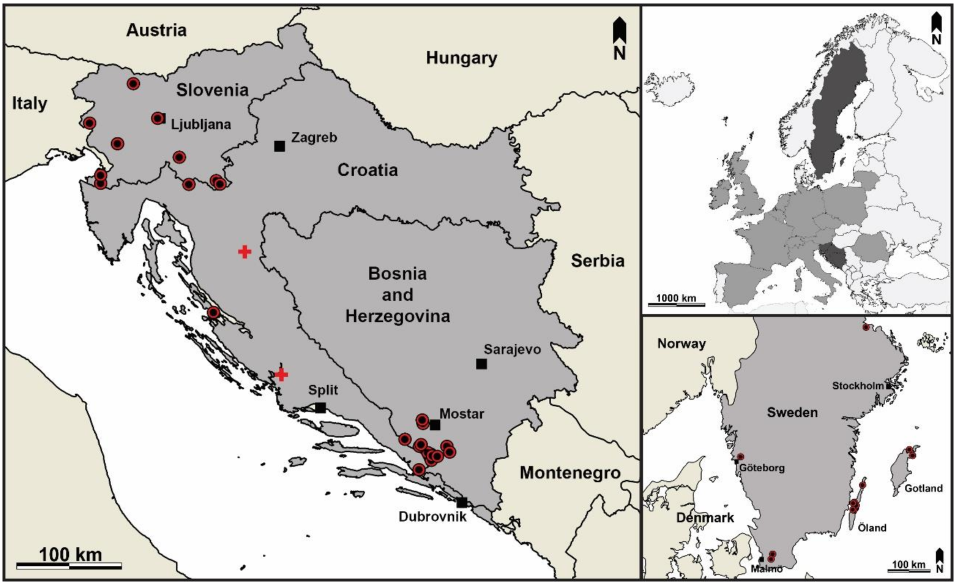

Figure 1. Known sites of *Forcipomyia paludis* in Slovenia, Bosnia and Herzegovina, Croatia (left) and Sweden (bottom right) with known European distribution (top right; countries with confirmed *F. paludis* presence are shaded). For the Balkans, new findings are shown with dots, and the previously published ones with crosses.
Slika 1. Lokacije kačjepastirske mušate (*Forcipomyia paludis*) v Sloveniji, Bosni in Hercegovini, na Hrvaškem (levo) in Švedskem (spodaj desno), z označeno razširjenostjo v Evropi (zgoraj desno: osenčene so države s potrjenimi najdbami kačjepastirske mušate). Za Balkan so s pikami označene nove najdbe, s križcem pa že znane iz objavljene literature.

Table 1. A list of the localities with records of *Forcipomyia paludis* in Slovenia, Croatia, Bosnia and Herzegovina and Sweden. For localities 4, 5, 27, 28 and 40, only approximate coordinates are given. Localities 27 and 28 are derived from Martens et al. (2008), locality 40 from Naturarv (2017). Abbreviations: L – number of locality, lat./lon. – latitude and longitude in WGS84 decimal degrees, Alt. – altitude, N_{Fp} – number of dragonflies infested with *F. paludis*.
Tabela 1. Seznam lokalitet v Sloveniji, na Hrvaškem, v Bosni in Hercegovini ter na Švedskem z najdbami kačjepastirske mušate (*Forcipomyia paludis*). Za lokalitete 4, 5, 27, 28 in 40 je podan približen geografski položaj. Lokaliteti 27 in 28 sta povzeti po Martens et al. (2008), lokaliteta 40 po Naturarv (2017). Okrajšave: L – številka lokalitete, lat./lon. – geografska širina in dolžina v WGS84 decimalnih stopinjah, Alt. – nadmorska višina, N_{Fp} – število kačjih pastirjev, okuženih s *F. paludis*.

L	Nearest city	Exact locality	Coordinates (lat./lon.)	Alt. [m]	N_{Fp}
Slovenia					
1	Bled	Šobčev bajer Lake, next to Šobec Autocamp	46.353804, 14.150758	425	1
2	Črnomelj	Meadow in Nerajske luge Nature Reserve	45.509402, 15.194878	150	1
3	Črnomelj	Kršeljivec Karst pond, near Hrast pri Vinici	45.477386, 15.240999	250	2
4	Kočevje	Hiking trail near Topli jarek Stream on Planina Kosa Mountain	45.476130, 14.853554	295	1
5	Kočevje	Ribnica River Valley	45.713331, 14.735428	485	1

L	Nearest city	Exact locality	Coordinates (lat./lon.)	Alt. [m]	N_{Fp}
6	Koper	Pinjevec Stream near Koštabona	45.477177, 13.759042	90	1
7	Koper	Brackish swamp in Škocjanski zatok Nature Reserve	45.548932, 13.755418	0	1
8	Ljubljana	Glinščica Stream	46.052821, 14.460757	300	1
9	Nova Gorica	Small pond at Podsabotin	46.002844, 13.607091	280	1
10	Vipava	Gacka Stream at Mlake pri Vipavi	45.827799, 13.962007	115	1
11	Vipava	Fishpond at Mlake pri Vipavi	45.827465, 13.962174	115	1
Bosnia and Herzegovina					
12	Čapljina	Nature Park Hutovo blato, Svitava	43.030278, 17.744167	5	4
13	Čapljina	Nature Park Hutovo blato, Karaotok near Hotel	43.063889, 17.755278	5	6
14	Čapljina	Nature Park Hutovo blato, Karaotok north-east from Hotel	43.068979, 17.755726	0	2
15	Čapljina	Nature Park Hutovo blato, above Lake Deransko	43.058619, 17.824150	55	2
16	Čapljina	Nature Park Hutovo blato, Škrka Lake	43.084431, 17.741653	5	3
17	Čapljina	Neretva River at Struge	43.094009, 17.707071	5	1
18	Ljubuški	Kravice Waterfall	43.156363, 17.608618	45	1
19	Ljubuški	Muratovac Canal at Ljubuško polje	43.216531, 17.436043	65	1
20	Ljubuški	Studenci, spring	43.169555, 17.627819	35	2
21	Mostar	Mostarsko blato, Lištica River and pond near the football camp	43.353056, 17.659167	235	1
22	Mostar	Mostarsko blato, Gnjilište, small lake	43.384167, 17.653333	280	1
23	Stolac	Hodovo, pond	43.147500, 17.935278	405	1
24	Stolac	Bregava River near Stolac	43.095556, 17.967500	80	2
Croatia					
25	Opuzen	Above Kuti Lake	42.951389, 17.595556	190	5
26	Pag	Velo blato Lake	44.356321, 15.156547	0	1
27	Plitvički Ljeskovac	Plitvička jezera National Park	44.879597, 15.617581	540	1
28	Šibenik	Krka National Park	43.803081, 15.964437	45	2

L	Nearest city	Exact locality	Coordinates (lat./lon.)	Alt. [m]	N_{Fp}
Sweden					
29	Öland, Färjestaden	Vanserums dammar Pond	56.689165, 16.654557	20	6
30	Öland, Färjestaden	Fishponds near Vanserum	56.691332, 16.640837	20	1
31	Öland, Färjestaden	Stream south from Spjutterumsvägen, near Vanserum	56.696767, 16.630516	20	1
32	Öland, Färjestaden	Tveta, Skogslund, stream	56.645941, 16.589547	30	1
33	Öland, Färjestaden	Meadows and forest edge at Hönstorp	56.658179, 16.547722	40	1
34	Öland, Högby	Several sites around Hornsjön Lake, the lake and its outflow	57.183654, 16.959556	5	18
35	Öland, Långlöt	Vitkärret Alby Pond	56.746978, 16.684886	25	2
36	Öland, Rälla	Greby stenbrott dammar Ponds	56.816323, 16.607173	35	2
37	Öland, Rälla	Forrest edge at Halltorps hage	56.794664, 16.570755	5	1
38	Skåne, Lund	Several sites close to Stensoffa by Krankesjön Lake	55.697734, 13.449883	20	13
39	Skåne, Lund	Peatbog south from Genarp	55.587637, 13.389524	60	1
40	Gotland, Fleringe	Bästeträsk Swamp	57.903233, 18.898806	10	NA
41	Gotland, Furilden	Furillen kalkbrott Ponds	57.772098, 19.011244	10	1
42	Göteborg, Angered	Lärjeåns dalgång, stream	57.773928, 12.068767	45	1
43	Gävle	Skandiavägen, stream	60.588166, 17.313653	25	1

Altogether, nine species of Zygoptera and 26 species of Anisoptera from nine families were found to harbour *F. paludis* (Tab. 2). The majority of these species are typical of lentic ecosystems. First evidences of six new dragonfly species being hosts are presented here: *Aeshna affinis*, *Lindenia tetraphylla*, *Cordulegaster heros*, *Libellula depressa*, *Selysiothemis nigra* and *Sympetrum flaveolum* (Tab. 2). Out of 98 host individuals, 63 were males and 33 females (the majority being mature imagines and only 11 juveniles, no parasitized teneral individual was found). Two parasitized females were caught in the mating wheel (copula), where only the females were infected. We were not able to identify the sex of two hosts as only a small portion of their body was visible on the photographs.

Table 2. A list of dragonfly species from Slovenia, Bosnia and Herzegovina, Croatia and Sweden with *Forcipomyia paludis* attached. Abbreviations: Fp – number of *F. paludis* parasitizing a dragonfly individual, L – number of locality in Tab. 1. Asterisk (*) denotes species included in Martens et al. (2008). Dragonfly species noted for the first time to host *F. paludis* are printed in bold.
Tabela 2. Seznam vrst kačjih pastirjev iz Slovenije, Bosne in Hercegovine, Hrvaške ter Švedske, ki so gostitelji kačjepastirske mušate (*Forcipomyia paludis*). Okrajšave: Fp – število kačjepastirskih mušat na posameznem osebku, L – številka lokalitete kot v Tab. 1. Z zvezdico (*) sta označeni vrsti iz Martens et al. (2008). Nove vrste kačjih pastirjev kot gostiteljev kačjepastirske mušate so zapisane v krepkem tisku.

Host species	Fp	L	Date	Dragonfly observed (photo) by
LESTIDAE				
Lestes sponsa	2	34	25.6.2014	Mats Aldrin
Sympecma fusca	2	29	19.7.2016	Damjan Vinko
CALOPTERYGIDAE				
Calopteryx splendens	3	38	NA	Sandhall (2000)
C. s. balcanica	2	12	19.5.2011	Dejan Kulijer
Calopteryx virgo	1	42	12.6.2014	Leif Andersson
	1	43	4.7.2015	Göran Persson
COENAGRIONIDAE				
Coenagrion ornatum	1	8	16.6.2016	Damjan Vinko
Coenagrion puella	1	5	11.6.2010	AK (2016)
	1	16	9.5.2012	Dejan Kulijer
Coenagrion pulchellum	1	13	15.5.2013	Dejan Kulijer
	1	29	19.7.2016	Dejan Kulijer
	1	34	7.7.2013	Mats Aldrin
Ischnura elegans	2	16	8.7.2012	Jan-Joost Mekkes
	1	29	19.7.2016	Dejan Kulijer
	1	41	7.7.2014	Raimo Neergaard
PLATYCNEMIDIDAE				
Platycnemis pennipes	1	2	25.7.2008	Dušan Klenovšek
*	1	28	July 2003	Roland Bönisch
*	1	28	July 2003	Roland Bönisch
AESHNIDAE				
Aeshna affinis	1	13	27.6.2013	Dejan Kulijer

Host species	Fp	L	Date	Dragonfly observed (photo) by
Aeshna cyanea	1	34	12.7.2013	Mats Aldrin
	3	37	2.7.2015	Gunnar Bohman
	18	38	25.7.2012	Raimo Neergaard
	3	38	11.7.2016	Sven Jönsson
	3	38	11.7.2016	Sven Jönsson
Aeshna grandis	46	29	14.7.2010	Staffan Kyrk
	2	30	19.7.2016	Damjan Vinko
	4	34	12.7.2014	Mats Aldrin
	16	34	12.7.2014	Mats Aldrin
	5	38	14.6.2014	Sven Jönsson
	8	38	14.6.2014	Sven Jönsson
Aeshna isoceles	3	9	25.7.2014	Bojan Zadravec
	2	13	27.6.2013	Dejan Kulijer
	1	13	27.6.2013	Dejan Kulijer
	5	34	7.7.2013	Mats Aldrin
	3	34	12.7.2013	Mats Aldrin
	2	34	27.6.2014	Mats Aldrin
	2	36	28.6.2016	Leif Dehlin
	7	36	28.6.2016	Leif Dehlin
Anax imperator	3	1	5.6.2015	Bojan Bratož
	4	7	16.7.2013	Miroslav Kastelic
	1	11	31.5.2015	Damjan Vinko
	1	23	10.6.2011	Dejan Kulijer
	2	25	30.8.2012	Dejan Kulijer
Brachytron pratense	2	15	26.5.2014	Damjan Vinko
	4	24	7.6.2012	Dejan Kulijer
GOMPHIDAE				
Gomphus vulgatissimus	1	12	1.5.2017	Dejan Kulijer
	6	18	5.6.2008	Ilija Šarčević
Onychogomphus forcipatus	1	6	22.6.2013	Miroslav Kastelic
Lindenia tetraphylla	5	25	30.8.2012	Dejan Kulijer
	2	26	27.6.2015	Bedjanič (2015)

Host species	Fp	L	Date	Dragonfly observed (photo) by
CORDULEGASTRIDAE				
Cordulegaster heros	5	4	6.7.2015	Bojan Bratož
	1	10	12.7.2011	Damjan Vinko
	4	20	21.6.2014	Ivana Sučić
	3	20	7.7.2012	Jan-Joost Mekkes
CORDULIIDAE				
Cordulia aenea	2	34	12.7.2013	Mats Aldrin
Somatochlora flavomaculata	3	34	7.7.2013	Mats Aldrin
	2	38	9.7.2010	Magnus Persson
LIBELLULIDAE				
Crocothemis erythraea	9	14	25.5.2014	Ana Tratnik
Libellula quadrimaculata	4	34	7.7.2013	Mats Aldrin
	5	39	13.7.2012	Sigvard Svensson
Libellula depressa	1	22	8.6.2012	Dejan Kulijer
Libellula fulva	35	15	26.5.2014	Damjan Vinko
	2	19	21.6.2014	Dejan Kulijer
Orthetrum albistylum	1	3	23.7.2015	Damjan Vinko
	6	12	19.5.2011	Dejan Kulijer
Orthetrum cancellatum	1	3	28.7.2015	Damjan Vinko
	1	14	25.5.2014	Ana Tratnik
	1	17	24.6.2014	Dejan Kulijer
	1	21	8.6.2012	Dejan Kulijer
	1	34	7.7.2013	Mats Aldrin
	8	34	27.6.2014	Mats Aldrin
	1	38	1.6.2014	Stefan Cherrug
Orthetrum coerulescens	12	12	24.5.2017	Dejan Kulijer
*O. c. anceps**	1	27	July 2003	Roland Bönisch
Leucorrhinia pectoralis	1	35	18.6.2013	Ingemar Alenäs
	2	35	18.6.2013	Ingemar Alenäs
	1	38	16.7.2006	Erland R. Nielsen
	3	38	20.6.2008	Billqvist (2014)
	15	38	18.6.2016	Magnus Billqvist

Host species	Fp	L	Date	Dragonfly observed (photo) by
Selysiothemis nigra	1	25	29.8.2012	Dejan Kulijer
Sympetrum danae	1	31	19.7.2016	Damjan Vinko
Sympetrum flaveolum	2	32	4.7.2005	Jörg Adelmann
	1	33	30.6.2015	Gunnar Bohman
	1	34	2.8.2013	Lars Bergendorf
Sympetrum fonscolombii	2	25	30.8.2012	Dejan Kulijer
Sympetrum sanguineum	3	13	27.6.2013	Dejan Kulijer
	1	34	7.7.2013	Mats Aldrin
	3	34	7.7.2013	Mats Aldrin
	1	34	8.7.2013	Mats Aldrin
	1	34	11.7.2014	Lars Bergendorf
	3	38	12.7.2011	Martin Andersson
	6	38	21.6.2014	Sven Jönsson
Sympetrum striolatum	6	12	24.5.2017	Dejan Kulijer
	1	16	25.5.2014	Damjan Vinko
	1	24	7.6.2012	Dejan Kulijer
	1	25	7.9.2012	Dejan Kulijer
	5	29	19.7.2016	Dejan Kulijer
	1	29	19.7.2016	Dejan Kulijer

In Zygoptera, the maximum number of midges per host individual was three, whereas in Anisoptera the number reached 35 (Fig. 2) and 46 midges per host. The parasite load proved to be mostly low; 44% of infested individuals were harbouring one parasitic midge. Only in rare cases (14%) more than five midges were observed on a single host. With one exception, all midges were attached to the host's wings. One individual was positioned on the male secondary genitalia and was not considered as attached. As the picture of an infested *Aeshna grandis* from 14.7.2010 was not acquired in high resolution, not all of *F. paludis* were accounted for in the analysis of the parasites' position, as possible wrong outputs could be made. In both suborders, the majority of midges (95%) were facing towards the wing base, 3% faced towards the posterior edge of the wing. The position on the wings differed between the suborders. In Anisoptera the upper side of the wing and the hind wing was predominant (62% of the midges were found on the upper side of the wings and 56% on the hind wings), while in Zygoptera the fore wing was favoured (91% of the midges were found on the front wings) and 88% of the midges were found on the upper side of the wing. In Anisoptera, 89% of the midges were located in the basal half of the wings, 3% were in the distal half and 8% were around the nodus. In Zygoptera, almost half of all midges were located in the basal half of the wing (46%) (Fig. 3), while the other two positions on the wings were more equally represented (distal half 25% and around the nodus 29%).

Figure 2. Three *Forcipomyia paludis* on *Sympetrum sanguineum* (L.13, Tab. 1) (top left), 15 on *Leucorrhinia pectoralis* (L.38, Tab. 1) (top right), 35 on *Libellula fulva* (L.15, Tab. 1) (bottom left) and 12 on *Orthetrum coerulescens* (L.12, Tab. 1) (bottom right) (photos: D. Kulijer, M. Billqvist, D. Vinko).
Slika 2. Tri kačjepastirske mušate (*Forcipomyia paludis*) na krilih krvavordečega kamenjaka (*Sympetrum sanguineum*) (L.13, Tab. 1) (zgoraj levo), 15 na dristavičnem spreletavcu (*Leucorrhinia pectoralis*) (L.38, Tab. 1) (zgoraj desno), 35 na črnem ploščcu (*Libellula fulva*) (L.15, Tab. 1) (spodaj levo) in 12 na malem modraču (*Orthetrum coerulescens*) (L.12, Tab. 1) (spodaj desno) (foto: D. Kulijer, M. Billqvist, D. Vinko).

Figure 3. Two *Forcipomyia paludis* on *Sympecma fusca* on Öland Island in Sweden (L.29, Tab. 1) (photo: D. Vinko).
Slika 3. Kačjepastirski mušati (*Forcipomyia paludis*) na prisojnem zimniku (*Sympecma fusca*) na otoku Öland na Švedskem (L.29, Tab. 1) (foto: D. Vinko).

Discussion

The known distribution of *F. paludis* in Europe is still scattered, since observations are made by chance encounters such as the reports in this paper. However, *F. paludis* is already known from several European countries, with most records coming from the areas between southern France, Switzerland and southern and eastern Germany (Martens et al. 2008, 2012, Wildermuth 2012), from where more than 50 observations are reported from each respective country. In the rest of Europe, except for the countries examined in this report, the records are scarcer. The species has been recorded in France, Germany, England, Austria, Sweden, Switzerland and Croatia (Martens et al. 2008, 2012, Martens 2012, Telfer 2009, Wildermuth 2012) as well as in Poland (Dominiak & Michalczuk 2009), the Czech Republic (Černý 2014), Ireland (Donnithorne 2010), the Netherlands (Manger & Martens 2013, Manger & van der Heijden 2016), Belgium (Claerebout 2013), Lithuania (Leuthold & Wildermuth 2014), Sardinia in Italy (Dell'Anna et al. 1995) and the Balearic Islands in Spain (Nielsen et al. 2014). In Sweden, two published records from Krankesjön in the southwestern part of the country exist (Sandhall 2000, Billqvist 2014). *Forcipomyia paludis* was also recorded in Romania, although as an individual and not as a parasite on a dragonfly's body (Remm 1988). Prior to this report, the known European distribution of *F. paludis* as a parasite on dragonflies is shown by Manger & van der Heijden (2016).

In Slovenia, the species is currently known from seven different (micro)regions spreading from the northwest to the southeast of the country: Gorenjska, Goriška Brda, Vipava River Valley, the Coastal-Karst region, Central Slovenia, Kočevska region and Bela krajina. Interestingly enough, despite the fact that the dragonfly photographs from all parts of Bosnia and Herzegovina were examined, only records from southern Herzegovina, a Mediterranean part of the country, have been known so far. In Croatia, *F. paludis* is known from three sites in Dalmatia and one in continental Croatia. In Sweden, it is known from the southern provinces of Skåne and Västergötland, from Öland and Gotland Islands, and from the middle part of the country from the northern border of Uppsala province. From this site near Gävle (L.44, Tab. 1), the northernmost siting of *F. paludis* in Europe comes from. In seven sites (L.12, L.13, L.16, L.20, L.29, L.34, L.38, Tab. 1), *F. paludis* was between the years discovered on several occasions, therein we conclude it successfully reproduces in at least those sites.

Our results represent the first evidence of six additional dragonfly species as hosts. In Europe up to now, 67 dragonfly species have been known as hosts of *F. paludis* (Martens et al. 2008, Martens 2012, Wildermuth 2012, Manger & van der Heijden 2016). Among them, 53 species are also represented in the fauna of Slovenia and 48 in Bosnia and Herzegovina. In Slovenia, nine dragonfly species are currently proven to be hosting biting midges, 18 species in Bosnia and Herzegovina and in Sweden, and seven in Croatia. Although no significant preference for any dragonfly taxon as host of *F. paludis* can be demonstrated (Martens et al. 2008), new host species will certainly be included in future reports. In Europe, a total of 73 dragonfly species are now documented with *F. paludis* attached to their body.

Forcipomyia paludis has been observed in Europe from the beginning of May until the beginning of September, although one record in March exists (Wildermuth 2012). This corresponds to the main flight season of dragonflies in the greater part of Europe, which lasts from early May to late September. Our findings coincide with this period. The frequencies of different positions and orientations of midges on the dragonfly wings observed in this study are similar to those described by Martens et al. (2008), where the reasons for these positions and orientations are also described briefly.

Except for four records from Slovenia and two from Sweden, all sightings of *F. paludis* in Slovenia, Bosnia and Herzegovina, Croatia and Sweden are based on photographs. Reference material still has to be collected and stored into 70% ethanol. In the near future, we hope to sample *F. paludis* from dragonflies to formally identify the species and to store specimens in museum collections. As observations of *F. paludis* are mostly coincidental, the species is likely to be found in more areas. We suspect that checking additional dragonfly photo archives will yield additional records of *F. paludis*. Thanks to our report, we are approaching the complete picture of the species distribution in Europe, where reports from Norway, Denmark, Finland, Estonia, Latvia, Belarus, Ukraine, Hungary, Slovakia, other countries on the Balkan Peninsula, Portugal as well as from other regions with already confirmed presence of *F. paludis* are still anticipated. Therefore, we invite everyone to carefully re-examine their photographic archives.

Povzetek

Kačjepastirska mušata *Forcipomyia paludis* (Macfie, 1936) (Diptera: Ceratopogonidae) je začasen zunanji zajedavec na odraslih kačjih pastirjih in edina vrsta mušate v Evropi, katere samice se hranijo s hemolimfo specifično na tej skupini žuželk (Martens et al. 2008). Na posameznem kačjem pastirju največkrat najdemo od par do ducat kačjepastirskih mušat. Zaradi njihove majhnosti (1,8 mm) jih pri terenskem delu pogosto spregledamo. Večino podatkov o njihovi razširjenosti tako dajejo fotografije, kar velja tudi za našo raziskavo. Kačjepastirske mušate na fotografijah prepoznamo kot ovalne temnejše pike na krilih kačjih pastirjev. Njihov abdomen je temno rjav, s spodnje strani pa rjav do rdečkasto-rjav. Imajo črno glavo in dvoje nepigmentiranih kril, ki bistveno ne presegajo dolžine zadka. Antene so krajše. Na krilih kačjih pastirjev lahko sicer najdemo tudi druge dvokrilce (Diptera) in pršice (Acarina).

Na podlagi terenskih in fotografskih podatkov avtorjev ter pregleda javno objavljenih fotografij ali zbirk podatkov (Foto-narava, BioPortal, Podatkovna zbirka fotografij nevretenčarjev, Artportalen, Naturarv, ipd.) poročamo o 95 novih podatkih o kačjepastirski mušati v štirih državah. Vrsto smo zabeležili na 11 lokacijah v Sloveniji, 13 v Bosni in Hercegovini, kjer je vrsta nova za favni držav, ter na 15 lokacijah na Švedskem in na dveh novih lokacijah na Hrvaškem. Podajamo tudi natančnejše informacije o petih že objavljenih podatkih vrste za Hrvaško in Švedsko. Osebki kačjih pastirjev so na sebi imeli od enega do 46 zajedavcev. V Sloveniji smo kačjepastirsko mušato zabeležili na Gorenjskem, v Goriških Brdih, Vipavski dolini, obalno-kraški regiji, osrednji Sloveniji, na Kočevskem in v Beli krajini. Navkljub pregledu več tisoč fotografij kačjih pastirjev s celotnega območja Bosne in Hercegovine smo kačjepastirsko mušato zabeležili le v južni Hercegovini. Na Švedskem je bila vrsta najdena v več delih na jugu države (Skåne, Göteborg, otoka Öland in Gotland) in v njenem osrednjem delu (Gävle), iz Hrvaške pa je znana s treh lokacij v Dalmaciji in ene v celinskem delu države. O pojavljanju kačjepastirske mušate poročajo še iz Anglije, Irske, Belgije, Nizozemske, Francije, Nemčije, Poljske, Češke, Litve, Švice, Balearskega otočja v Španiji, Romunije, Sardinije v Italiji in iz Avstrije (Martens et al. 2008, Manger & van der Heijden 2016). Poročamo o najbolj severnem pojavljanju vrste, ki se sicer po Evropi pojavlja med majem in septembrom.

Pred tem poročilom je bilo v Evropi znanih 67 vrst kačjih pastirjev, ki jih zajeda ta mušata (Martens et al. 2008, Martens 2012, Wildermuth 2012, Manger & van der Heijden 2016). V okviru prispevka poročamo o šestih novih gostiteljskih vrstah – višnjevi devi (*Aeshna affinis*), veliki peščenki (*Lindenia tetraphylla*), velikem studenčarju (*Cordulegaster heros*), modrem ploščcu (*Libellula depressa*), temnem slaniščarju (*Selysiothemis nigra*) in rumenem kamenjaku (*Sympetrum flaveolum*). V Sloveniji smo zabeležili devet vrst gostiteljev, 18 v Bosni in Hercegovini, enako število na Švedskem in sedem na Hrvaškem. Ker kačjepastirska mušata zajeda kačje pastirje vrstno neznačilno, so najdbe novih gostiteljskih vrst pričakovane. V prispevku poročamo tudi o položaju zajedavcev na krilih kačjih pastirjev.

Ob pregledu dodatnega fotografskega gradiva in večji pozornosti pri terenskem delu lahko pričakujemo še večje število podatkov o razširjenosti te vrste. Za Slovenijo, Bosno in Hercegovino, Hrvaško ter Švedsko je treba nabrati tudi referenčno gradivo.

Za slovensko ime vrste predlagamo ime »kačjepastirska mušata«.

Acknowledgements

It is a pleasure to thank Hansruedi Wildermuth, Špela Gorički, and both reviewers for additional comments for the improvement of this paper. Thanks to Simon Kovačič, Duša Vadnjal, Bojan Škerjanc, Ana Tratnik, Barbara Zakšek, Ali Šalamun, Matjaž Bedjanič, Alja Pirnat, Maja Vrhovnik, Tomi Trilar, Ivana Sučić, Jan-Joost Mekkes, Ilija Šarčević and Helena Lager, who examined their photos taken in included countries for potential records of *F. paludis* and in some cases contributed the data. Thanks to all the authors from Table 2 who allowed us to include their photos in this study. Aleš Likar helped us with the contacts of some authors from Foto-narava. Additionally, we would like to thank Ana Tratnik for her company during the field work in Nature Park Hutovo blato in 2014 and at the ECOO 2016 south-east post congress trip. Thanks to Ali Šalamun and Matjaž Bedjanič for the discussion on the Slovene name for *F. paludis* and Nastja Majerič for improving the language.

Part of the fieldwork in Bosnia and Herzegovina was funded by the following project supported by the Critical Ecosystem Partnership Fund (CEPF): »Karst freshwater habitats: identification and participatory conservation planning of threatened invertebrate and fish species«, which was led by the BIO.LOG Society (Bosnia and Herzegovina) and the Slovene Dragonfly Society (Slovenia). A part of the fieldwork of the second author in Bosnia and Herzegovina was carried out under the projects supported by the Rufford Foundation, the International Dragonfly Fund (IDF) and the Environmental Fund of the Federation of Bosnia and Herzegovina. Some of the material from Sweden in 2016 and Bosnia and Herzegovina in 2012 was collected during the two post congress trips of the respective European Congress on Odonatology (ECOO).

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Recommendations for a consistent use of vernacular names for *Proteus anguinus* in English and Slovenian scientific texts

Priporočila za enotno rabo angleških in slovenskih imen za vrsto *Proteus anguinus* v znanstvenih besedilih

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Ever since its description two and a half centuries ago, the proteid urodelan *Proteus anguinus* Laurenti, 1768 (Fig. 1) has been known to the wider, scholarly audience by several different common names, German as well as English. The number of local Slovenian names, originating from times before the scientific description, is still much larger (Zois 1807, Freyer 1850, Aljančič 1989, Parzefall et al. 1999). There are probably several reasons for this terminological richness including the linguistic and cultural diversity in the Dinaric Karst area of the western Balkan Peninsula, as well as a long history of research and high public attention (Aljančič et al. 1993). On the other hand, the animal is still not common enough and generally known for a single, globalized English name to become generally accepted and to prevail in popular use. We regard the diversity of vernacular names for *Proteus anguinus* as valuable elements of cultural heritage that we do not wish to diminish in any way. Since all these names, e. g. človeška ribica, močeril, bela kačica or protej in Slovenian, and Grottenolm or Olm in German are unique, there is no danger of ambiguity. Likewise, we acknowledge that 'človeška ribica' is the most widely used Slovenian name by which people relate to this important flagship species in spoken language as well as popular, scholarly and legal texts. What is to be supported in everyday use may not be so desirable in scientific or legal texts. Our motivation to write down these nomenclatural recommendations is multifold. From an editorial perspective, it is a matter of consistency and style to refer to the same phenomenon under the same name as much as possible. Searches in electronic databases, cross-referencing and indexing become much easier when fewer words are involved. As representatives of the three Slovenian laboratories working scientifically on *Proteus anguinus*, we are often asked to render our opinion; we need to give recommendations to students writing their theses and wish to be consistent in our own use. Finally, certain names or forms of names are to be preferred over others. For example, the monomial form has the advantage of allowing for a more flexible addition of extensions when new subordinate taxa are being described (Sket & Arntzen 1994). The English names 'blind cave salamander' and 'European cave salamander' are ambiguous as several other blind urodelan species are known from North American caves, and other European urodeles (genus *Speleomantes*) are referred to as cave salamanders. Surely, all of these issues could be resolved simply by using the scientific name all the time. However, for reasons that may not be purely scientific, authors sometimes prefer to use names that are more attractive or just shorter and simpler than the scientifically most justified *Proteus anguinus*.

When shaping our proposal of vernacular names we followed several criteria. They are listed loosely by importance.
(1) Ambiguous or potentially ambiguous names should be avoided. This criterion eliminates names like 'blind cave salamander' and 'European cave salamander'.
(2) Misleading names should be avoided. This does not apply as much to the obviously nonsensical 'človeška ribica' as it does to the literal English translation 'human fish' and a number of older names such as bela kačica in Slovenian and the German folk name Fischotter formerly used in the region of Kočevje, Slovenia (Aljančič 1989).
(3) Widely used and accepted names should be preferred. By this criterion, 'blind cave salamander' and 'človeška ribica' come into conflict with the first and the second criterion, respectively. But we believe that unambiguity and truthfulness should be given priority.
(4) Simple names, in particular monomial ones, are better than complex names. This rule eliminates

the technically unambiguous but cumbersome ‘European blind cave salamander’.

These considerations narrow down the choice of suggested replacements for *Proteus anguinus* in scientific texts to a couple of names in each language. **‘Olm’**, from the Thuringian vernacular for Molch, or newt (Neri & Ziegler 2012), and first used by the German naturalist Lorenz Oken (1817), is a well-established name that avoids the ambiguity issues with various versions of ‘cave salamander’. Above all, it is wonderfully simple, unique and memorable. The Slovenian **‘močeril’** comes close both in etymology and by being fostered by a 19th century naturalist, the Slovenian Henrik Freyer (Freyer 1842, 1850, Aljančič 1989). Besides these two names that meet all the above criteria, we recommend the use of the vernacularized generic name, **‘proteus’**, in both English and Slovenian. This is the oldest name, by which the species became internationally renowned. It exists in several variants, such as the archaic Slovenian ‘proteuz’ and the scarcely used ‘protej’, as well as the common French form ‘le Protée’, the Italian ‘il Proteo’. However, ‘proteus’ is the most universal one and therefore to be preferably used in scientific communication.

Acknowledgements

We thank (in alphabetical order) Lilijana Bizjak Mali, Špela Gorički, Katja Poboljšaj, Mitja Prelovšek, Boris Sket, David Stanković, Valerija Zakšek and Anamarija Žagar for their constructive comments, suggestions and views on the terminology of *P. anguinus*.

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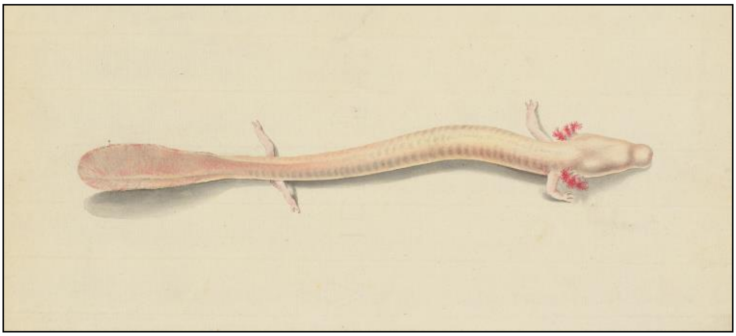

Figure 1. First picture of proteus based upon a live animal, known at that time to the locals as ‘bela riba’ [white fish] or ‘zhloveshka riba’ [human fish] (Vincenc Dorfmeister/ Sigismund Zois, around 1805, archive of SAZU).

Slika 1. Prva slika proteusa po živi živali iz časa, ko so jo domačini poznali po imenu ‘bela riba’ ali ‘zhloveshka riba’ (Vincenc Dorfmeister/ Sigismund Zois, okrog 1805, arhiv SAZU).

NATURA SLOVENIAE 19(1): 25-26
»SOS *Proteus*« – EDITORIAL

Prejeto / Received: 10.5.2017
Sprejeto / Accepted: 6.6.2017

Second International Meeting SOS *Proteus*: »Conservation of black *Proteus* and its habitat – 30 years after its discovery« / Drugi mednarodni posvet SOS *Proteus*: »Varstvo črne človeške ribice in njenega habitata – 30 let po odkritju«

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Upon the 30th anniversary of the discovery of the black proteus *(Proteus anguinus parkelj)*, the Tular Cave Laboratory (Society for Cave Biology) organised the second International Meeting SOS *Proteus*: »Conservation of the black *Proteus* and its habitat – 30 years after its discovery«, held on 10.–11. 12. 2016 in Črnomelj, Bela krajina, Slovenia.

The first day of the meeting gathered 72 researchers and experts on proteus, speleobiology, karstology, herpetology, conservation and public outreach from Slovenia, Italy, Croatia, Hungary, Germany, Portugal and Romania. The keynote lecture with the title »The black cave salamander (*Proteus anguinus parkelj*) – its history and some other curiosities« was held by Prof. Boris Sket. The program of the meeting was divided into four sessions (Habitat and Ecology; Veterinary and Developmental Studies; Conservation and Field Research; Discussions, Conclusions and Next Steps) and included 13 presentations of the latest findings in the field of research and conservation of proteus and threats to karst groundwater. The presentation part of the meeting was concluded by two short documentaries on the vulnerability of proteus (Tular 2016).

In the closing session, participants joined a discussion on current environmental problems in the habitat of the black proteus. Niko Šuštarič, Andrej Hudoklin and Gregor Aljančič presented the initiative of the Society Proteus on the urgent protective measures in the proteus habitat in Bela krajina, which was submitted to the Slovenian Ministry of the Environment and Spatial Planning. The discussion was centred on the most critical threats such as the overuse of biogas-slurry, in search for solutions and advices with the help of the assembled international audience.

The second day of the meeting was dedicated to the public presentation of threats to the black proteus and sources of drinking water in Bela krajina. The general public and media were invited to a short presentation of arguments and to participate in discussions on the urgent protective measures in the affected area habitat of black proteus and the most important source of drinking water for Bela krajina. The public hearing was attended by 35 participants.

Figure 1. Participants of the 2nd International Meeting SOS *Proteus*: »Conservation of the black *Proteus* and its habitat – 30 years after its discovery«, first day (photo: Boris Grabrijan).
Slika 1. Udeleženci 2. Mednarodnega posveta SOS *Proteus*: »Varstvo črne človeške ribice in njenega habitata – 30 let po odkritju«, prvi dan (foto: Boris Grabrijan).

At the meeting the assembly honoured Professors Boris Sket, Boris Bulog and Dušan Plut for their outstanding contributions to the study and conservation of the black proteus and its habitat.

Extended abstracts of most lectures are presented on the following pages of this issue of *Natura Sloveniae*.

Acknowledgements

We wish to thank the honourable host of the meeting, Mrs Mojca Čemas Stjepanovič, the Mayor of Črnomelj. We also thank the Society Proteus, Museum Collection of Črnomelj, and the Zupančič family for their support in the organisation of the meeting.

The 2nd International Meeting SOS *Proteus* was supported by the Slovenian Ministry for Environment and Spatial Planning (2550-16-300010) in partnership with the Societas Herpetologica Slovenica and the Slovenian Odonatological Society.

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Discovering the black proteus *Proteus anguinus parkelj* (Amphibia: Caudata)

Kako smo odkrivali črnega močerila *Proteus anguinus parkelj* (Amphibia: Caudata)

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The famous proteus was first mentioned in literature as 'a vermin' by Valvasor (1689), and scientifically described by Laurenti (1768). The animals were highly troglomorphic, without dark pigmentation and with eyes covered by skin. Later, very similar animals were found along the Dinaric karst from the Italian Carso/Kras through Slovenia, Croatia, down to the extreme SE of Herzegovina (Sket 1997) and probably molecularly detected in Montenegro (Gorički et al. 2017). *Proteus anguinus*, človeška ribica, čovječja ribica, močeril, protej, (German) Olm, became a very popular animal, a kind of a mascot of the Dinaric underground; its occasional findings have been often mentioned even in newspapers. Additional troglomorph and troglobiotic amphibians were found in North America; they belong to another family, Plethodontidae, while proteids are represented in North America by epigean relatives of proteus, the *Necturus* spp.

But it was only in the 20th century that a non-troglomorph proteus was found. The first specimen was caught by researchers of the Inštitut za raziskovanje krasa (from Postojna) during a pumping experiment on the Dobličica spring near Črnomelj, in Bela krajina, SE Slovenia (Aljančič et al. 1986). The specimen was given to the Biology Department, University of Ljubljana, for study purposes. The animal caught our attention since it greatly differed from the white protei. As even the white proteus is very popular, this finding caused much attention and resulted in numerous newspaper articles: immediately, as well as in the following year(s).

The black proteus, beside nearly black pigmentation and well developed eyes, exhibited all characters which one would expect from the epigean ancestor of the troglobiotic *Proteus anguinus*. But we were cautious; instead of immediately describing a new species, or even a new genus, as was often the case with cave animals and their eyeless epigean relatives (compare the case *Anoptichthys* – *Astyanax*), we were first trying to obtain additional specimens. Scuba diving in the lakelet and epigean drains of the Dobličica spring did not provide any specimens, which suggested the trogobiotic nature of the 'black proteus'. Later, an additional specimen was seen and photographed in a spring at Jelševnik and finally, after a strong rain, we found more than ten specimens creeping from holes through the thin turf, evidently covering a porous karst rock, in another spring, Na trati, at Jelševnik.

After that, a coworker was engaged, able to find out phylogenetic relations by molecular means. The result was a description of a new subspecies *Proteus anguinus parkelj* by Sket & Arntzen (1994). Our caution seemed justified. Instead of being an outgroup to populations of the white proteus, the black one from Jelševnik appeared to be more closely related to the white populations from SE Slovenia than those were to the white proteus from SW Slovenia. These analyses, grounded on allozymes, were later followed by DNA analyses with a higher number of samples (Gorički & Trontelj 2006), which only confirmed the black proteus as a distally split branchlet of the species' tree. Its morphological characters are most probably plesiomorphies, preserved during a number of (repeated) innovations in other populations. Beside dark pigmentation and evidently functional eyes, these are a normally shaped head without a snout elongation, differently shaped skull bones, lower teeth number, shorter legs, shorter tail (with lower vertebrae number), and longer trunk with higher vertebrae number. Another view was expressed by Sessions et al. (2015), who consider this animal a victim of secondary 'de-troglomorphisation'. In spite of near- absence of troglomorphies, *P. a. parkelj* is evidently a troglobiont, appearing outside caves not more often than the white proteus.

Beside proteus, the springs at Jelševnik are draining also some epigean and even terrestrial (Diplopoda, Chilopoda) animals, which indicates a relatively good food supply, resulting in a comparatively weak selection pressure for this non-troglomorph troglobiont. Beside that, maintaining of the non-troglomorph appearance might be a result of a late immigration underground; note that the presence of the black proteus is paralleled by the only cave shrimp race with still present and pigmented eye rudiments (*Troglocaris anophthalmus ocellata* Jugovic et al. 2012) living in the same area.

The distribution area of the black proteus seems not to reach beyond several kilometers in diameter, SW to W of Črnomelj in SE Slovenia.

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Cultivation and morphology of blood cells of the olm *Proteus anguinus*

Gojenje in morfologija krvnih celic močerila *Proteus anguinus*

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Sex identification in *Proteus anguinus* using external morphological characteristics is not possible because of the lack of sexual dimorphism. We can identify their sex only during the reproductive season when mature eggs are visible through the non-pigmented skin of females and the cloaca is swollen in males. Accurate sex identification is crucial for demographic studies in natural populations, and would also be important for the establishment of a successful captive breeding program that could be used for evolutionary developmental studies as well as conservation measures.

We have optimized a non-destructive method, derived from human cytogenetics, to visualize chromosomes in order to search for potential dimorphism in sex chromosomes (Gredar 2016). We initially assumed that *P. anguinus* has similarly heteromorphic X and Y sex chromosomes as its closest living relative the North American mudpuppy, *Necturus maculosus* (Sessions 1980). Our cell culture approach was based on a previously described method used in *N. maculosus* (Seto et al. 1964) involving *in vitro* cultivation of blood cells in primary cell culture, but extensive modifications of *in vitro* cell culturing conditions are required. The first step is optimization of growth conditions, including the correct choice of culture medium and its osmolality, and the optimal incubation temperature. It was also necessary to determine the optimal concentration and time course of treatment with mitogen (phytohemagglutinin or. PHA-M) and antimitotic (colchicine) for the adequate stimulation of cell divisions of lymphocytes in cell culture and subsequent arrest in metaphase. We determined that the concentrations of mitogen and antimitotic in *P. anguinus* must be at least 3 or 4 times higher, with much longer treatment times, than are routinely used in human cytogenetics. One of our most surprising results is that the cultivation of cells was successful at an incubation temperature of 25°C indicating that *P. anguinus* cells can tolerate *in vitro* higher temperatures than previously believed. We successfully established a viable blood cell culture and were able to visualize the chromosomes. However, a recent study in our laboratory showed that *P. anguinus* is characterized by a translocation between the X and Y sex chromosomes so that both males and females have identical-looking chimeric sex chromosomes (Sessions et al. 2016). Consequently it is not possible to identify their sex chromosomally. Nevertheless, the establishment of successful cell culture methodologies potentially enables other kinds of studies including cell biology, cell physiology, genetics, biochemistry, toxicology and others in a way that does not require sacrificing the animal. This is especially important for protected and vulnerable species like *P. anguinus*.

Very little is known about the morphology of blood cells and hematological parameters in *P. anguinus*. Therefore we used blood smears for a detailed morphological description of proteus blood cells as well as for differential counts. The relative abundance of different types of white blood cells (WBC) can provide important information about the physiological condition of animals and, by extension, problems with the surrounding environment (Allender & Fry 2008).

In almost all Urodeles the erythrocytes (red blood cells or RBC) mature in the peripheral blood because of lack of bone marrow (Turner 1988). The same is true for *P. anguinus*, and the number of erythroblasts, that are immature RBC, is quite high (3.9–10.5% of all cells; N = 3) in comparison with axolotl (0.1–0.35%; N = 3) (Gredar 2016). The RBCs of *P. anguinus* are relatively large (52.7 ± 0.48 µm × 29 ± 0.24 µm; N = 300) and are among the largest in amphibians due to the large genome size, which is about 15 times that of the human genome. RBC sizes in vertebrates show a positive correlation with genome size (Gregory 2001) and a negative correlation with metabolism in animals, thus it is not surprising that metabolic rate in *P. anguinus* is considerably lower than that of the most surface dwelling amphibians (Hervant et al. 2001). The differential count of leukocytes (WBC) in *P. anguinus* shows that lymphocytes and neutrophils are the predominant types (53.5–90.1% and 6.9–37.2%, respectively (N = 3)), while the eosinophils and monocytes are less numerous (0.0–7.0% and 1.7–3.0%, respectively (N = 3)) and basophils are almost absent. The low ratio between neutrophils and lymphocytes (0.20) indicates that animals were not under stress during captivity (Davis & Maerz 2008). This research was done on a small sample size and further studies are needed to obtain baseline values of WBC in *P. anguinus*. Also, more analyses are needed for accurately interpreting hematological data and for identifying abnormalities. Future research will focus on deviations from baseline values of the WBC profile, which can signal various physiological problems.

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Microbial and parasitic threats to proteus

Mikrobna in parazitska ogroženost močerila

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Although proteus encounters various microorganisms and parasites in its natural environment, the records on them are scarce, while the only comprehensive work on the topic dates to over half a century ago (Vandel et al. 1965). The known data on specialized parasites comprise the protozoan *Chloromyxum protei* described in kidney channels (Joseph 1905) and the trematode *Plagioporus protei* (Prudhoe 1945) found in the small intestine of proteus, while the records on other parasites are less specific. These include an unidentified trematode found in the digestive tract (Matjašič 1956), monogenean flatworms and ciliate protozoans attached to its outer surface (Sket B., personal communication) and encysted nematodes in the pancreas (Schreiber 1933). The peritoneal cavity and gut lumen are commonly colonized by free-living nematodes (Fig. 1), while several other peculiar parasites in various developmental stages were observed in kidneys, liver and in the gut wall (Fig. 2). Despite frequent occurrence of parasites in their tissues, the parasitized proteus individuals remain in good shape, indicating the effectiveness of its defensive mechanisms and the ability to cope with naturally occurring parasites and pathogens.

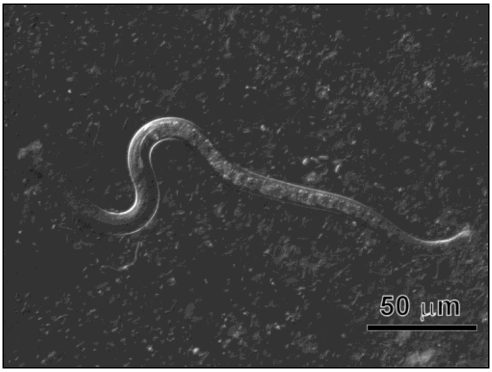

Figure 1. Nematode in the gut content of proteus from Stobe cave (photo: R. Kostanjšek).
Slika 1. Glista v črevesni vsebini močerila iz jame Stobe (foto: R. Kostanjšek).

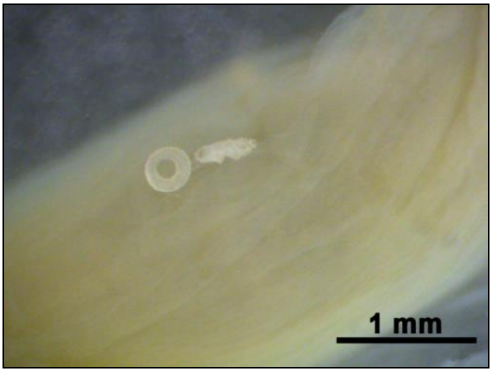

Figure 2. Unknown parasite with ring-shaped adhesion structure on the gut surface of proteus from Planinska jama (Photo: L. Bizjak Mali).
Slika 2. Nepoznani parazit z obročasto pritrjevalno strukturo na površini črevesa močerila iz Planinske jame (foto: L. Bizjak Mali).

On the other hand, animals kept in artificial or semi-natural environments for scientific, exhibition or recovery purposes (Aljančič et al. 2016) appear prone to opportunistic microbial infections. Among others, these include the infections of the outer surface with oomycoete water molds from the genus *Saprolegnia* sp. (Kogej 1999), black yeasts and amoebae (Gunde Cimerman, unpublished data). The most frequent bacterial etiological agent implicated with the opportunistic infections of proteus in captivity is *Aeromonas hydrophila*, causing dermatosepticemia or »red leg syndrome«, a generalized systemic bacterial disease associated with cutaneous erythema and hemorrhagic changes of the other tissues (Densmore & Green 2007). In addition, various fungi and oomycetes were identified in the outer jelly coat of proteus eggs laid in captivity (Zalar et al. 2016). The increased susceptibility of proteus to pathogens and parasites in controlled conditions simulating its natural environment indicates the sensitivity of its immune system even to moderate stressors. In that view, we can only speculate on proteus susceptibility to pathogens under constant pressure of pollutants and other stressors in their natural environment (Bulog 2007).

Beside the factors compromising their defensive mechanisms, newly introduced pathogens pose yet another threat to native amphibians, including proteus. Among the wide array of etiological agents of amphibian diseases, the most severe threat to amphibian diversity on the global scale is currently posed by chitrydiomycosis, a lethal disease caused by chytrid fungi from the genus *Batrachochytrium*, and viral infections caused by ranaviruses from the family Iridioviridae (Densmore & Green 2007, Latney & Klaphake 2013). These two highly virulent pathogens are held responsible for the global decline and extinction of several hundred amphibian species (Latney & Klaphake 2013). Despite their severity and rapid spread throughout Europe, the fungal and viral threat to native amphibians in Slovenia, including proteus, is far from being appropriately monitored, although their possible introduction to the underground water systems of the Dinaric Karst could be devastating for entire proteus populations.

In conclusion, aside to newly emerged and highly virulent microbial pathogens, the most severe pathogenic threat to proteus is most probably related to pollution and other changes in the subterranean waters that potentially compromise the immune response of proteus and its innate ability to cope with the parasites and pathogens in its natural environment.

Acknowledgements

We wish to thank Monika Novak, Martina Turk, Polona Zalar and Jerneja Ambrožič Avguštin for the isolation and preliminary identification of fungi and bacteria, as well as Valerija Zakšek, Peter Trontelj and Žiga Fišer for their contribution to the identification of the helminthes.

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Conservation genetics of proteus in the Postojna-Planina Cave System

Varstvena genetika močerila v Postojnsko-planinskem jamskem sistemu

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Proteus is the largest obligate cave animal in the world. It inhabits subterranean waters of the Dinaric Karst. It is an endangered species protected under the European Habitats Directive and national legislations. During the last reporting period under the EU Habitats Directive in 2013, the conservation status of proteus in Slovenia was estimated and reported as unfavourable (Hudoklin 2011, 2016). In recent years, considerable effort has been invested to improve conservation-relevant knowledge of the species, e. g. new approaches to monitoring presence using environmental DNA (Gorički et al. 2017), monitoring of habitat quality parameters (Hudoklin 2016), and genetic monitoring of quantitative population parameters (Trontelj & Zakšek 2016).

Here we present our progress on the conservation genetic research conducted within an applied research project under the title »Toward the conservation of the European cave salamander (*Proteus anguinus*): monitoring guidelines, current status estimation and identification of evolutionarily significant units«. In this project, we are estimating population parameters (e.g. heterozygosity, gene flow, possible population bottlenecks, effective population size, migration rates), genotyping individuals, and evaluating the taxonomic and conservation status of proteus populations. The greater part of the population-level work has been carried out in the Postojna-Planina Cave System, a large subterranean system, which hosts high numbers of proteus that can be accessed without the need of deep sump technical diving. In Planina Cave, we sampled proteus from the inner 1.8 kilometres of the subterranean Pivka River. In order to have reference points for the geographic position of the caught animals, the studied part of the cave was divided into 23 sections that were between 50 and 100 m long (Fig. 1). In Postojna Cave, proteus were caught in a 200 m long sidearm of the same river in the part called Črna jama.

For the purpose of genetic monitoring, we developed and characterized the first microsatellite markers for proteus (Trontelj & Zakšek 2016, Zakšek et al. accepted). We amplified 23 microsatellites from populations sampled in the caves of Postojna and Planina. The number of alleles per locus varied from 3 to 9. The loci were largely unlinked and conformed to Hardy-Weinberg frequencies. Genetic equilibrium and an F_{ST} value of only 0.0024 suggest a nearly panmictic population in both caves, even though they are separated by some 10 kilometres of subterranean river course. Bayesian clustering detected a weak genetic structure, but failed to unambiguously assign individuals to either of the two caves. These microsatellites will be applied in a genetic mark-recapture study and population monitoring in the near future.

In the summers of 2015 and 2016, 775 and 824 animals, respectively, were caught, using hand nets during snorkelling and scuba-diving. Skin swabs were taken to obtain DNA. In both years a very similar number of animals were caught per individual sections (Fig. 2). Large-scale genotyping and genetic identification of individuals is still in progress, but we already have data on 73 individuals that were genetically marked in 2015 and recaptured the following year. Out of the 73 recaptured animals, most (77%) were found strictly in the same cave section in both years, 16% in adjacent sections, and only 7% were recaptured in non-contiguous cave sections, although never more than 200 m away. These data suggest that at least some animals are strongly territorial and do not change their positions more than some tens of meters within one year. The finding is in agreement with Balázs et al. (2015) who reported extreme philopatry of visually tagged proteus over several years in southern Herzegovina. Further support of a constant territorial structure is the similarity between patterns of caught animals by section (Fig. 2). On the other hand, the near lack of genetic structure between Postojna and Planina Cave means that there must be substantial migration going on over a distance of about 10 kilometres. It is possible that only some individuals are territorial, while others, perhaps sexually immature ones, are more mobile and inclined to disperse. Genotyping of additional animals and reconstructing their relatedness will hopefully give more conclusive answers.

Acknowledgements

We are grateful to numerous enthusiastic friends and colleagues (see Acknowledgement in Fišer et al., this issue) for their help with the fieldwork. The study was funded by the Slovenian Research Agency (Project number L1-6731), and co-funded by the Slovenian Ministry of the Environment and Spatial Planning and the Centre for Cartography of Fauna and Flora (CKFF). Samples were collected under the permits No. 35601-132/2014-4 and No. 35601-26/2016-4 issued by the Slovenian Environment Agency.

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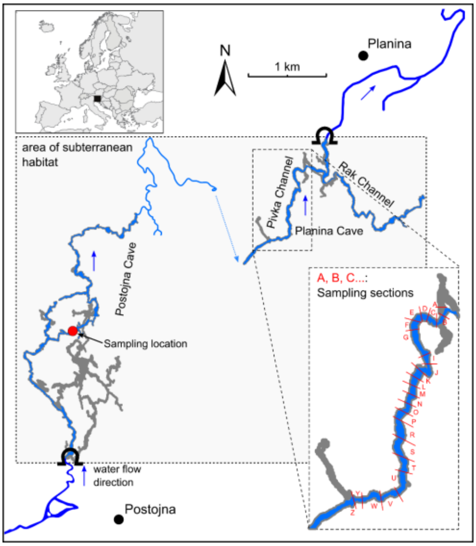

Figure 1. Map of the study area with the two sampling areas (marked by red dot: Črna jama and by red letters: Pivka River in Planina Cave) of proteus populations within the Postojna and Planina Cave System.
Slika 1. Shematski prikaz vzorčenja močerila v Postojnsko-planinskem jamskem sistemu. Rdeči krogec označuje območje vzorčenja v Črni jami, rdeče črke pa odseke v Pivškem rokavu v Planinski jami.

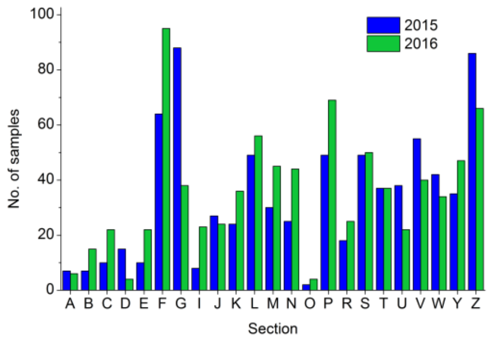

Figure 2. Number of caught and genetically marked animals by sections of the subterranean Pivka River in Planina Cave in 2015 and 2016.
Slika 2. Število ujetih in genetsko označenih močerilov v posameznih odsekih vzdolž podzemeljske Pivke v Planinski jami v letih 2015 in 2016.

The utility of non-genetic data collected during genetic monitoring of proteus populations

Uporabnost ne-genetskih podatkov, pridobljenih med genetskim monitoringom močerila

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Proteus anguinus is the only obligate cave vertebrate of the European continent (Aljančič et al. 1993, Parzefall et al. 1999) and a Dinaric Karst endemic (Sket 1997). In the past decades, population declines of this charismatic species have been reported from a number of localities (Sket 1997, Hudoklin 2011). However, only recently initiatives have been undertaken to develop and establish schemes for monitoring population numbers and distribution. As a part of these initiatives, various new methodological approaches have been tested: a) *in situ* underwater animal tagging (Balázs et al. 2015), b) detection via environmental DNA (Aljančič et al. 2014, Gorički et al. 2017, Vörös et al. 2017), and c) genetic monitoring (Trontelj & Zakšek 2016).

Genetic monitoring as described in Trontelj & Zakšek (2016) requires catching substantial numbers of live proteus. In order to maximize the amount of scientific information obtained once an individual is caught, besides DNA, a number of other biological data are collected. These include body length and mass as well as observations on anomalies such as injuries, pigmentation, gravidity, parasites, etc. Body size can be used to assess population demography and, conjointly with data on injuries and parasites, even health of populations. Taking into account possible fixed differences in body size between proteus phyletic lineages, ecological conditions of cave systems can be inferred. Long term monitoring of animals' body size reveals life-history traits, which have been so far investigated mostly in captive individuals bred in cave laboratories (e.g. Briegleb 1962, Durand & Delay 1981, Juberthie et al. 1996, Aljančič & Aljančič 1998). In this contribution, we present first results based on non-genetic data obtained during the fieldwork for the genetic monitoring of proteus.

In 2015 and 2016, we extensively sampled proteus in several Slovenian caves. These were selected to represent populations from all major mitochondrial DNA lineages of proteus (Trontelj et al. 2009). Each year, we caught about 900 individuals, most of them in the Postojna-Planina Cave System. This is probably the largest dataset ever assembled for natural populations of proteus. Animals were caught using diving equipment and hand nets. First, a DNA sample was non-invasively taken from each individual using a skin swab. Then, body length and mass were measured, the animal was thoroughly examined for anomalies, photographed, and released back to the cave river.

The demographic structure of populations was tentatively assessed by estimating the proportion of reproductively capable individuals in a population. Relying on data from proteus bred in captivity (Durand & Delay 1981, Juberthie et al. 1996, Voituron et al. 2011), we assumed that 20 cm long animals are at least 18 years old and almost certainly already sexually mature. According to preliminary results, the proportion of reproductively capable individuals differed between our samples from different proteus populations. For example, in the Črna jama population we caught almost exclusively sexually mature animals, while in the cave Vetrovna jama pri Laški kukavi these represented only about one quarter of all individuals found.

The relationship between body length and mass was statistically modelled using a power function (see legend to Fig. 1 for more details). Preliminary results on the length-to-mass relationship in four proteus populations for which we had roughly similar sample sizes are shown in Fig. 1. Substantial differences were found between some of these populations. The populations from the subterranean Pivka and Reka Rivers differed most. At the maximal length of about 30 cm, animals from the subterranean Reka River were almost twice as heavy as animals from the subterranean Pivka River. Whether this difference is genetically determined or the consequence of differences in food abundance and quality between cave systems, remains to be investigated.

Some individuals, whose mass was much lower than expected for animals of their length, were found to be infested with parasites. If a connection between infestation and low body mass is confirmed by additional cases, such outliers could be used to estimate population health. In Planina Cave, about 6% of individuals had some minor body injuries, mostly cuts in the tail fin and missing toes. This percentage was similar in both years of sampling, as was the percentage of individuals with at least some darkly pigmented patches of skin (about 7%).

Genetic identification of captured individuals provides an opportunity to assess changes in body size. Within a year, none of the 73 so far identified recaptured individuals from Planina Cave has grown more than one centimeter in length. Their change in mass was much more variable, showing increase and decrease of up to 8 g. As body mass can markedly change already after feeding or defecation, this variability needs to be taken into account before making conclusions about changes in body mass.

Taken together, the fieldwork undertaken in our genetic monitoring scheme produces, as a side product, a number of additional data that are relevant for the biology and conservation of this unique and enigmatic amphibian.

Acknowledgements

We are grateful to Aja Zamolo, Ana Miglioranca, Andrej Hudoklin, Anja Kos, Borut Lozej, Ciril Mlinar Cic, Claudio Bratoš, Daniela Eilers, Dare Hribar, Darja Kolar, Edvard Gregorčič, Ester Premate, Franc Kljun, Frank Körner, Gabriela Enache, Gregor Bračko, Jan Gojznikar, Jerneja Rovtar, Kaja Vukotić, Katarina Novak, Katarina Tušar, Katja Leban, Lilijana Bizjak Mali, Lisa Fisler, Ljerka Lah, Lucija Slemenšek, Luka Mrzelj, Maja Jelenčič, Maja Sever, Manca Velkavrh, Marjeta Konec, Matej Simčič, Mike Patton, Mitja Prelovšek, Nataša Sivec, Nika Šumer, Primož Gnezda, Rok Kostanjšek, Rožle Kaučič, Ryan Riegg, Sandi Vidrih, Sašo Weldt, Sebastjan Kovač, Selena Trontelj, Špela Borko, Špela Gorički, Tadeja Balanč, Tajda Gredar, Tatjana Kordiš, Tobias Trontelj, Urška Ratajc, Vinko Kukman, Žan Kuralt, and Živa Hanc for their help with the fieldwork. This study was funded by the Slovenian Research Agency (Project number L1-6731), and co-funded by the Slovenian Ministry of the Environment and Spatial Planning and the Centre for Cartography of Fauna and Flora (CKFF). The research was performed in accordance with permits No. 35601-132/2014-4 and No. 35601-26/2016-4 issued by the Slovenian Environment Agency.

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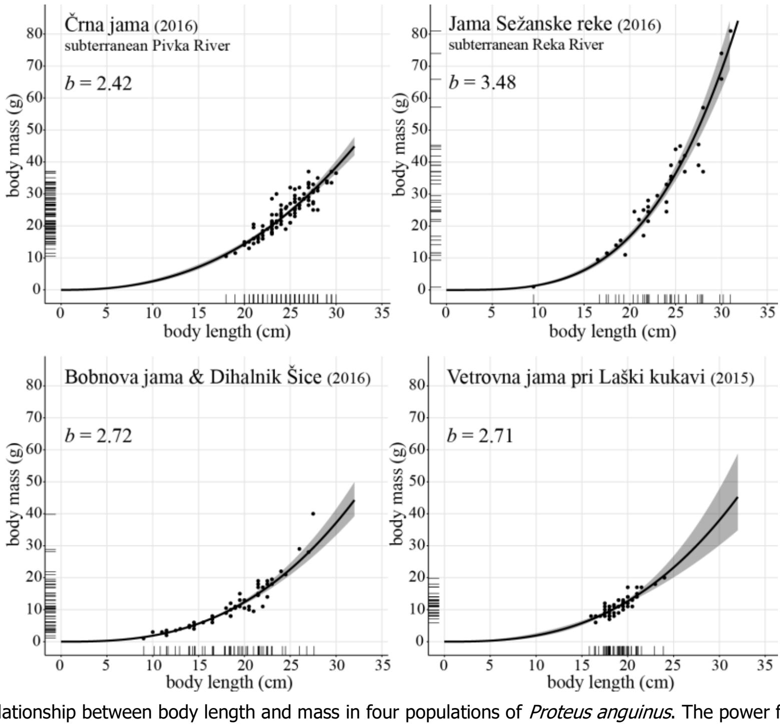

Figure 1. Relationship between body length and mass in four populations of *Proteus anguinus*. The power function $M = a L^b$ (M – mass, L – length, a – intercept, b – slope) was linearized and fitted to the log-transformed data. When length increases by 1%, mass increases by b%.

Slika 1. Odnos med telesno dolžino in maso pri štirih populacijah močerila (*Proteus anguinus*). Odnos opisuje potenčna funkcija $M = a L^b$ (M – masa, L – dolžina, a – presečišče, b – naklon), ki smo jo najprej linearizirali, nato pa ocenili vrednosti parametrov a in b za logaritemsko transformirane podatke. Pri povečanju telesne dolžine za 1 % se masa poveča za b %.

Observations on the olm *Proteus anguinus* population of the Vrelo Vruljak System (Eastern Herzegovina, Bosnia and Herzegovina)

Opazovanja populacij močerila *Proteus anguinus* v sistemu izvira Vruljak (vzhodna Hercegovina, Bosna in Hercegovina)

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The Vruljak System is one of the most valuable and vulnerable *Proteus anguinus* habitats in the Trebišnjica River Basin (Bosnia and Herzegovina, Eastern Herzegovina). The amazing density of aquatic invertebrates results in a healthy population of olms. The easy logistics and the high density of animals make the Vruljak System a good location for trial experiments. The highest known location where the water enters the Vruljak System is the Ždrijelovići ponor zone of the Ljubomirsko Polje, from where the water flows down to the level of Popovo Polje and emerges again at the north-west end of Popovo Polje (sometimes referred to as Trebinjsko Polje). Most of this water surfaces from temporary springs in the comparatively small closed depression of Vruljsko Polje during floods, forming an intermittent lake and then disappearing into numerous swallow-holes. The system then joins the Trebišnjica River in the Gorica district through two larger and several smaller spring caves (Lewarne et al. 2010). Most of the studies and ideas presented here are at an early stage and, therefore, the preliminary results are not enough for solid scientific conclusions.

The accessible lower end of the Vruljak System (Vruljak 1 Cave and Vruljak 2 Cave) is situated right under the edge of the city on the right side of the Trebišnjica River and has side passages that are most probably directly connected with the surface and are, therefore, vulnerable to anthropogenic influence (Fig. 1.). To prove such information, the traditional field method is dye testing, which gives precise data on the source locations and also provides some information on the nature of the connecting passages. Dye testing needs a lot of manpower, special conditions and perfect timing. In the case of a complex system, the results can be largely affected by the chosen water conditions and therefore can only be interpreted according to those water conditions. To gain long term information, we installed water temperature loggers (Tinytag Aquatic 2 by Omni Instruments) in various but well defined passages. A one-year long trial with a sampling frequency of

Figure 1. Positions of the Vruljak 1 Cave and Vruljak 2 Cave in Trebinje, Gorica District. The white arrows represent flow directions.
Slika 1. Položaj jam Vruljak 1 in Vruljak 2 v Trebinju, območje Gorica. Bele puščice prikazujejo smer toka.

one reading every 16 minutes showed that water temperatures differ in different branches (Lewarne 2016). The main passages showed a balanced curve, while the side passages reacted more promptly to surface weather conditions, indicating a direct connection to the surface. Based on the preliminary results, we plan to install more loggers to increase the resolution of the data. Although the temperature data cannot prove direct swallow-hole connections and cannot provide information on the exact location of the water origin, with precise cave and surface maps to support the hydrological data, we believe we will be able to assume more accurately the possible water and pollution sources affecting the water quality in the caves.

Over the last decade of monitoring the Vruljak 1 Cave, we have witnessed several occasions when trout (mostly brown trout *Salmo trutta*), entrapped in the cave, actively hunted for olms utilizing the help of diver lights (Fig. 2.). We have no information on how successfully they can hunt in complete darkness, but since the cave is regularly visited by divers we considered it as a serious threat. As an obvious solution, each year we eliminated the trout from the cave by hunting them with a speargun. In this way we could keep the number of trout inside the cave at a minimum level, but a permanent solution was needed since the artificially regulated surface water regime allowed new fish to enter the cave year by year. In 2015, we eliminated eight trout from the cave and examined their stomach contents, in which we found: *Troglocaris* sp. (n = 29), *Niphargus balcanicus* (n = 11), *Niphargus vjetrenicensis* (n = 2), *Metohia carinata* (n = 1), *Theodoxus subterrelictus* (n = 16), *Asellus aquaticus* (n = 3). The result of the stomach content analysis showed that direct predation is not the only threat, but by eating troglobionts, trout are also strong competitors of the natural top predators. The Vruljak 1 Cave is connected with the Trebišnjica River via an approximately 50 metre long surface stream that serves as a natural corridor for fish from the river to the cave during high water levels. In 2015, we constructed two dams from the stones found in the streambed. The dams are loose so they do not elevate the natural water level in the cave, but are relatively wide (0.5 metres) so they make a hard-to-pass barrier for big fish like adult trout. In 2016, we could not detect any newcomer trout in the cave, which means that the stone dams in such cases can be a good solution with minimal alteration of the natural environment, although better conclusions can only be drawn after a longer monitoring period.

Figure 2. Trout grabbing the olm *Proteus anguinus* (photo: B. Lerner).
Slika 2. Postrv, ki drži močerila *Proteus anguinus* (foto: B. Lerner).

The common minnow *Phoxinus phoxinus* inhabits the cave entrances especially during dry periods, but can also be seen in deeper parts of the caves. During a monitoring dive in 2016 we witnessed an olm eating a common minnow (Fig. 3.). We did not witness the actual hunting and catching, but as the fish looked intact and fresh we can assume that it was actively caught while still alive. This observation shows that the surface dwelling common minnow or other small-sized fish can at least occasionally play an important role in the diet of olms (Lewarne & Balázs 2016).

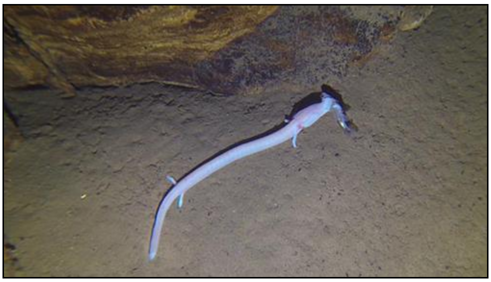

Figure 3. The olm *Proteus anguinus* eating a Common minnow (photo: M. Mede).
Slika 3. Močeril *Proteus anguinus*, ki je pisanca (foto: M. Mede).

The Vruljak 2 Cave starts with approximately 15 metres of dry passage and the underwater part of the cave begins with a lake where abundant hypogean fauna can be found, including olms. This setup makes the cave a perfect location for an observatory. Our idea was to build a camera system capable of recording the behaviour of olms without disturbance. Therefore we installed an underwater infrared (IR) camera system in the lake in spots where olms are always present. An obvious technical problem with the usage of IR technology underwater is that the longer the wavelength of the light, the greater its adsorption by the water, therefore the IR spectrum fades away quickly. To overcome this problem we installed strong IR lights in waterproof casings together with the cameras. By 2016, the camera system was ready for testing and the test was successful. The final goal is to have the system permanently running and to connect it to the internet so researchers and even the wider public can have a view on the natural behaviour of the olms.

We started a mark and recapture study on the olm population of the Vruljak 1 Cave in 2010 using a Visible Implant Elastomer (VIE) tagging system (by NorthWest Marine Technology) (Balázs et al. 2015). We chose this tagging method because it does not require removing the animals from their original positions. Since we were highly concerned about the possible impact of the method on the health of the olms, we only tagged seven individuals for a five-year trial period. During these five years we observed the tagged individuals several times and did not detect any problems. Therefore, in 2015 we considered to continue the experiment by increasing the number of tagged animals. In May 2016 we tagged 19 additional adult olms (Fig. 4). In total, we now have 26 tagged individuals in a well-defined 200 metre long passage. We already have some recapture data suggesting high site fidelity, but even with this increased number of tagged individuals it will take some more years to gather enough data to draw firm conclusions.

Figure 4. Tagging with Visible Implant Elastomer (photo: B. Lerner).
Slika 4. Označevanje s tehniko Visible Implant Elastomer (foto: B. Lerner).

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The problematics of cave pollution in Bela krajina

Problematika onesnaženosti jam v Beli krajini

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According to the Underground Cave Protection Act (Ur. l. RS 2004), caves in Slovenia are defined as natural heritage of national importance and are owned by the state. The law defines the activities in caves, mostly outlining the prohibited impacts on cave environment. Some regulations have also been applied for tourist activities, due to the commercial use and adaptation of the show-caves inventory (Ur. l. RS 2004). Although the legislation strictly prohibits the pollution of caves in any form, monitoring and remediation activities are not officially being practiced. Nevertheless, some caves have been cleaned up in the past, mostly on the initiative by cavers (Prelovšek 2011).

Anthropogenic impacts on caves were quite common already in the past, when used for residence, shelter, religious ceremonies, extraction of natural resources and tourism. However, with the socio-economic development, the emergence of unusable household waste and the unregulated waste management, the pressures on caves led to the increase of cave pollution in Slovenia, especially after World War II (Prelovšek 2011). Subsequently, it has been estimated that around 20% of caves in Slovenia are polluted, especially in the lower karst areas close to the settlements (Čekada 2015).

Several studies have already shown the problematic of communal landfills in karst areas of Slovenia, which greatly contribute to contamination of karst waters (e.g. Kogovšek & Petrič 2007, 2010). Beside communal landfills, illegal waste disposal is problematic in karst areas, due to their vulnerability and low self-purification processes (Ford & Williams 2007). Waste dumped in caves is washed by precipitation into groundwater, and once the groundwater is contaminated it is very difficult to remediate it (Kaçaroğlu 1999). Due to the quantity, toxicity and poor degradability of the dumped waste, practically none of the karst springs can be used for water supply without prior additional physical or chemical processing. This type of pollution is threatening the cave biota and at the same time poses a threat to the public health (Prelovšek 2011).

Cave pollution is negatively affecting the groundwater dependent ecosystems important for the survival of species (Mezga et al. 2016). In the long term, it might cause decline of one of the most important symbols of the subterranean biodiversity, the white olm (*Proteus anguinus anguinus*), as well as the black olm (*Proteus anguinus parkelj*), cave-dwelling tube worm (*Marifugia cavatica*) and troglobiotic 'living fossil' bivalve (*Congeria kusceri*). *Proteus anguinus* is endemic to Dinaric karst (Sket 1997), whereas the subspecies *P. anguinus parkelj* is known as an endemic of Bela krajina (Sket et al. 2003). Cave pollution can also indirectly affect the surface biodiversity, affecting the species that are groundwater dependent, such as the European pond turtle (*Emys orbicularis*), the presence of which was reassessed in Bela krajina (Vamberger et al. 2013). Even though the habitats of endangered and endemic species are included in the Natura 2000 network and the main aim of the Natura 2000 Management Programme for Slovenia is to ensure a favourable conservation status of Europe's significant species and habitat types (Vlada RS 2015), many polluted caves are also found within Natura 2000 sites.

The prime aim of this study was to find the main drivers of cave pollution/degradation, which can provide important data for the identification of potential effects on species, habitats and even public health in Bela krajina.

Bela krajina is located in the southeast of Slovenia and covers around 600 km^2 (Plut 2008). The central part of the region is characterized by the low area of the Črnomelj Plain that is surrounded by the hilly terrain of Gorjanci, Kočevski Rog and Poljanska gora. Due to the predominance of limestone and dolomite rocks, most of the region is karstified. The hilly terrain is characterized by the presence of distinct pits and shafts with an abundance of springs at the foothills, while on Črnomelj Plain horizontal caves prevail (Stepišnik & Natek 2014, Cave Registry 2016). The share of Natura 2000 network in Bela krajina is around 46% (Hudoklin 2014).

With more detailed analysis, we studied the distribution of polluted caves in the region of Bela krajina, their interdependence with the proximity to roads, proximity to settlements, proximity to water sources and land-use types.

Data on caves was obtained from the Cave Registry (2016), which includes location, type of entrance and state of the cave. Road infrastructure data were obtained at The Surveying and Mapping Authority of the Republic of Slovenia (GURS 2014), the same as data on selected buildings with potential waste production (GURS 2015). Land use data was acquired at the Ministry of Agriculture, Forestry and Food (MKGP 2016).

Using ArcGIS (ver 10, ESRI 2010), with the operation »Near«, we calculated the shortest Euclidean distance between caves and roads, as well as between caves and potential producers of waste (e.g. buildings, industries, and restaurants). We identified the land use type where the cave was located, and assessed the relationship between cave state (polluted, destroyed or clean) and cave entrance (horizontal or vertical entrance).

According to the Cave Registry (2016), 622 caves were registered in Bela krajina by 2015, of which 118 were defined as polluted and/or destroyed. We categorized the caves according to the estimated volume of waste: 55 low polluted (0.1–0.9 m^3), 19 medium polluted (1.0–4.9 m^3), 35 high polluted (more than 5 m^3) and 9 destroyed (not accessible anymore). The total amount of waste in affected caves in Bela krajina is estimated at around 1,000 m^3. The results show that the 10 most polluted caves (Tab. 1) hold more than 60% of all waste. Polluted caves are especially problematic in the catchment areas of springs and the areas under protection of Natura 2000. For example, 13 polluted caves are located within the catchment area of the Dobličica spring, which poses as the main water supply for the region. Overall, we identified 70 polluted and 3 destroyed caves within Natura 2000 sites.

The results of the GIS analysis revealed that clean caves are more distant from roads or settlements than the polluted ones, so we assume that these two indicators have high impact on the pollution of caves. The trend is even more obvious within the subcategory of medium polluted caves, where the distance from the roads and settlements diminishes with the increase of the amount of waste in the caves. According to the results, land use is also one of the relevant drivers of cave pollution. The share of polluted caves is higher in forests (73%) than in other land-use types: woodland (9%), grasslands (9%), urban areas (3%), extensive orchards (3%) and abandoned farmland (3%).

We noted differences in the relationship between cave pollution and type of cave entrance. Caves with vertical entrances are usually more difficult to reach by local people, and are thus mostly limited to cave explorers. Nevertheless, these sites present a favourable hidden locality for illegal waste disposal.

To conclude, this study shows which caves are more polluted and therefore pose a threat to both the environment and public health. Hence with this research, we intend to draw attention and raise awareness of the general public for the problems associated with cave pollution in Bela krajina.

Table 1. List of the ten most polluted caves in Bela krajina (source: Cave Registry 2016).
Tabela 1. Seznam desetih najbolj onesnaženih jam v Beli krajini (vir: Kataster jam JZS 2016).

Reg. no.	Name of the cave	Municipality	Estimated amount of waste [m^3]
2356	Jama pri Vranovičih	Crnomelj	100
3947	Brezno Planina	Crnomelj	100
854	Kadiševa jama	Metlika	100
853	Kipina jama	Metlika	100
8006	Brezno v Vogrju	Crnomelj	50
6676	Jama dobrega pajka	Crnomelj	50
9751	Brezno nad Jugorjem	Metlika	50
10312	Brezno 1 v Koprivni dolini	Crnomelj	50
5217	Kotlovnica	Crnomelj	32
9612	Sikara	Crnomelj	30

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GIS analysis to assess the groundwater habitat pollution of black proteus

GIS-analiza za oceno onesnaženosti podzemne vode v habitatu črnega proteusa

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Karst areas are among the world's most vulnerable landscapes to environmental impacts (Veni 2004). Most of pollution in karst areas is due to unsustainable anthropogenic activities (intensive agriculture and industry, unregulated urbanization) that are reflected in the decline of subterranean biodiversity and in the loss of drinking water resources. Slovenia may be particularly vulnerable in this respect, as it is a prime hotspot of subterranean biodiversity (Culver & Sket 2000), and its ground waters constitute the drinking water supply for 97% of its residents (Kranjc et al. 2009). Slovenia is thus facing urgent conservation challenges, and among the critically affected organisms is *Proteus anguinus*, a global symbol of subterranean biodiversity.

Since the 1986 discovery of a unique, darkly pigmented population of proteus, the black proteus (*Proteus anguinus parkelj*) within a single cave system of less than 30 km^2 in Southeast Slovenia, the protection of karst underground habitats has become even more important. This most distinct and rare of all proteus populations is highly endangered; because of its extremely limited distribution, even a small local pollution could have a devastating impact on the entire population and could also destroy the overlapping regional drinking water supply.

The main threats for the black proteus relate to agriculture, particularly the overuse of pesticides and fertilizers on farming land and vineyards, and after 2009, the production of biogas slurry distributed as free fertilizer (Hudoklin 2011). Industry's side products, likewise, pollute the groundwater, e.g. foundry sand contaminated with heavy metals dumped into a doline 700 m from the Jelševnik Springs (a local system comprising the permanent spring, Jezero, and two groups of temporary springs, Na trati, and Jamnice), harbouring the black proteus. After alarming accumulation of zinc in the tissues of the black proteus from Jelševnik Springs (i.e., Na trati 2) was shown (Bulog et al. 2002), the washing out of the waste disposal was minimized by a clay cover; however, this remained *in situ* as a long-lasting threat. Furthermore, villages in the catchment area lack a central sewage system and continue to use primitive individual household septic tanks, from which waste waters enter the karst groundwater. Researchers have detected increased levels of phosphates and nitrates in the Jelševnik Springs (i.e., Na trati 1 and 2; Bulog et al. 2002).

Recently, detection of proteus environmental DNA (eDNA) traces (Gorički et al. 2017) has helped obtain a more precise information on the distribution of the black proteus. Although the number of its known localities was doubled, the expected distribution area of the black proteus (i.e., the area between confirmed localities of the black proteus, without the plausible continuation under the high plateau of Kočevski Rog) is now estimated at less than 3 km^2 (Gorički et al. 2017), extending into the near shallow karst plain of the Črnomelj area. Unfortunately, this newly discovered part of the black proteus habitat lies under the most intensive agriculture areas (about 2/3 of land use type above the black proteus habitat comprise fragmented cultivated fields and vineyards), adding to our inference that the black proteus is severely endangered.

Based on proteus eDNA survey, we employed GIS both to draw the new distribution of the black proteus as well as to test whether any long-term pollution trends within its habitat may be assessed despite the general scarcity of prior data on groundwater quality. Recent data are available at the Dobličica Spring (drinking water control) and the Jelševnik Springs (monitoring of the black proteus habitat since 2000; Bizjak Mali & Bulog 2016). We additionally employed historic data on physical and chemical parameters of groundwater in Bela krajina from an extensive survey of the Karst Research Institute ZRC SAZU (Habič et al. 1990), which also led to the unexpected discovery of the black proteus.

Limited by only a basic set of pollutants measured by Habič et al. (1990), we analyzed concentrations of nitrates and orthophosphates in groundwater in order to reconstruct their quantity during the time of black proteus discovery, and thus to assess the trends in the threat to the black proteus habitat (1987 vs. 2014).

We employed the IDW (Inverse Distance Weight) interpolation tool from ArcGIS 10.3.1 (Esri 2015) to visualize the distribution of nitrates and orthophosphates in 1987 within the expected distribution area of the black proteus and the adjacent white proteus localities (i.e., measurements belong to 7 karst springs, of which 5 are already confirmed localities of proteus; hereafter area of interest, AOI). Unfortunately, most of these springs were not included in subsequent investigations; hence we were able to illustrate pollution trends in the 1987–2014 period only for two of the springs: Dobličica and Jelševnik (i.e., Jezero; ARSO 2014). Three main categories were used in IDW analysis to assess the pollution with nitrates and orthophosphates in AOI, defined as: *low* (< 2.22 mg NO_3^- /l, < 0.045 mg PO_4^{3-} /l), medium (2.22–13.3 mg NO_3^- /l; 0.045–0.25 mg PO_4^{3-} /l) and high (> 13.3 mg NO_3^-/l; > 0.25 mg PO_4^{3-}/l) (USGS 1999).

The map of interpolated values of pollution in 1987 (Fig. 1) shows medium concentrations of nitrates (Fig. 1a) and orthophosphates (Fig. 1b) in the majority of the AOI, while high concentrations of nitrates are exhibited only in the buffer areas of two springs (Obršec and Pački Brežiček – both verified localities of the black or white proteus, respectively). Low concentrations of nitrates are present in a small area around the Dobličica Spring, while low concentrations of orthophosphates covered less than half of the expected habitat of the black proteus.

The comparison of pollution in two analyzed springs (Dobličica and Jelševnik/Jezero) in 1987 vs. 2014 revealed a concerning growing trend for both nitrates and orthophosphates (Tab. 1). The critical increases of nitrates (280% for Dobličica Spring and 272% for Jelševnik Springs/Jezero) and orthophosphates (150% for Dobličica Spring and 111% for Jelševnik Springs/Jezero) implicate intensified use of fertilizers in agriculture and unregulated sewage disposal in the settlements within AOI.

The results of this pilot GIS analysis indicate an urgent need for implementation of a monitoring scheme for the black proteus and its habitat. Immediate action is needed to reverse the pollution trends and to prevent population declines. Moreover, the highly increased levels of nitrates and orthophosphates in the Dobličica Spring do not only affect subterranean biodiversity and the unique black proteus, but also raise public health concerns.

Acknowledgements

We thank Andrej Hudoklin for help and advice during the field survey and GIS data collection. The research was performed during the project »With *Proteus* we share dependence on groundwater«, financially supported by the EEA Financial Mechanism and the Norwegian Financial Mechanism 2009–2014 (SI03-EEA2013/MP-17). GIS analyses were performed at the Biological Institute ZRC SAZU.

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Table 1. Concentrations of nitrates and orthophosphates from Dobličica and Jelševnik Springs (i.e., Jezero) in 1987 and 2014, with their corresponding percentage of increase.
Tabela 1. Koncentracije nitratov in ortofosfatov iz izvira Dobličice ter izvira v Jelševinku (Jezero) v letu 1987 in 2014, z odgovarjajočim odstotkom povečanja.

Spring	Nitrates mg NO_3^-/l		NO_3^- increase 1987 vs. 2014 (%)	Orthophosphates mg PO_4^{3-}/l		PO_4^{3-} increase 1987 vs. 2014 (%)
	1987	2014		1987	2014	
Dobličica	0.9	3.42	280	0.01	0.025	150
Jelševnik (Jezero)	1	3.72	272	0.009	0.019	111

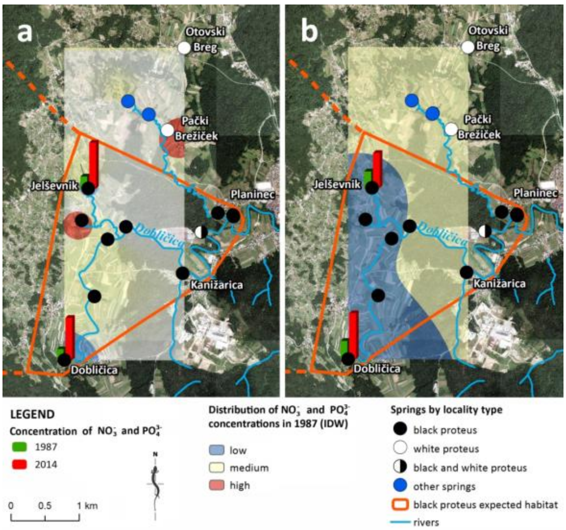

Figure 1. Pollution of *Proteus* habitat indicated by the distribution of interpolated concentration ranges of pollutants (NO_3^- (a) and PO_4^{3-} (b)) for the year 1987 within the AOI in Bela krajina, Slovenia; the bar charts show the concentration of pollutant in analyzed springs for 1987 and 2014.
Slika 1. Onesnaženost habitata človeške ribice, prikazana z interpolacijo porazdelitve koncentracije onesnažil (NO_3^- (a) in PO_4^{3-} (b)) za leto 1987 na preučevanem območju Bele krajine; stolpci prikazujejo koncentracije onesnažil v analiziranih izvirih v letu 1987 in 2014.

The olm of Hermann's Cave, Harz Mountains, Germany – eggs laid after more than 80 years

Močerili iz jame Hermannshöhle v Nemčiji po več kot 80 letih odložili jajca

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Since 1931, live individuals of *Proteus anguinus,* the proteus or olm (Fig. 1), have been kept in an artificial environment in the show cave Hermannshöhle (Hermann's Cave) in Rübeland, Harz Mountains, Sachsen-Anhalt, Germany. In 1956, more animals were brought to Rübeland. Originally all animals came from the Postojnska jama and were brought unlawfully to Rübeland (Völker 1981). A total of 20 animals were taken to Rübeland over a period of 25 years (Knolle et al. 2016). In 2015, almost 60 years after the last animal had been put into the artificial lake in the cave, nine of the animals were still alive.

Figure 1. The olm in Hermann's Cave (photo: Harald Schütz).
Slika 1. Močeril iz jame Hermannshöhle (foto: Harald Schütz).

The situation in January 2015 was as follows: the total number of animals in Rübeland had dropped below 10. The sex of the animals was unclear, but there seemed to be only males. Reproduction or sexual activity had never been observed during more than 80 years. A decision was made to try to improve the conditions in order for the animals to reproduce. Since the olm is severely endangered, reproduction of the olms seemed an important objective. We also wanted to take the chance to inform the general public about this fascinating species, its origins and importance. First of all, gender determination of the animals was necessary. With the support of Olivier Guillaume from Moulis, France, we identified five females and four males. The determination of the sex was possible because all females bore eggs and males displayed aggressive behaviour. Due to nature conservation there was no authorization to mark the animals individually. All animals were in good shape (Knolle et al. 2016). According to these results, it was decided to find a grant for the further investigation of the olms in Hermann's Cave and for the optimization of the conditions of keeping it.

The objectives of the project, which was supported by Lotto Sachsen-Anhalt and Tourismusbetrieb Stadt Oberharz am Brocken and was conducted by the Harz Speleological Working Group, were: accurate observation and care of the animals, improvement of conditions for the olms in Hermann's Cave, documentation of the situation in the cave (films, photos), establishment of contacts and cooperation with experts abroad, and scientific exchange, all serving to preserve the animals in Rübeland. The project started in November 2015 (Knolle et al. 2016).

In the winter of 2015/2016, new hiding places were created in the pond. The animals started to display sexual behaviour (territorial, slightly aggressive, the females became larger) and we embarked on checking for eggs and spermatophores. In early February 2016, the first spermatophore was found and at the end of February the first egg was detected (Fig. 2).

The eggs were distributed into small basins. Altogether, six eggs were found (Knolle et al. 2016). Unfortunately, the eggs did not develop, with even the last egg drying out in late October 2016. But the animals currently show territorial behaviour and seem to be ready to reproduce.

We would like to cooperate more closely with the laboratories in charge of the olm. With the help of experts from abroad we hope to further improve the conditions in Hermann's Cave, if necessary. Our aim is to raise awareness among the general public for the needs of protecting this species. In the process we intend to promote the protection of other cave animals as well as the conservation of their habitats. We have applied for additional funding in order to study the olms more thoroughly, for example the animals could become the subject matter of a doctoral thesis.

In the meantime the continuation of the project was ensured by finding a sponsor for another year of work. We will enhance our collaboration with the University of Halle-Wittenberg and other experts.

Acknowledgements

Gregor Aljančič, Hans-Christian Anger, Michael K. Brust, Holger Fraustein, Ute and Uwe Fricke, Dr. Wolf-Rüdiger Große, Olivier Guillaume, Wolfgang Hase, Cathleen Hensel, Ingeborg Kopp, Dr. Dieter Mucke, Michael Müller, Fritz Reinboth, Ernst Schuhose, Harald Schütz, Hannes Tschorn †, Christel and Reinhard Völker, Günter Volmer, Siegfried Wielert, Marion von Oppeln, Stefan Zaenker as well as the archives of ArGeKH, VdHK, Tourismusbetrieb der Stadt Oberharz am Brocken – Rübeländer Tropfsteinhöhlen, and the State Archives of Slovenia.

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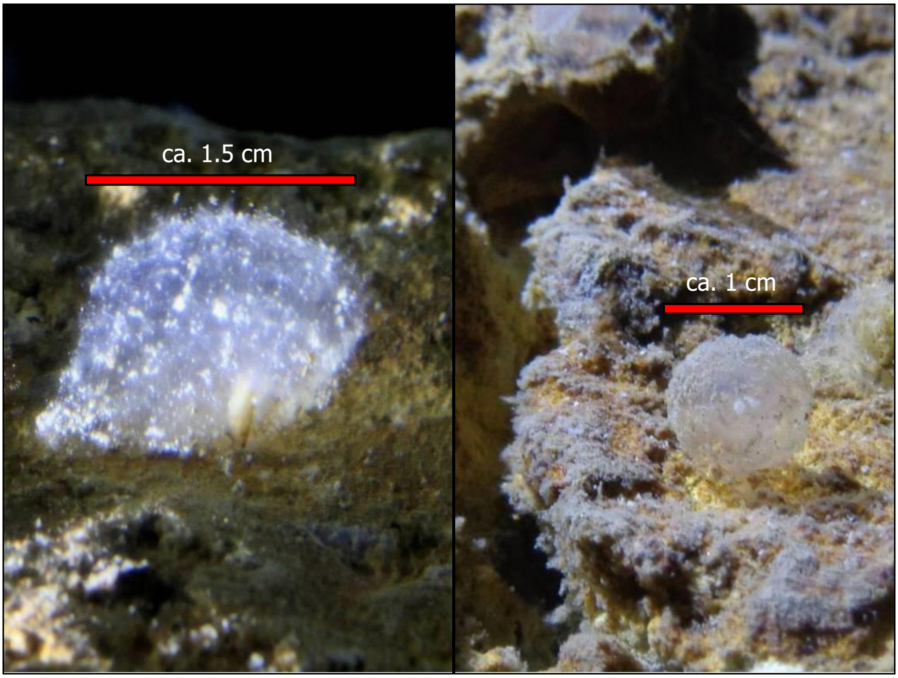

Figure 2. Spermatophore and egg of the olm in Hermann's Cave (photo: Ute Fricke).
Slika 2. Spermatofor in jajce močerila v jami Hermannshöhle (foto: Ute Fricke).

NAVODILA AVTORJEM

NATURA SLOVENIAE objavlja izvirne prispevke, ki imajo za ozadje terensko delo s področja biologije in/ali prispevajo k poznavanju favne in flore osrednje in jugovzhodne Evrope. Prispevki so lahko v obliki znanstvenih člankov, kratkih vesti ali terenskih notic.

Znanstveni članek je celovit opis izvirne raziskave in vključuje teoretično ozadje tematike, območje raziskav in metode uporabljene pri delu, podrobno predstavljene rezultate in diskusijo, sklepe ter pregled literature. Dolžina naj ne presega 20 strani.

Kratka znanstvena vest je izvirni prispevek, ki ne vsebuje podrobnega teoretičnega pregleda. Njen namen je seznaniti bralca z delnimi ali preliminarnimi rezultati raziskave. Dolžina naj ne presega petih strani.

Terenska notica je krajši prispevek o zanimivih favnističnih ali florističnih opažanjih in najdbah na področju Slovenije. Dolžina naj ne presega treh strani.

Vsi prispevki bodo recenzirani. Avtorji lahko v spremnem dopisu sami predlagajo recenzente, kljub temu pa urednik lahko izbere tudi kakšnega drugega recenzenta. Recenziran članek popravi avtor oz. avtorji sami. V primeru zavrnitve se originalne materiale skupaj z obrazložitvijo glavnega urednika vrne odgovornemu avtorju.

Prispevki, objavljeni v reviji Natura Sloveniae, ne smejo biti predhodno objavljeni ali sočasno predloženi in objavljeni v drugih revijah ali kongresnih publikacijah. Avtorji se s predložitvijo prispevkov strinjajo, da ob njihovi potrditvi, ti postanejo last revije.

Prispevke lahko oddate na naslov Natura Sloveniae, Večna pot 111, SI-1111 Ljubljana, Slovenija (telefon: (01) 423 33 70, fax: 273 390, E-mail: maja.zagmajster@bf.uni-lj.si).

FORMAT IN OBLIKA PRISPEVKA
Prispevki naj bodo napisani v programu Word for Windows, v pisavi "Times New Roman CE 12", z levo poravnavo in 3 cm robovi na A4 formatu. Med vrsticami naj bo dvojni razmak, med odstavki pa prazna vrstica. Naslov prispevka in naslovi posameznih poglavij naj bodo natisnjeni krepko v velikosti pisave 14. Latinska imena rodov in vrst morajo biti pisana ležeče. Uredniku je potrebno prispevek oddati v primerni elektronski obliki (disketa, CD, elektronska pošta) v Rich text (.rtf) ali Word document (.doc) formatu.

Naslov prispevka (v slovenskem in angleškem jeziku) mora biti informativen, jasen in kratek. Naslovu naj sledijo celotna imena avtorjev in njihovi naslovi (vključno z naslovi elektronske pošte).

Izvleček v slovenskem jeziku mora na kratko predstaviti namen, metode, rezultate in zaključke. Dolžina izvlečka naj ne presega 200 besed za znanstveni članek oziroma 100 besed za kratko znanstveno vest. Pod izvlečkom naj bodo ključne besede, ki predstavljajo področje raziskave. Njihovo število naj ne bo večje od 10. Sledi abstract in key words v angleškem jeziku, za katere velja enako kot za izvleček in ključne besede.

Glavnina prispevka znanstvenega članka in kratke znanstvene vesti je lahko pisana v slovenskem jeziku čeprav je bolj zaželjen angleški jezik. Prispevek, ki je pisan v slovenskem jeziku mora vsebovati obširnejši angleški povzetek - summary, prispevek pisan v angleškem jeziku pa obširnejši slovenski povzetek (200-500 besed). Terenska notica je v celoti napisana v angleškem jeziku, brez izvlečka, ključnih besed in povzetka. Pri oblikovanju besedil naj se avtorji zgledujejo po zadnjih številkah revije.

SLIKE IN TABELE
Skupno število slik in tabel v prispevku naj ne bo večje od 10, njihovo mesto naj bo v članku nedvoumno označeno. Posamezne tabele z legendami naj bodo na ločenih listih. Naslovi tabel naj bodo nad njimi, naslovi slik in fotografij pa pod njimi. Naslovi in legenda slik in tabel naj bodo v slovenskem in angleškem jeziku. Pri navajanju slik in tabel v tekstu uporabljajte okrajšave (npr. angl: Tab. 1 ali Tabs. 1-2, Fig. 1 ali Figs. 1-2 in slo.: Tab. 1 in Sl. 1).

NAVAJANJE LITERATURE
Navajanje literature v besedilu mora biti na ustreznem mestu. Kadar citiramo enega avtorja, pišemo Schultz (1987) ali (Schultz 1987), če sta avtorja dva (Parry & Brown 1959) in če je avtorjev več (Lubin et al. 1978). Kadar navajamo citat večih del hkrati, pišemo (Ward 1991, Pace 1992, Amman 1998). V primeru, ko citiramo več del istega avtorja objavljenih v istem letu, posamezno delo označimo s črkami (Lucas 1988a, b). Literatura naj bo urejena po abecednem redu.

Primeri:

- članke iz revij citiramo:

Schultz J.W. (1987): The origin of the spinning aparatures in spiders. Biol. Rev. 62: 123-134.

Parry D.A., Brown R.H.J. (1959): The hydraulic mechanism of the spider leg. J. Exp. Biol. 36: 654-657.

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

Lucas S. (1988a): Spiders in Brasil. Toxicon 26: 759-766.

Lucas S. (1988b): Spiders and their silks. Discovery 25: 1-4.

- knjige, poglavja iz knjig, poročila, kongresne povzetke citiramo:

Foelix R.F. (1996): Biology of spiders, 2. edition. Harvard University Press, London, pp. 155-162.

Nentwig W., Heimer S. (1987): Ecological aspects of spider webs. In: Nentwig W. (Ed.), Ecophysiology of Spiders. Springer Verlag, Berlin, 211 pp.

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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FORMAT AND FORM OF ARTICLES
Papers should be written with Word for Windows using "Times New Roman CE" size 12 font, align left and margins of 3 cm on A4 pages. Double spacing should be used between lines and paragraphs should be separated with a single empty line. The title and chapters should be written bold in font size 14. The latin names of all genera and species must be written italic. All submissions should be sent to the editor in the appropriate electronic version on diskette, CD or via e-mail in Rich text format (.rtf) or Word document (.doc) format.

Title of paper should be informative, understandable, and concise. The title should be followed by the name(s) and full adress(es) of the author(s), including E-mail adresse(s).

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ILLUSTRATIONS AND TABLES
Papers should not exceed a total of ten illustrations and/or tables, with their positon amongst the text clearly indicated by the author(s). Tables with their legends should be submitted on separate pages. Titles of tables should appear above them, and titles of illustrations and photographs below. Illustrations and tables should be cited shortly in the text (Tab. 1 or Tabs. 1-2, Fig. 1 or Figs. 1-2).

LITERATURE
References should be cited in the text as follows: a single author is cited, as Schultz (1987) or (Schultz 1987); two authors would be (Parry & Brown 1959); if a work of three or more authors is cited, (Lubin et al. 1978); and if the reference appears in several works, (Ward 1991, Pace 1992, Amman 1998). If several works by the same author published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc. (Lucas 1988a, b). The literature should be arranged in alphabetical order.

Examples (use the the following forms):

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Schultz J.W. (1987): The origin of the spinning aparatures in spiders. Biol. Rev. 62: 123-134.
Parry D.A., Brown R.H.J. (1959): The hydraulic mechanism of the spider leg. J. Exp. Biol. 36: 654-657.
Lubin Y.D., Eberhard W.G., Montgomery G.G. (1978): Webs of Miagrammopes (Araneae: Araneaidae) in the neotropics. Psyche 85: 1-13.
Lucas S. (1988a): Spiders in Brasil. Toxicon 26: 759-766.
Lucas S. (1988b): Spiders and their silks. Discovery 25: 1-4.

- for books, chapters from books, reports, and congress anthologies:

Foelix R.F. (1996): Biology of spiders, 2. edition. Harvard University Press, London, pp. 155-162.
Nentwig W., Heimer S. (1987): Ecological aspects of spider webs. In: Nentwig W. (Ed.), Ecophysiology of Spiders. Springer Verlag, Berlin, 211 pp.
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.