CEPF Final Project Completion Report

Instructions to grantees: please complete all fields, and respond to all questions, below.

Organization Legal Name	Društvo za jamsko biologijo [Society for Cave Biology]
Project Title	Assessment of the endangered subterranean biodiversity of the Skadar/Shkodra Lake Basin
CEPF GEM No.	189
Date of Report	13th January 2017

CEPF Hotspot: Mediterranean Basin Biodiversity Hotspot

Strategic Direction:
3.3 Raise awareness of the importance of priority key biodiversity areas, including those that have irreplaceable plant and marine biodiversity

Grant Amount: $19,992

Project Dates: 1st May – 31st December 2016

1. **Implementation Partners for this Project *(list each partner and explain how they were involved in the project)***

Biospeleological Society of Montenegro, Podgorica, Montenegro (Miloš Pavićević):
- standard speleobiological survey and *Proteus* eDNA sampling in Montenegro and Albania;
- preparing the database on caves and springs of Skadar Lake Basin;
- basic groundwater quality analysis in Montenegro, documenting sources of pollution in karst;
- meetings with local administration and public outreach in Montenegro;
- staff for field work.

Evolutionary Zoology Laboratory of the Institute of Biology at the Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia, http://ezlab.zrc-sazu.si (Prof. Matjaž Kuntner):
- *Proteus* eDNA isolation;
- facilities and staff of the Evolutionary Zoology Lab;
- counterpart funding for DNA analysis materials and supplies;
- DNA isolation equipment (microcentrifuge, autoclave sterilizer, deep freezers, consumables);
- IT system, digital and classical libraries;
- software license for GIS (ESRI ArcGIS 10.3.1 with extensions).

Department of Life Sciences, University of Trieste, Trieste, Italy, http://dsv.units.it/en (Prof. Alberto Pallavicini, qPCR and NGS supervisor; Dr. David Stanković, qPCR and NGS analysis key expert).

- qPCR analysis (*Proteus* eDNA);
- facilities and staff of the Department of Life Sciences.

Department of Biology and Chemistry at the Faculty of Natural Sciences, University of Shkodra "Luigj Gurakuqi", Shkodër, Albania, http://unishk.edu.al/en/node/90 (Denik Ulqini):
- host of the workshop "Conservation of cave biodiversity in southeast Dinaric Karst";
- digital and classical libraries;
- sterilizing materials for *Proteus* eDNA filtering (Prof. Nevila Bushati);
- networking for protection of Skadar Lake ecosystems.

Network for the Affirmation of NGO Sector (http://www.mans.co.me), **NGO Center for Protection and Research of Birds of Montenegro** (http://www.birdwatchingmn.org/) and **NGO Green Home** (http://www.greenhome.co.me/):
- networking for protection of the Morača River and Skadar Lake ecosystems;
- sharing information and nature conservation arguments.

Other stakeholders
Department of Biology, University of Ljubljana, Slovenia, http://www.bf.uni-lj.si/en/biology/department/ (Prof. Cene Fišer); **Karst Research Institute at the Scientific Research Centre of the Slovenian Academy of Sciences and Arts**, Postojna, Slovenia, http://izrk.zrc-sazu.si/en#v (Prof. Tanja Pipan); **Montenegrin Academy of Sciences and Arts, Montenegro**, http://www.canuorg.me/cmsen/index.htm **(**Acad. Gordan Karaman):
- determination of collected subterranean taxa.

Conservation Impacts

2. Describe how your project has contributed to the implementation of the CEPF investment strategy set out in the ecosystem profile

The project was contributing to the implementation of the CEPF investment strategy set out in the ecosystem profile through the following Strategic Directions:

3.3. Raise awareness of the importance of priority key biodiversity areas, including those that have irreplaceable plant and marine biodiversity

Detailed investigation brought solid evidence on *Proteus* distribution in Montenegro. Sampling of cave fauna and contributed to the knowledge on its diversity.

4.1. Build a broad constituency of civil society groups working across institutional and political boundaries toward achieving the shared conservation goals described in the ecosystem

Slovenia–Montenegro–Albania trans-border cooperation (NGOs, local communities and GOs) for the conservation of the endangered subterranean biodiversity and protection of groundwater was established through organizing events and meetings, study visits and courses.

3. Summarize the overall results/impact of your project

3. 1. **Assessment of the endangered subterranean biodiversity**

- detailed survey of the distribution of *Proteus anguinus* by environmental DNA sampling in the Skadar Lake Basin and at the selected localities in Western Montenegro (25) and Northern Albania (11);
- *Proteus* eDNA first time fully confirmed in groundwater of Montenegro (2), reaching the Skadar Lake. Less clear but present was the eDNA trace found in additional karst springs in Montenegro (3);
- pilot sampling of *Proteus* eDNA in the Dinaric Karst of Albania was negative (2);
- pilot inventory of subterranean biodiversity and establishment of the cave registry draft of the Skadar Lake Basin (29 caves and karst springs).

3.2. **Public promotion and academic outreach**

- first international speleobiological workshop "Conservation of the cave biodiversity in Southeast Dinaric Karst " organized in Albania;
- academic dissemination on international scientific and conservation meetings (4);
- popular science lecture on *Proteus* and threats to drinking water for pupils coming from rural primary schools (1);
- the mission of the project and CEPF strategic directions explained to general public within promotion of the project on internet (6) and social media (Montenegro, Albania, Slovenia).

3.3. **Extending the Trans-Balkan conservation alliance, capacity building**

- specialized training for caving and cave fauna sampling techniques (5 days, 14 participants);
- study visits by conservationists from Montenegro (3) and Albania (3) at the Tular Cave Laboratory.

Planned Long-term Impacts - 3+ years (as stated in the approved proposal)

- New eDNA method to detect endangered aquatic subterranean biodiversity
- Public promotion and academic outreach
- Extended Trans-Balkan conservation alliance & capacity building

4. Actual progress toward long-term impacts at completion

Planned Short-term Impacts - 1 to 3 years (as stated in the approved proposal)

- Assessment of the subterranean biodiversity in Skadar Lake Basin
- Geographic Information System (GIS) analysis
- Public education, capacity building and academic outreach
- Extending the Trans-Balkan conservation alliance, capacity building

5. Actual progress toward short-term impacts at completion

5.1 - Assessment of the subterranean biodiversity in Skadar Lake Basin

The assessment was made through an information table of all localities (36 caves and karst springs) visited and/or analysed in Montenegro and Albania, including WGS84 coordinate data and sampling type (*Proteus* eDNA, other cave fauna) and a distribution map of *Proteus*.
The survey revealed new localities, but also confirmed weak traces of *Proteus* eDNA discovered during the previous project (see map on Fig. 13).
The results extended the previous known habitat of *Proteus* within the Dinaric karst of Montenegro, based on eDNA traces, as well as documenting new springs and caves with high importance for groundwater resources and as a habitat for diverse cave fauna.
The proposed test of a method based on next generation sequencing techniques (NGS) was cancelled after a dry summer season and low level of groundwater in Montenegro delayed the plan, and was realized that the hard task could not be achieved in the already very short time given in this project.

5.2 Geographic Information System (GIS) analysis
An updated GIS database in Esri format, ready to use, containing the eDNA results for *Proteus* analysis as well as all localities visited, sampled and/or analysed in Montenegro and Albania is now available (an XML format was used in order to share the GIS data - in Supplement materials; the XML format allows the easy conversion in Esri geodatabase by the end user). The new distribution map with eDNA results (classified as positive, plausible, suspicious, and negative results) is now available, along with the distribution map of eDNA results from 2014. The same area of interest was used on both maps in order to better visualise the progress of the discoveries of eDNA traces in Montenegro and the new sites visited and/or analysed from Albania (details in COMPONENT 1, Activity no. 5).

For the reasons explained at the COMPONENT 1, Activity no. 3, GIS database does not contain any result from the NGS method proposed initially to assess the overall groundwater biodiversity of Skadar/Shkodra Lake. Also, the distribution map of overall presentation of the cave biodiversity in the studied areas in Montenegro and Albania was not delivered, since the gathered samples from caves are in the process of determination at taxonomy experts.

5.3 - Public education, capacity building and academic outreach
During the project the following activities were successful completed:
- Workshop (1) "*Conservation of cave biodiversity in Southeast Dinaric Karst*", on 29th October 2016 in Shkoder, Albania (details in COMPONENT 3, Activity no. 12);
- Courses for NGO capacity building in Albania (2): 5-day basic course in caving techniques (safety regulations, horizontal caving skills) combined with 3-day basic course in cave fauna sampling techniques with introductory caving trips (details in COMPONENT 3, Activity no. 13-14);
- Public education (1): one-day popular science lecture on *Proteus* and protection of its habitat trips (details in COMPONENT 2, Activity no. 8);
- Interviews and articles in media (6), social network posting (over 12): details in COMPONENT 2, Activity no. 10;
- Academic outreach (4) – presentation of the project within international specialized scientific meetings (details in COMPONENT 2, Activity no. 11).

Table 1: Project outputs (proposed vs. achieved)

Project outputs/ Results	Planned (direct, measurable changes of activities will achieve)	Proposed measure of these results? (indicators)	Achieved measure of these results? (indicators)	How can we verify you have achieved these results? (means of verification)	Where are the achieved results
1	Terrestrial and aquatic subterranean invertebrate biodiversity surveyed in the Skadar Lake Basin (Montenegro and Albania), using standard sampling methods at 30 sites (caves, karst springs and wells), of which approximately 25 are in Montenegro and 5 in Albania	Number of caves examined (total of 30 sites)	Number of caves visited (total of 36 sites)	Photo and data submitted	In final report + supplement materials
2	*Proteus* distribution by eDNA sampling surveyed in selected localities in Montenegro (25) and for the first time in Albania (5), with focus on potential localities in Skadar Lake Basin	The results will be mapped and classified as positive, plausible, suspicious, and negative results	The results are mapped and classified as positive, plausible, suspicious, and negative results	Results of analysis submitted	In final report + supplement materials
3	New eDNA method to detect the traces of the invertebrate aquatic subterranean fauna living in caves or karst springs based on next generation sequencing techniques (method tested on 10 samples from researched caves in Slovenia, applied on 20 samples from localities in Montenegro and Albania	Method developed and tested	Method was not developed due to under-explored region contain taxa whose sequences are not deposited into GenBank.	Results of test submitted	No results submitted
4	Cave and Speleobiological Registry of Skadar Lake Basin (Montenegro and Albania): database with basic data of caves and karst springs	GIS database in Esri data format with necessary attribute table, in English	All visited localities (caves and springs) are included in the same GIS database as the eDNA results	GIS database submitted	GIS database submitted as XML file in Supplement materials
5	Integrated GIS database and distribution map of present cave biodiversity in the studied areas in Montenegro and Albania	Analysis completed	Partial results – only sites are mapped, not the overall cave biodiversity	Resulted maps and database submitted	GIS database submitted as XML file in Supplement materials
6	Basic groundwater quality analysis in Montenegro at 5 selected localities	Analysis completed	Analysis not realized (samples not collected)	Results of analysis submitted	Results of analysis not submitted

7	Meetings with local administration	Number of meetings	Meeting at the Environment Protection Agency of Montenegro	List submitted	List submitted
8	Presentations (3) in selected rural primary schools in Montenegro	Presentation performed	One day presentation for more than 150 pupils and teachers from all over Montenegro	Photo submitted	Poster of the event + link with the program submitted in the report and Supplement materials
9	Educative leaflets (Montenegro and Albania editions; 3,000 copies)	Number of printed leaflets	Output not realized	Leaflets delivered	Leaflets not delivered
10	Promotion of project goals on social media, local and national media (Montenegro, Albania, Slovenia; 6)	Published articles	6 published articles in online media and TV, and more than 12 publications in social media	Link	Links provided in this final report
11	Academic dissemination: presentations on international scientific and conservation meetings (1)	Abstract submission, registration on conference	4 presentations in the international scientific meetings	Published abstract	Links to abstracts, links to photos of the events (in the final report), Poster (in Supplement materials)
12	International workshop in Shkodër/Skadar (Albania) on conservation of subterranean biodiversity of Montenegro and Albania	Invitations, online registration of lectures and participants, delivering the program of the workshop	Activity realized	Photo submitted, abstracts collection, weblink to event	Photos, activity description and link to event; invitations, first circular, detailed program in the Supplement materials
13	Meetings with local administration (3) in Montenegro and Albania	Number of meetings	2 meetings realized	List submitted	List submitted
14	NGO capacity building in Albania: 5-day basic course in caving techniques (safety regulations, horizontal caving skills)	Invitations, online registration of participants, delivering the program of the course	Activity realized	Number of participants, photo submitted	14 participants, activity description and weblink to event in the final report; invitations, first circular, detailed report in the Supplement materials

15	NGO capacity building in Albania: 3-day basic course in cave fauna sampling techniques	Invitations, online registration of participants, delivering the program of the course	Activity realized	Number of participants, photo submitted	14 participants, activity description and weblink to event in the final report; invitations, first circular, detailed report in the Supplement materials
16	Visit of the Tular Cave Laboratory (Slovenia) by speleobiologists from Montenegro and Albania	number of participants	Activity realized	Photo submitted	6 participants (3 from each country); activity description and weblink to event, invitations, first circular, detailed report on Albanian conservationists visit in the Supplement materials

6. Describe the success or challenges of the project toward achieving its short-term and long-term impacts

Success:

- eDNA method developed and tested in the previous project (2013-2014) was this time replicated in a detailed survey of Montenegro, extended to a pilot search in the northern Albania along the Shkodra Lake;
- eDNA analyses recorded first evidence of *Proteus* in Montenegro, supporting the plausible detections established in the previous project. One of the positive sites detected in this project represents the most southern presence of the globally threatened cave amphibian *Proteus*, hidden in the Dinaric Karst resurgence of the Skadar/Skodra Lake (see map on Fig. 13);
- first international workshop on cave biodiversity organized in Albania and cave training for Albanian conservationists to develop new capacity in this important key biodiversity area;
- search for contacts and networking was the key to successful collaboration during our field work, as well as in the outreach activities and dissemination.
- involving Albanian and Montenegrin participants into the project to collaborate in writing reports of their activities, press release invitations, to give presentations in workshop on their recent research.

Challenges

- Lack of any contact or information regarding speleobiology or caving in Albania critically delayed the preparation of fieldwork activities on the Albanian side of the Skadar/Shkodra Lake. The initial specialized contact came from Dr. Philippe Theou, a French researcher studying bats of Albania,

opened urgently needed contacts with the Albanian nature conservation NGOs, which became later actively involved in the project.

- Development of sampling and filtering methodology during field work, in improvised laboratories, and transportation of delicate samples back to Slovenia for eDNA isolation. With the growing network, support of a specialized laboratory in Montenegro and Albania were used to sterilize filtering equipment, an important step to avoid contamination and in achieving accurate results.

- Lack of accurate and homogeneous topographical, hydrogeological and geological data (particularly in Albania) obstructed the fieldwork and building the distribution maps. In order to address this challenge within the GIS analyses and statistics, we used mainly the European environmental and terrain open data, since most of such data are not publicly available in Albania and Montenegro.

The gathering data process was slow and mainly done during the fieldwork, reflected in lower number of water samples of *Proteus* eDNA in Albania (only for 2 localities) and standard sampling for cave invertebrates.

7. **Were there any unexpected impacts (positive or negative)?**
   The effort put in communication and networking during field-trips and workshop in Albania received high attention and involvement of local communities and regional nature conservation NGO sector. This unexpectedly strong support was reflected the success of events, trainings and collaborations with Albanian participants and Montenegrin partner and hopefully better awareness towards long-term protection of cave biodiversity.

Project Components and Products/Deliverables

List each component and product/deliverable from Grant Writer

8. **Describe the results from Component 1 and each product/deliverable**
9. **Repeat point 8 above for each Component in your approved proposal**

COMPONENT 1: ASSESSMENT OF THE ENDANGERED SUBTERRANEAN BIODIVERSITY IN SKADAR/SHKODRA LAKE BASIN (MONTENEGRO AND ALBANIA)

Context of the project

In 2013-2014, SCB successfully implemented the project "Monitoring of *Proteus anguinus* by environmental DNA sampling", in Bosnia and Herzegovina and Montenegro, within a project co-financed by the Critical Ecosystem Partnership Fund, BirdLife International and DOPPS (see project outcomes:
http://www.cepf.net/SiteCollectionDocuments/mediterranean/FinalReport_SCB.pdf)
This grant highlighted to CEPF and the RIT the current gap of knowledge on the subterranean biodiversity within the Mediterranean Ecosystem Profile, and the need for greater data collection on this fragile and often-overlooked habitat. Further work is now needed, focusing especially in the Skadar Lake Basin (Montenegro and Albania).

Activity 1: **TERRESTRIAL AND AQUATIC SUBTERRANEAN INVERTEBRATE BIODIVERSITY STANDARD FIELD SURVEY**

Terrestrial and aquatic subterranean fauna was sampled using standard methods (see Schneider & Culver, 2004) at 8 localities in Montenegro and at 8 localities in Albania. Sampling was not

successful because of dry summer season and low level of groundwater in Montenegro, for which cave fauna was rarely present in the less favorable seasonal conditions of the accessible parts of caves.

Fig. 1. Karst terrain above Trnovo, Montenegro.
Photo: G. Aljančič

Fig. 2. Collecting cave fauna in Cetinjska pećina.
Photo: M. Pavićević

During each visit, caves and its habitats were photographed; temperature of air and groundwater was taken. Traces of pollution and other negative anthropogenic impacts were also documented. Cave was visually inspected; any terrestrial or aquatic organisms were recorded and photographed when possible, only specimens of potential subterranean taxa were collected for determination. Terrestrial specimens were collected by hand, aquatic by water net. No traps were used during this project, except during a pilot survey of the epikarst fauna in the Grbočica Cave. Presence of bats and fish was checked particularly in caves of Albania. At the time of writing this final report, specimens of cave fauna collected during this project are in the process of determination by taxonomists specialized on the taxa in question. Finally, these specimens will be deposited at the national natural history museums in Podgorica and Tirana.

Montenegro

Fig. 3. Rijeka Crnojevića flows into the Skadar/Shkodra Lake. Photo: G. Aljančič

Speleobiological investigation of Montenegrin caves was organized by the Biospeleological Society of Montenegro. List of investigated caves was ere obtained from the previous records of the Biospeleological Society of Montenegro, several karst springs were identified during the project by support of the SCB and local communities.

Fig. 4. Setting traps for epikarst fauna. Photo: Photo: G. Aljančič.

Fig. 5. Cave Obodska Pećina. Photo: M. Pavićević.

Montenegro field-work was organized in five rounds, 16 days spent in total:

- July 27 – August 3, 2016 (Biospeleological Society of Montenegro, Society for Cave Biology),
- August 30 (Biospeleological Society of Montenegro),
- September 15–20, 2016 (Biospeleological Society of Montenegro),
- October 25, 2016 (Biospeleological Society of Montenegro, Society for Cave Biology),

- December 4, 2016 (Biospeleological Society of Montenegro).

From 25 localities (caves and karst springs) visited in Montenegro, the data on cave fauna was collected from 8 caves, while 12 localities were tested for *Proteus* eDNA.

Three filtering devices to collect epikarst fauna, mostly copepods, from drips were set in Grbočica Cave (sampling containers kindly provided by Prof. Tanja Pipan). These traps were set under three distinct drips from the cave ceiling, continuously collecting organisms washed from the epikarst by percolating water from 21st October to 4th December 2016.

Albania

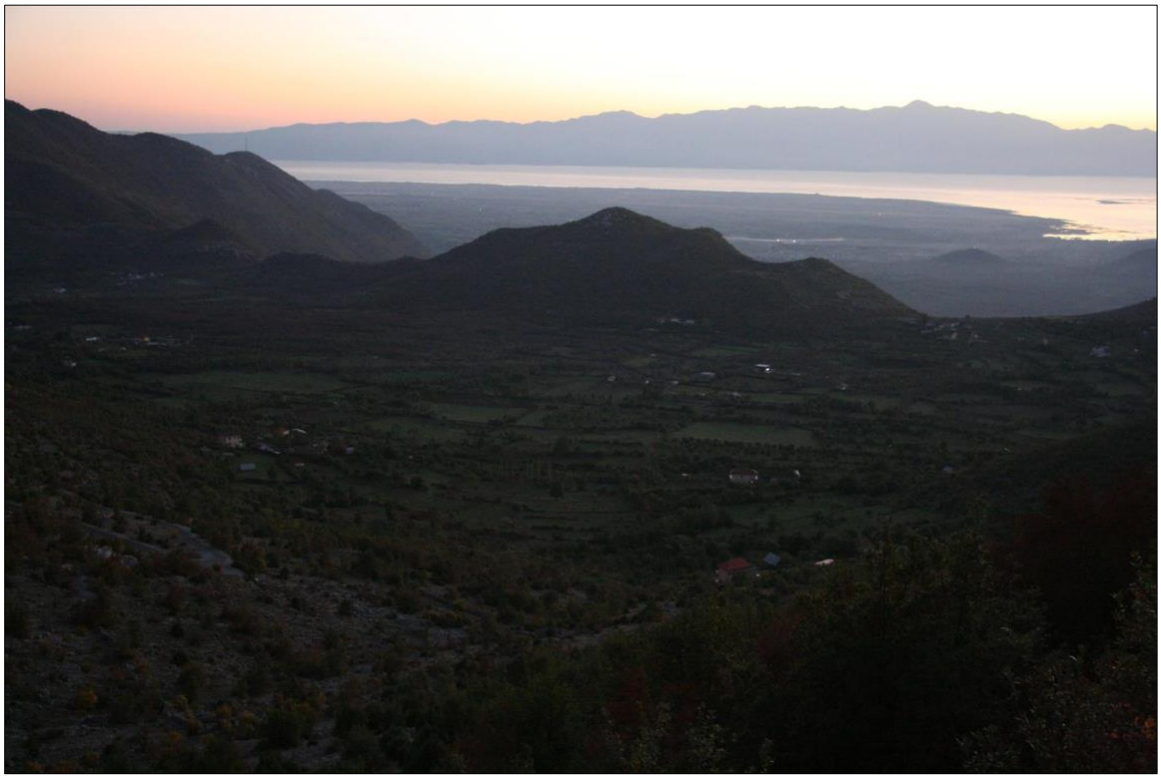

Fig. 6. Bratosh above the Skadar/Shkodra Lake. Photo: G. Aljančič

Although very short time available to plan and execute the fieldwork in Albania, with limited information on caves and karst springs in the Shkoder area, we consider this activity very successful and productive. Due to the previous unknown karst area and lack of available information on potential localities, we registered two water caves, previously not known to researchers.

Albania fieldwork was organized in two rounds, during 7 days, as following:
- October 19–23, 2016 (Society for Cave Biology, Biospeleological Society of Montenegro),
- October 24–30, 2016 (Society for Cave Biology).

From 11 localities visited in Albania (Cave Shpella e Krevenices, Cave Shala e Kastratit, Cave Shpella e Pëllumbave, Cave Shpella e Zef Toma, tunnels near Shpella e Zef Toma 1-3, Spring Syri i

Sheganit, Cave Shpella e Pëllumbave, Cave Shpella e Gurres, Cave Shpella e Gjules (Shpella e Gjolave), tunnels near Bratosh, Spring Gruemire), the data on cave fauna was collected in 7 localities, while 2 caves were tested for *Proteus* eDNA (Cave Shpella e Krevenices, Cave Shala e Kastratit).

Fig. 7 & 8. Entrance to water cave Shpella e Krevenices (left); pumping of groundwater through vertical entrance. Photo: G. Aljančič.

First information on caves came from previous Bulgarian-Albanian caving expeditions during 1990', recent data was kindly contributed by Dr. Philippe Théou. Information on caves was gathered also from local communities during the fieldwork, hence two water caves were discovered and documented (caves Shala e Kastratit and "Shpella me pëllumba" near Vukpalaj – Bajzë), and large chambers were explored in one of the previously known vertical caves (Cave Shpella e Zef Toma near Vukpalaj – Bajzë). Special attention was put into checking for the presence of bats, small mammals and fish in caves, data on observation was shared with the NGO Protection and Preservation of Natural Environment in Albania (PPNEA) and the Department of Biology and Chemistry at the Faculty of Natural Sciences, University of Shkodra.

Fig. 9. *Apfelbeckia* in the cave Shpella e Zef Toma.
Photo: G. Aljančič

Fig. 10. Siphon in the cave Shala e Kastratit.
Photo: G. Aljančič

Details on sampled and visited localities from both countries in Table 3 in the supplement, available at the corresponding address of the Society for Cave biology upon request.

Activity 2: **eDNA *Proteus* SURVEY (SAMPLING, FILTERING, ISOLATING DNA, qPCR ANALYSIS)**
The present project replicated the eDNA method developed within the project "A survey of the distribution of *Proteus anguinus* by environmental DNA sampling" (2013–2014) for a detailed examination of the previously detected trace of *Proteus* in Montenegro, and to extend the sampling towards the Skadar Lake Basin. The eDNA *Proteus* survey followed the protocol

described in the previous project (see Final report, p. 8–10). Using such DNA-based identification tool, we rechecked the first traces of *Proteus* eDNA discovered during a pilot test in our previous project.

Results of this project for the first time unequivocally established the presence of *Proteus* in groundwater of Montenegro at two sites, and found its likely traces at additional four sites. *Proteus* was confirmed in the Boka Kotorska Bay in Adriatic, and Rijeka Crnojevića at the beginning of the Skadar/Shkodra Lake (see map on Fig. 13). The SYBR chemistry-coupled real-time PCR approach was again shown to be very successful and time-efficient method for detection and monitoring of *Proteus,* now applied with fidelity at its most southeastern range of occurrence.

From 36 caves or karst springs, 16 localities from Montenegro and 2 from Albania were sampled, from 10 to 20 l of water per locality. Finally, 12 caves or karst springs were analyzed from Montenegro and 2 from Albania. Water samples were collected and filtered by the Biospeleological Society of Montenegro and the Society for Cave Biology, in field laboratories in Podgorica and Shkoder. Rigorous controls for preventing contamination were employed, including commercial decontamination solutions (DNA-ExitusPlus solution, AppliChem), chlorine-, autoclave- and UV-based sterilization of all re-usable materials, and workspace decontamination with DNA-ExitusPlus solution.
Filters with eDNA were transported to Slovenia, where DNA was extracted using bead beating and the PowerWater DNA isolation kit (MO BIO Laboratories), with the final elution volume at 50 µl per sample by Gregor Aljančič. Extraction was conducted at the Evolutionary Zoology Laboratory of the Institute of Biology at the Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia (Dr. Ren-Chung Cheng, assistant; Prof. Matjaž Kuntner, supervisor).
Finally, samples were analyzed in SYBR chemistry based real-time PCR assay by Dr. David Stanković at the Department of Life Sciences, University of Trieste, Italy (Prof. Alberto Pallavicini, supervisor). Two sets of specific primers were used to amplify short conserved fragments of *Proteus anguinus* mitochondrial DNA (16S rDNA gene, 153 bp and in the control region, 106 bp) if present in the sample. To prevent contamination, DNA extraction was conducted in a building where no post-PCR work or extraction of DNA from *Proteus* tissue samples is conducted.

Table 2: Results of the eDNA survey in Montenegro and Albania.

Country	Visited localities	Analyzed samples	eDNA positive locality	eDNA plausible/ suspicious	eDNA negative locality
MONTENEGRO	25	16	2	4	10
ALBANIA	11	2	0	0	2
Total	**36**	**18**	**2**	**4**	**12**

Montenegro field-work was organized in four rounds, 14 days spent in total:
- July 27 – August 3, 2016 (Biospeleological Society of Montenegro, Society for Cave Biology),
- August 30 (Biospeleological Society of Montenegro),
- September 15–20, 2016 (Biospeleological Society of Montenegro),
- October 21–25, 2016 (Biospeleological Society of Montenegro, Society for Cave Biology),

Albania field-work was organized in two rounds, 9 days spent in total:

- October 19–23, 2016 (Society for Cave Biology, Biospeleological Society of Montenegro),
- October 24–30, 2016 (Society for Cave Biology).

Activity 3: **CALIBRATING NEW eDNA METHOD TO DETECT THE TRACES OF THE INVERTEBRATE AQUATIC SUBTERRANEAN FAUNA**

The proposed pilot of a *next generation sequencing* (NGS) based method was the most challenging activity of this project. Though the available 8 months of the project and funds were not sufficient for completing such cutting-edge task, first models revealed that the present knowledge on subterranean biodiversity in Montenegro, particularly in Albania is insufficient, with little up-to-date information on of what is living in caves. While recently Montenegro started to be more involved in biospeleological research, Albania is critically lacking molecular phylogeny research on subterranean fauna. Without basic GenBank library for the taxa identification, monitoring with NGS methods would give only higher taxonomic category (i.e., genus, family, order).

Although both countries are harboring a potential subterranean hotspot, the current gap of DNA sequences of cave organisms from Albania in GenBank did not support further development of this method in available time of the project. For these reasons, we decided to cancel this activity and to focus on more on success of eDNA sampling.

Activity 4: **PREPARING THE CAVE AND SPELEOBIOLOGICAL REGISTRY OF SKADAR LAKE BASIN**

During this project we were piloting the cave and speleobiological registry of the Skadar Lake Basin. Through our fieldworks and literature research we identified and visited 29 caves and karst spring (18 in Montenegro, 11 in Albania). As reported under Activity 2 some of them were sampled for *Proteus* eDNA detection, while others we sampling for cave fauna with standard methods (samples in the process of determination by taxonomists).

Table with caves and springs identified during this project, their coordinates, approximate length, status of cave fauna sampling, eDNA results and comment on pollution (in the supplement, available at the corresponding address of the Society for Cave biology or the Biospeleological Society of Montenegro upon request).

Activity 5: **CONCEIVING AND BUILDING AN INTEGRATED GIS DATABASE AND DISTRIBUTION MAP OF PRESENT CAVE BIODIVERSITY**

GEOGRAPHIC INFORMATION SYSTEM (GIS) *ANALY*SIS: GIS database and maps of *Proteus* distribution based on eDNA traces in key areas.

The GIS analysis was carried out in two phases, contributing to the project field work planning, data visualization and results assessment. The database and the maps were produced progressively in ArcGIS 10.3.1 software by Magdalena Năpăruş-Aljančič.

5.1 Inventory of existing data on distribution of *Proteus* and hydrogeology

5.1.1. Montenegro.

We used the updated distribution maps of *Proteus* in Montenegro resulted from the project "A survey of the distribution of *Proteus anguinus* by environmental DNA sampling" (2013-2014) to choose the potential sampling sites. The hydrogeological connections were mapped according with the available hydrogeological map for Dinaric karst

(http://diktas.iwlearn.org/im/hydrogeological-map-of-the-dinaric-karst).

Several localities which showed *Proteus* eDNA signal in previous survey were resampled, potential areas identified during the past project were included in the present survey. The Biospeleological Society of Montenegro initially proposed 16 caves and springs from Montenegrin side of the Skadar Lake Basin to be sampled, and due to the lack of publications on Albanian karst, only one locality for Albania (Fig. 11).

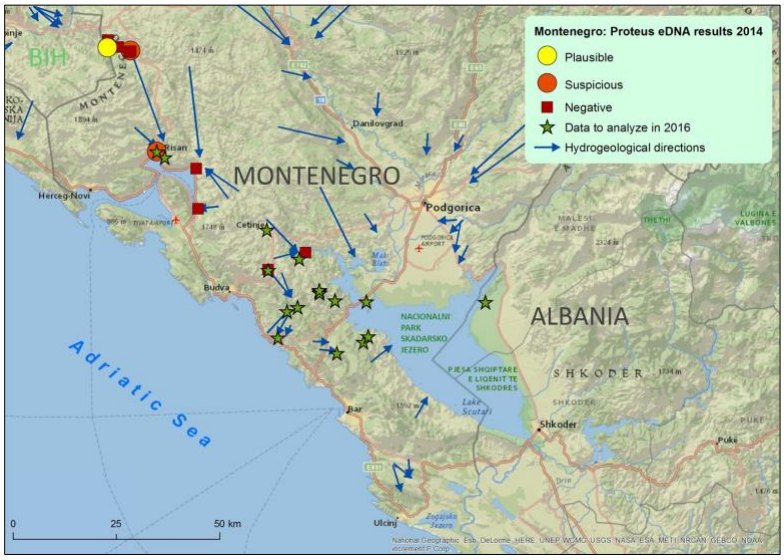

Fig. 11: Proposed localities for sampling in 2016. Map: Magdalena Năpăruş Aljančič.

5.1.2. Albania.

While the amount of available data on caves and springs is insufficient in Montenegro, the Albanian part of the Dinaric Karst is critically under-investigated. The main difficulty we encountered was lack of any specialized contact person.

The first coordinates to upgrade our GIS database within the area of interest were obtained with the help of Dr. Philippe Theou, a French researcher studying bats in Albania. Another problem we encountered in Albanian data analysis was the lack of details for terrain check and the road infrastructure to access them.

The GIS database for Albania had to be updated mainly during the fieldwork, by taking accurate GPS coordinates and/or discovering new localities while checking for known ones. The missing available information on hydrogeology from Albanian maps was another impediment of an accurate search for localities to be included in the eDNA sampling process. Most of the localities included in the GIS database were potential only for cave invertebrates sampling.

5.1.3. The GIS database and results

The locality data contain the basic information including the name of the locality, location, WGS coordinates expressed in decimal degrees, type of locality (cave, spring, well), elevation, water temperature, eDNA results 2016 ('+' positive on both genes; '?' plausible on one gene; '(-)' suspicious on one gene; '-' negatives results; only visited).

The final GIS database contains the point feature classes to map the visited localities and results of the eDNA analysis is available as .xml format in the *Supplemental materials*. This data format permits easy import in *ArcGIS File Geodatabase* format to be further used or visualised.

The 2016 distribution map of *Proteus* eDNA finds in Montenegro and Albania contain the eDNA results for *Proteus* eDNA (green = positive; orange = plausible; yellow = suspicious and red = negative); visited localities with no eDNA analysis (grey) and hydrogeological connections (blue arrows). The results are overlapped on World Open Street basemap. In order to better identify the progress of discoveries from 2014 to 2016, we choose to represent the same area, including Albanian side of area of interest (Skadar/Shkodra Lake) (Fig. 11 & 12).

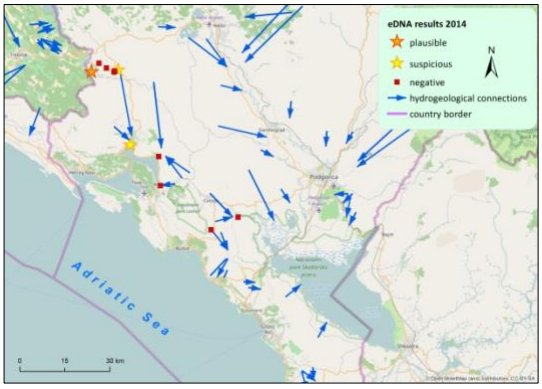

Fig. 12. 2014 distribution map of *Proteus* eDNA results in Montenegro. Map: Magdalena Năpăruş Aljančič.

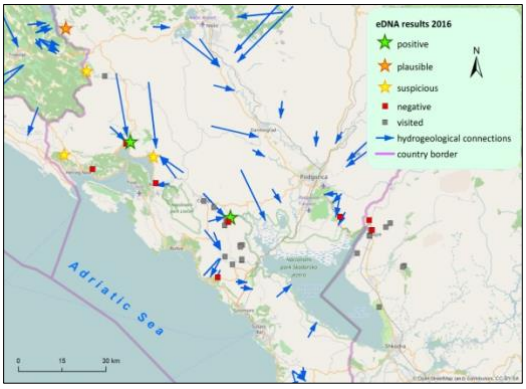

Fig. 13. 2016 distribution map of *Proteus* eDNA results in Montenegro and Albania. Map: Magdalena Năpăruş Aljančič.

Within the GIS database we gather the following data:

Montenegro

From 25 localities (caves and karst springs) visited in Montenegro, 12 localities were tested for eDNA, while the data on cave fauna was collected in 8 caves.

Albania

From 11 localities visited in Albania, 2 were tested for eDNA, while the data on cave fauna was collected in 7 localities.

Activity 6: **BASIC GROUNDWATER QUALITY ANALYSIS IN MONTENEGRO, DOCUMENTED SOURCES OF POLLUTION**

By monitoring the quality of water the Geological Survey of Montenegro documented significant change of quality compared to previous years. More attention has been given to major karst springs in the background of the Skadar Lake. As it has been shown before, concentration of Na, phosphates, sulphates, nitrates and Cl are raising. In the cave Obodska pećina and spring of Obodska River it has been caused by direct inflow of waste waters into underground by underground channels from Cetinje. Springs of Podgorska also show increased concentrations of these substances. The rising water in the Skadar Lake south edge originates from Dinaric Mountains and is contaminated by the dirty waters of Cetinje. Increased concentration of sulfates and phosphates comes from fertilizers that are used for treatment of several vineyards, for which this area is recognized. Increased concentration of these chemical compounds has been measured in lake's water and water's inflow. The dramatic pollution has been detected during intensive

short-term rains. Results of these researches show the changing level of natural characteristics of water. It's been concluded that this state is caused by inflow of dirty waters from land areas of confluential surfaces. Changing level of natural attributes of water is also shown by values of used indicator.

The cave Obodska pećina mentioned above has also been positive on Proteus eDNA, hence more attention should be put on urgent detecting and removing these sources of pollution. The activity was not accomplished because the partner Biospeleological Society of Montenegro did not collect water samples for the analysis.

COMPONENT 2: PUBLIC PROMOTION AND ACADEMIC OUTREACH

Activity 7: **MEETINGS WITH LOCAL ADMINISTRATION**

During the project Biospeleological Society of Montenegro organized 2 meetings with Duško Mrdak at the Environment Protection Agency of Montenegro. The topics of the meetings were covering the current situation of biospeleological research in Montenegro and the necessary steps towards a proper legislation framework in order to preserve the potential cave biodiversity and to include as soon possible among the highly protected species the *Proteus.* Discussions were productive and open new doors for further communication towards the urgent protection of caves and their endangered biodiversity.

Activity 8: **PRESENTATIONS IN SELECTED RURAL PRIMARY SCHOOLS**

This activity was planned to be conducted in rural primary schools.

On 29th of September, during the annual national event dedicated to science: "Dani nauke" [Days of science] (www.daninauke.me), organized at the Biology Department, Faculty of Natural Sciences, University of Podgorica, Jelena Popović from Biospeleological Society of Montenegro was giving popular science lectures on *Proteus*: "Zmajeva beba: Čovječja ribica-endem područja Dinarida. Kako je dobila ime? Ima li je u CG?" [Dragon baby: the human fish – endemic species of Dinarids. How it get the name? It lives also in Montenegro?] (Fig. 14; http://daninauke.me/dani-nauke-2016-cudesni-svijet-biologije-hemije/).

The 30 minutes lecture was repeated during whole day and attended by more than 150 pupils and their teachers from primary to secondary schools from all over Montenegro. The lecture was focused on popular science facts on *Proteus*, karst and clean drinking water, received excellent feedback from audience with many questions and interesting comments. The discovery of *Proteus* eDNA traces in Montenegro from 2014, as well as this year continuation of the project were mention during the presentation.

Fig. 14. Poster at Days of Science in Podgorica (Jelena Popović).

Activity 9: **PREPARING AND DELIVERING EDUCATIONAL LEAFLETS**
The activity was initially postponed towards the end of the project, in order to establish collaborations with Albanian NGOs, to prepare a cross boundary message on conservation of cave biodiversity. Based on the experience from previous project, when our Montenegrin partner helped the preparation of leaflets, we encouraged them to overtake this activity together with the Albanian colleagues. Due to unexpected personal tragic events of our partner, the activity was not finalized until the end of the project.

Activity 10: **PROMOTION IN MEDIA (SOCIAL MEDIA, LOCAL AND NATIONAL MEDIA)**
The promotion of the project was made mainly via Tular Cave Laboratory' FB and Twitter page and FB pages of project partners.
The project was presented in Slovene national TV, within a popular science show on *Proteus* and karst, "Ugriznimo znanost" [Let's Bite science] (http://4d.rtvslo.si/arhiv/ugriznimo-znanost/174440505; https://www.facebook.com/176703559080895/videos/1147498122001429/?hc_ref=PAGES_TIMELINE), and popular magazine Jana (http://govori.se/zanimivosti/pazimo-na-crno-clovesko-ribico/). The project was also presented in a popular Slovene scientific podcast "Metamorfoza" dedicated to *Proteus* and Tular Cave Laboratory:
http://metinalista.si/metamorfoza-022-cloveska-ribica/

One of the best promotions with very high impact in scientific community was made in Science Magazine online journal of the eDNA method developed by SCB during 2014 CEPF project, linking directly to the previous project Final report from at CEPF webpage.
http://www.sciencemag.org/news/2016/06/crime-solving-technique-maps-underground-lair-slovenian-dragon

Other international media linking this year project or previous project (2013-2014):
Conservation International: http://blog.conservation.org/2016/04/dna-test-confirms-presence-of-cave-giants-in-europe/
Regional Environmental Center Albania: http://mjedisisot.info/index.php/natyre/2185-ne-kerkim-te-peshkut-njeri-dhe-ne-shqiperi

Promotion on social media:
Tular Cave Laboratory FB page:
Tular Cave Laboratory FB page:
October 10: https://www.facebook.com/TularCaveLaboratory/posts/1281593981923342
October 11, conference in Sofia, Bulgaria:
https://www.facebook.com/TularCaveLaboratory/posts/1284789301603810
October, 24, conference in Ohrid, Macedonia:
https://www.facebook.com/TularCaveLaboratory/posts/1297486730334067
Cave training in Albania:
https://www.facebook.com/824521317630613/photos/?tab=album&album_id=1309315675817839
https://www.facebook.com/TularCaveLaboratory/posts/1288260814589992
Workshop in Shkoder, Albania: https://www.facebook.com/events/317981931914193/
https://www.facebook.com/824521317630613/photos/?tab=album&album_id=1310344019048338
Overall project activities in Montenegro and Albania:
https://www.facebook.com/824521317630613/photos/?tab=album&album_id=1312563625493044
https://www.facebook.com/TularCaveLaboratory/posts/1296963957053011
Visit of Albanian conservationists in Slovenia
https://www.facebook.com/TularCaveLaboratory/photos/?tab=album&album_id=1370506803032059
Promotion of project activities from other NGOs:
Lakuriqët e [Natës-Bats-in-Albania]
https://www.facebook.com/Lakuriqët-e-Natës-Bats-in-Albania
PPNEA: https://www.facebook.com/ppnea/posts/1207645535948786

Activity 11: **ACADEMIC DISSEMINATION**
11.1. One of the main achievement during this year project was the submission of the scientific article "Extended range and putative new species of the olm *Proteus* revealed by environmental DNA" (Špela Gorički, David Stanković, Aleš Snoj, Matjaž Kuntner, William R. Jeffery, Peter Trontelj, Miloš Pavićević, Zlatko Grizelj, Magdalena Năpăruş-Aljančič and Gregor Aljančič) at the Scientific Reports [Nature] journal (now in review). The article also describes the eDNA methods developed by the SCB and their partners during 2013-2016 projects. The potential publication in this high scientific open access journal will facilitate the visibility of the project results, the contributing researchers and organizations/institutions involved in the development of the innovative eDNA method with very high impact for the conservation of subterranean biodiversity within the CEPF Mediterranean Basin Biodiversity Hotspot program.

11.2. Presentation of the project at 3 international scientific and nature conservation meetings, on innovative eDNA method and recent research of the Tular Cave Laboratory in the Skadar/Shkodra Lake Basin.

The objectives and proceedings of the project were communicated to international and local nature conservation organizations at the following meetings:
11.2.1. Presentation "*Combining GIS and eDNA to assess the groundwater habitat of black* Proteus *in Slovenia*" (Magdalena Năpăruș-Aljančič, Špela Gorički, David Stanković, Matjaž Kuntner, Gregor Aljančič) at the **Third European SCGIS Conference - Geoinformation technologies for natural and cultural heritage conservation**, Sofia, Bulgaria (11-12 October 2016; http://conf2016.scgisbg.org/conf2016/conf-p4/)
Dr. Magdalena Năpăruş-Aljančič presented the benefits of eDNA method combined with the facilities offered by GIS technologies which results in a powerful tool to assess distribution of *Proteus* and its present conservation status and will better guide future management of karstic groundwater resources. The progress of this year project was also presented to the audience (http://conf2016.scgisbg.org/conf2016/wp-content/uploads/2013/09/Program-3rd-European-SCGIS-Conf.pdf).
Photos from the event here:
https://www.facebook.com/TularCaveLaboratory/photos/a.824527474296664.1073741828.824521317630613/1284785304937543/?type=3&theater

11.2.2. Poster "*With the Olm* (Proteus anguinus) *we share dependence on clean groundwater*" (Tajda Turk, Magdalena Năpăruş-Aljančič and Gregor Aljančič) presented by Tajda Turk at **the 5th Congress of Ecologists of Macedonia, Ohrid, Macedonia**, 19-22 October 2016 organized by Macedonian Ecological Society (http://mes.org.mk/nov-sajt/?p=4365&lang=en; poster in **Supplement materials**).
Photos from the meeting here:
https://www.facebook.com/TularCaveLaboratory/posts/1297486730334067?pnref=story

11.2.3. Presentation "*Searching for* Proteus *at the South-eastern edge of the Dinaric Karst*" (Gregor Aljančič, Špela Gorički, David Stanković, Magdalena Năpăruș-Aljančič, Miloš Pavićević, Aleš Snoj, Matjaž Kuntner) at the International workshop "**Conservation of cave biodiversity in Southeast Dinaric Karst**" (October 29, Shkoder, Albania)
Photos from the event here:
https://www.facebook.com/824521317630613/photos/?tab=album&album_id=1310344019048338

11.2.4. Presentation "*GIS analysis to assess the groundwater habitat pollution of black* Proteus*"* (Magdalena Năpăruș-Aljančič, Špela Gorički, David Stanković, Matjaž Kuntner & Gregor Aljančič) at the **2nd International meeting SOS *Proteus "*Conservation of black *Proteus* and its habitat: 30 years after its discovery"** (December 10, Črnomelj, Slovenia) with mention of this year project.
Photos from the event here:
http://invazivke.weebly.com/novice/the-2nd-international-meeting-sos-proteus-has-ended

COMPONENT 3: EXTENDING TRANS-BALKAN CONSERVATION NETWORKS

Activity 12: **ORGANIZING AN INTERNATIONAL WORKSHOP IN SHKODËR/SKADAR (ALBANIA), INVOLVING EXPERTS FROM SLOVENIA, MONTENEGRO AND ALBANIA**

Tular Cave Laboratory and Biospeleological society of Montenegro together with the host, the University of Shkodra "Luigj Gurakuqi", Albania, organized the workshop "***Conservation of cave biodiversity in Southeast Dinaric Karst***", on 29th October 2016 in Shkoder, Albania.

The workshop was held at the Library of the University of Shkodra "Luigj Gurakuqi", and gathered 20 participants involved in nature conservation. Researchers, students, representatives of NGOs coming from Albania, Montenegro, Czech Republic, Germany, Romania and Slovenia were presenting their experiences, methods and solutions, participated in the discussion on protection of the endangered cave and karst biodiversity of Montenegro and Albania, within topics:

- Distribution and richness;
- Present conservation status;
- Negative anthropogenic pressures on groundwater and environmental hazards;
- Concrete conservation actions and best practice, public outreach;
- Obligations of the national and international conservation legislation and conventions;
- Funding for research and conservation of cave biodiversity and karst.

Fig. 15. International workshop on cave fauna in Shkodër.
Photo: M. Pavićević

Fig. 16. Participants of the workshop.
Photo: G. Aljančič

The official invitation and the detailed program of the workshop are listed in the Supplement.
Photos from the event:
https://www.facebook.com/824521317630613/photos/?tab=album&album_id=1310344019048338

Activity 13: **NGO CAPACITY BUILDING IN ALBANIA (5-DAY BASIC COURSE IN CAVING TECHNIQUES: SAFETY REGULATIONS, HORIZONTAL CAVING SKILLS)**

Albanian conservationists were invited to attend a 5-day basic course to learn basic caving knowledge and skills, to meet colleagues from Montenegro and Slovenia and to contribute to conservation of cave biodiversity and karst around the cross-border area of the Skadar/Shkodra Lake Basin.

The training for cave techniques was successfully finished by 10 young Albanian researchers and professionals working in conservation. The course was taking place 3 days indoor (on artificial climbing walls in Tirana) and 2 days outdoor (in the karst area of Shkodra lake, Albania), with the following activities:

1. **14. 10. 2016**: introduction and meeting with candidates:

- presentation of the project and course
- introduction to biospeleology, history of research, geomorphology, vulnerability of karst.

2. **15.–16. 10. 2016**: training on artificial climbing wall
   - safety regulations and caving equipment,
   - single rope technique (ascending, descending),
   - anchoring, knots.
3. **22. –23. 10. 2016**: 3 caving excursions
   - practical training in caves,
   - terrain search for cave entrances,
   - cave fauna sampling techniques,
   - identifying local karst threats and vulnerability.

Supervisors of the training: **Gregor Aljančič** (Society for Cave Biology; instructor at Speleological Association of Slovenia, Cave Rescue Service of Slovenia), **Miloš Pavićević** (Biospeleological Society of Montenegro; instructor of the Mountain Rescue Service of Montenegro), **Leonid Merzlyakov** (Biospeleological Society of Montenegro; senior trainer and cave rescuer), **Madalena Aljančič** (Society for Cave Biology; karstologist) and **Tajda Turk** (Society for Cave Biology; caver).

Fig. 17. *Introduction to caving equipment*.
Photo: G. Aljančič

Fig. 18. Caving training at Rock Tirana climbing wall.
Photo: M. Aljančič

Photos from the cave training:
https://www.facebook.com/824521317630613/photos/?tab=album&album_id=1309315675817839

Activity 14: **NGO CAPACITY BUILDING IN ALBANIA (3-DAY BASIC COURSE IN CAVE FAUNA SAMPLING TECHNIQUES WITH INTRODUCTORY CAVING TRIPS)**

Within the 5-day basic course in caving framework we included a 3-day basic course in cave fauna sampling techniques with three introductory caving trips. The course was attended by 10 participants.

On 14th October Gregor Aljančič presented goals of the project, importance of the Dinaric Karst as the world's hotspot of subterranean biodiversity and the obligation of Albanians to study and protect it, and the mission of CEPF program in the Mediterranean Basin Biodiversity Hotspot.

On 22–23rd October, practical training on survey and protection of karst was included in the caving trips, emphasizing the problems of drinking water in karst areas and the importance of involving the local community in field work process, directly or through outreach activities.

Sampling techniques for cave fauna (especially cave invertebrates) and *Proteus* eDNA were also used during the last two days of the training.

Fig. 19. *Introduction to cave fauna*.
Photo: M. Aljančič

Fig. 20. Participants of the course.
Photo: G. Aljančič

Photos from the event:
https://www.facebook.com/TularCaveLaboratory/photos/a.1309315675817839.1073741859.824521317630613/1309338982482175/?type=3&theater

Activity 15: **BUILDING A TRANS-BOUNDARY ALLIANCE FOR CONSERVATION OF SUBTERRANEAN FAUNA (NGOs, LOCAL COMMUNITIES AND GOs)**
Trans-boundary *Proteus* conservation alliance extended to Albania, reaching five conservation NGOs and local communities.

NGO capacity building: training in the survey and protection of groundwater biodiversity, involving partners and local community in field work (documentation of caves and karst springs), laboratory monitoring of drinking water sources, as well as outreach activities. Conservation researchers from Albania advised to enforce and support legal protection of cave habitats and its biodiversity, to protect karst groundwater towards pristine drinking water.
The following NGOs from Albania joined the alliance:
- Protection and Preservation of Natural Environment in Albania (PPNEA)
- Albanian Society for Protection of Birds & Mammals (ASPBM)
- Albanian Ornithological Society (AOS)
- Rock Tirana
- Lakuriqët e Natës [Bats in Albania]
- GO: Faculty of Natural Sciences, University of Tirana

Activity 16: **VISIT OF THE TULAR CAVE LABORATORY (SLOVENIA) BY YOUNG BIOLOGISTS FROM MONTENEGRO AND ALBANIA**
As a contribution to the Critical Ecosystem Fund's SE Balkan capacity-building initiative, most perspective young Albanian (3) and Montenegrin (3) biologists involved in the project were invited for a short study visit to Slovenia. The program was adapted according to their field of interest in nature conservation, including a visit at the Tular Cave Laboratory, meeting people from nature conservation GOs and NGOs in Slovenia, with speleobiology, herpetology, bat and large mammal's conservation fieldwork.

1. **Visit of Montenegrin biologists to Slovenia** (17th – 26th July 2016)
Jelena Popović, Milica Jovanović and Miloš Pavićević
a) Visit of the Tular Cave Laboratory (17th July 2016)
b) Attendance at the 29th Biology Student Research Camp (17th – 26th July 2016)

Practice in fieldwork, meeting experts and students, exchanging ideas.

2. **Visit of Albanian biologists to Slovenia** (12th – 15th December 2016; detailed report in the supplement)
Eva Dulellari, Ervis Loçe and Enerit Saçdanaku
a) Meeting with herpetologists at the Slovenian Herpetological Society (12th December 2016):
Albanian herpetologist Enerit Saçdanaku gave a lecture on the conservation status of amphibians and reptiles in Albania.

b) Whole day excursion:
- Visit to the Škocjan Caves (http://www.park-skocjanske-jame.si/en/): introduction to the management and best practice in karst landscape (UNESCO world heritage site, Underground wetland site/Ramsar, Karst biosphere reserve/MBA, Natura 2000, Regional Park), meeting with one of the Park's ranger;
- Visit of the DOPPS' bird observatories at the Škocjan Zatok Nature Reserve.

c) Meetings with Mojca Jernejc Kodrič and Urška Kačar, curators of the Department of Vertebrate Zoology at the Slovenian Museum of Natural History in Ljubljana, and with researchers from Department of Biology, Biotechnical Faculty, University of Ljubljana.
Eva Dulellari gave a presentation to pupils and biology teachers of the high school in Kranj about the efforts and actions for conservation of birds and large mammals (bears) in Albania engaging also school pupils.
Visit of the Tular Cave Laboratory: the Albanian team received detailed presentation on ecology and biology of *Proteus*, on history of the laboratory, research and conservation efforts to protect *Proteus* in Slovenia and its karst habitat.

Photos from the event:
https://www.facebook.com/TularCaveLaboratory/photos/?tab=album&album_id=1370506803032059

10. If you did not complete any component or deliverable, how did this affect the overall impact of the project?

During the project, three of the activities were not finalized or we had to cancel them:
1. New eDNA method to detect the traces of the invertebrate aquatic subterranean fauna living in caves or karst springs based on next generation sequencing techniques
2. Basic groundwater quality analysis in Montenegro at 5 selected localities
3. Educative leaflets (Montenegro and Albania editions; 3,000 copies)

The reasons for not completing these activities were already explained above in section Project Components. If accomplished, the missing activities would increase the quality of the project, however, on level of project's components they were well balanced by those accomplished.

11. Please describe and submit any tools, products, or methodologies that resulted from this project or contributed to the results
(All products described in the section 'PROJECT COMPONENTS')

Benefits to Communities

12. Please describe the communities that have benefited from CEPF support

*Please report on the size and characteristics of communities and the benefits that they have received, as a result of CEPF investment. Please provide information for all communities that have benefited **from project start to project completion**.*

	Community Characteristics											Nature of Socioeconomic Benefit								
								Size of Community											
Community Name	Subsistence economy	Small landowners	Indigenous/ ethnic peoples	Pastoralists / nomadic peoples	Recent migrants	Urban communities	Other*	50-250 people	251-500 people	501-1,000 people	Over 1,001 people	Increased access to clean water	Increased food security	Increased access to energy	Increased access to public services (e.g. health care, education)	Increased resilience to climate change	Improved land tenure	Improved recognition of traditional knowledge	Improved representation and decision-making in governance forums/structures	Improved access to ecosystem services
								x												

*If you marked "Other" to describe the community characteristic, please explain:

Lessons Learned

13. Describe any lessons learned related to organizational development and capacity building.

The current project is part of a grant that highlighted to CEPF and the RIT the current gap of knowledge on the subterranean biodiversity within the Mediterranean Ecosystem Profile, and the need for greater data collection on this fragile and often-overlooked habitat. As further work was needed, focusing especially in the Skadar Lake Basin (Montenegro and Albania), the grant was mainly piloting several activities and actions, some on previous unknown territories.

The novelty of the project should came from two aspects:

1. *test of a new eDNA method*, *aiming to assess overall cave biodiversity of Southern Dinaric Karst*. This novel molecular method has not been previous tested in countries with well researched cave biodiversity, and the gene determination is not yet calibrated for all possible cave invertebrates. Such method is more time consuming for sampling and there is only one available expert (Dr. Tanja Pipan) to cover the full laboratory analysis. Together with the problem encountered for capacity building (details below), the eDNA proposed method and analysis had to be canceled.
2. *implementing new project capacity-building activities in Albania* – a country little known directly, and with no previous specialized contacts. When tried to contact directly Albanian NGOs having projects within the same donators (CEPF and BirdLife) we didn't get any reply. We searched more contacts and we found better access, precious help and feedback from the French researcher studying bats in Albania, Dr. Philippe Theou, that open many doors towards more specialized local help among conservationists and researchers. After this, everything went very well, gathering many contacts and we succeed in 90% of all activities planned for Albania.

   Though, the success of capacity building in Albania, placed Montenegro in a sensitive position, as we concentrated too much time and effort only for one country, and somehow expected that our Montenegrin partner has now enough independence and capacity building gained from the previous project. Unfortunately, similar to Albania, Montenegro lacks of active conservationists for cave biodiversity. The specialized society – Speološko Društvo Črna Gora has only one fully active member (Miloš Pavićević) and up to four quasi-active members – too little to could implement alone the part of a project involving extensive field and laboratory works and outreach activities. Both Montenegro and Albania need further investment in capacity building and constant supervising of their activities.

14. Describe any lessons learned related to project Design Process *(aspects of the project design that contributed to its success/shortcomings)*

Specifically, the project taught us how to better address the capacity building activities (details below, Q15). The idea of organizing training on cave techniques in Albania was in a way innovative and very productive, especially during the last part of the project.

On the other hand, as the project was granted as a pilot test for the assessment of cave biodiversity in the little known previous cross-border key biodiversity area of the Skadar/Shkodra Lake Basin, we over-evaluated the available human resources in Montenegro, and we had to cancel three of the activities and to increase the focus on other activities better related with capacity building.

15. Describe any lesson learned related to project Implementation *(aspects of the project execution that contributed to its success/shortcomings)*

The basic cave training and cave sampling training techniques helped us to build important bridges and to communicate better through the following stages of the project. The direct contact and demanding physical activities created a special relationship and energy among participants. Already at beginning of our activities in Albania, a strong working team was built with easy communication further on the project. The success of the trainings was reflected by the success of all fieldwork and laboratory activities, networking, visits of Albanian and Montenegrin conservationists in Slovenia, the organization of the international workshop in cave biodiversity.

Overall, the implementation of the project and its execution were successful at approx. 75%.
All changes and relocations in the project were successful managed thanks to permanent consulting, communication and support from the RIT and CEPF.

16. Describe any other lessons learned relevant to the conservation community

The main lessons we learned during this project were that interactive capacity building activities, dissemination and practical educational tools are crucial. In order for the project to be effective, the local partners and other NGOs, local community and other institutions had to be involved in the field-work process, laboratory work, and in writing and preparing promotion materials for presentations and events, writing reports. Such actions result in high credibility and trust between the project organizers and participants, provide sustainable and long-lasting knowledge and practice to the conservation community involved in the project.

Sustainability / Replication

17. Summarize the success or challenges in ensuring the project will be sustained or replicated

Successful replication and implementation of the eDNA method in nature will drive the studies on *Proteus* and other subterranean biodiversity in the next decade, especially in areas less researched – as those from Albania.

The highly vulnerable *Proteus* has become a symbol of a successful nature conservation management linkage. The Society for Cave Biology has made a first big step in assessing cave biodiversity and implementing cave biodiversity capacity building in Albania and Montenegro. New ideas, research perspectives and partnerships already arise to be implemented in future projects, with the conservationists from Albania, Montenegro and from whole Dinaric Karst range.

18. **Summarize any unplanned activities that are likely to result in increased sustainability or replicability**

Recently, Montenegro was started to be the focus of many Slovenian biospeleologists, as the recent researches were showing the presence of important hotspots for cave biodiversity. With the organizing of the international workshop for cave biodiversity in Shkoder, Albania, and the promotion of the project, more scientists from Slovenia turn their interest towards the high potential subterranean biodiversity from this country. Our rich finds on cave invertebrates still need to be determined in term of species by the scientists from Slovenia, and the possibility of the discovery of new species is very high. Such finds will encourage more research to be made in the area and new conservation projects to plan and implement.

Safeguards

19. If not listed as a separate Project Component and described above, summarize the implementation of any required action related to social and environmental safeguards that your project may have triggered

During the project implementation, constant lectures, thematic documentaries and trainings focused also on high vulnerability of karst and karst groundwater to pollution. We use this project opportunity to give awareness on importance of a clean karst environment and to direct link with clean drinking water resources.
The project respected all fundamental ethical principles and requirements valid in Slovenia, Montenegro and Albania, including those under the Charter of Fundamental Rights of the European Union. The care of animals was in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable federal laws, guidelines, and policies of the United States of America.
The care of captive animals in the Tular Cave Laboratory was in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of The National Institutes of Health of the United States of America. No human subjects were used in this project.
During the project, specimens of *Proteus anguinus* were observed in nature, as well as in captivity of the Tular Cave Laboratory, from a distance with infrared observation devices, with no physical interference to disturb them. These animals were not involved in any experiments. Housing permits are held by the Tular Cave Laboratory (Slovenian Environment Agency, permit #35601-79/2009-2 and permit #35601-79/2009-4)

Additional Funding

20. Provide details of any additional funding that supported this project and any funding secured for the project, organization, or the region, as a result of CEPF investment

Donor	Type of Funding*	Amount	Notes
/			

** Categorize the type of funding as:*

A Project Co-Financing (other donors or your organization contribute to the direct costs of this project)
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Additional Comments/Recommendations

21. Use this space to provide any further comments or recommendations in relation to your project or CEPF

This Final report was written by Magdalena Năpăruș-Aljančič, Gregor Aljančič, David Stanković, Leonid Merzlyakov and Miloš Pavićević, with reports by Eva Dulellari, Enerit Saçdanaku and Ervis Loce.

All project activities described in this report were performed in its entirety by a voluntary work.

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