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THE MORPHOLOGICAL VARIABILITY, DISTRIBUTION PATTERNS AND ENDANGERMENT IN THE OGULIN CAVE SPONGE *EUNAPIUS SUBTERRANEUS* SKET & VELIKONJA, 1984 (DEMOSPONGIAE, SPONGILLIDAE)

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The distribution and morphological variability of the troglobiotic freshwater sponge *Eunapius subterraneus* Sket & Velikonja, 1984 registered in Croatia as a threatened species in the IUCN category Endangered (EN) was investigated. The research area encompassed Velika and Mala Kapela Mountains as well as the upper catchment area of the Dobra and Mrežnica rivers on the NE side of Velika and Mala Kapela Mountains, mostly in the Ogulin–Plaški karst plateau. The species was registered in six localities, three previously known from the literature and three constituting new records: Mandelaja pit and the caves Crnačka špilja and Izvor špilja Gojak. For subspecies identification the morphology and dimensions of megascleres and gemmuloscleres of the collected samples were analyzed. Significant differences among sponge populations were indicated by one-way ANOVA ($p < 0.001$) in width and length of megascleres. The subspecies *E. s. mollisparspanis* Sket & Velikonja, 1984 was found only at its type locality, in the cave Rudnica špilja VI. The preliminary variability of other populations after discriminant function analysis of measured morphometric parameters such as the width and length of mega- and gemmulo-scleres indicates four groups, but it was not possible to separate them clearly in all cases. Subterranean sponges were not found in two localities known from the past, the Đula – Medvedica cave system and the cave Špilja u kamenolomu Tounj, both under great pressure of groundwater habitat changes because of water pollution, river direction changes and habitat destruction.

Key words: freshwater sponges, caves, springs, gemmules, spicules, distribution, Croatia

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Istraživana je rasprostranjenost i morfološka varijabilnost ogulinske špiljske spužve *Eunapius subterraneus* Sket & Velikonja, 1984, koja je prema kriterijima IUCN registrirana kao ugrožena vrsta. Područje istraživanja obuhvaća Veliku i Malu Kapelu te ogulinsko-plaščansku zavalu, odnosno gornje slivno područje rijeka Dobre i Zagorske Mrežnice na sjeveroistočnoj strani Male i Velike Kapele. Vrsta je zabilježena na šest lokaliteta, od kojih su tri nova: Mandelaja, Crnačka špilja i Izvor špilja Gojak. Za determinaciju podvrsta korištena su sljedeća morfološka obilježja: veličina i oblik gemosklera i megasklera. Značajne razlike između populacija pokazala je jednosmjerna analiza varijance širine i dužine megasklera. Podvrsta *E. s. mollisparspanis* je utvrđena samo na tipskom lokalitetu, u špilji Rudnica VI. Diskriminantna analiza mjerenih morfoloških parametara koji su uključivali dužinu i širinu mega- i gemosklera prikazala je odnose među populacijama svrstavajući ih u četiri grupe, ali ih nije potpuno razdvojila. Podzemne populacije spužvi nisu ponovno pronađene u špiljskom sustavu Đula-Medvedica i Špilji u kamenolomu Tounj. Ti su lokaliteti ugroženi zbog zagađenja vode, hidrotehničkih zahvata i devastacije uzrokovane radom kamenoloma.

Ključne riječi: slatkovodne spužve, špilje, izvor, gemule, spikule, Hrvatska, biogeografija

INTRODUCTION

Although sponges are abundant in marine caves, in groundwater habitats only stygoxene to eustygophile species have been previously reported (VACELET, 1994). The only specialised subterranean freshwater sponge known to date is *Eunapius subterraneus* Sket & Velikonja 1984, which includes two subspecies, *E. s. subterraneus* Sket & Velikonja 1984 and *E. s. mollisparspanis* Sket & Velikonja 1984.

Since the description of the species in 1984 and 1986 no sustained research has been conducted. At that time only three localities were known (the caves Tounjčica špilja, Rudnica špilja VI, Mikašinovića špilja). In the meantime, 5 additional localities have been found by cavers and cave divers exploring the area (the caves Izvor špilja Gojak, Mandelaja, Crnačka špilja, Špiljski sustav Đula–Medvedica, Špilja u kamenolomu Tounj). This has raised questions about the distribution area of the species, the taxonomical status of newly-discovered populations and the biology of the species in general.

This paper focuses on research performed in the framework of the project »Conservation of *Eunapius subterraneus*, the only subterranean freshwater sponge in the world« funded by the Rufford Small Grant to the Croatian Biospeleological Society. Investigations have been conducted in order to analyze the variability of some key morphological features, to identify newly recorded populations, to record distribution patterns as well as to examine the current state of habitat quality and population-threatening changes in the environment.

MATERIAL AND METHODS

Fieldwork consisted of collecting samples by scuba diving (B. Jalžić, I. Čukušić) in caves during the winter and late summer 2004 when water levels were at their lowest. Thirty caves were visited but observation of sponges was successful only in six: (1) Tounjčica špilja (31.01.2004); (2) Mikašinovića špilja (31.01.2004); (3) Izvor špilja Gojak (01.02.2004); (4) Rudnica špilja VI (27.08.2004); (5) Jama Mandelaja (28.08.2004) and (6) Crnačka špilja (12.09.2004). In 2004 no sponges were found in the cave Špiljski sustav Đula–Medvedica (leg. B. Jalžić, 15.02.1998, but material is lost) and cave Špilja u kamenolomu Tounj (observation of D. Bakšić, 22. 9. 2002).

For identification, only a few specimens were sampled, from Tounjčica špilja 3 specimens, from Mikašinovića špilja 5, from Izvor špilja Gojak 3, from Rudnica špilja VI 4, from Jama Mandelaja 4 and from Crnačka špilja 3. Sponges were fixed and stored in 96% ethanol.

Laboratory work was conducted in the Department of Zoology of the Faculty of Science, Zagreb University, and included isolation of gemmules and spicules (megascleres and gemmuloscleres). Gemmules were isolated from surrounding tissue by tweezers. Megascleres were isolated by boiling a fragment of the tissue in concentrated nitric acid until the cellular material was disintegrated and the liquid became clear. The sample tube was filled with distilled water and the spicules were left to settle on the bottom. Water was afterward removed and changed three times. After the last change, spicules were suspended in ethanol and poured onto a microscope slide. When ethanol evaporated, the slide was covered with Canada balsam and a cover slip. Gemmuloscleres were isolated by boiling nitric acid directly on a microscope slide.

The spicules were photographed on a Zeiss microscope equipped with an Olympus C-3040 Zoom digital camera. Length and width of the spicules were measured in the application Scion Image version Beta 4.0.2. (Fig. 1).

The following four morphometric parameters were examined: megasclere width, megasclere length, gemmulosclere width and gemmulosclere length. For each of

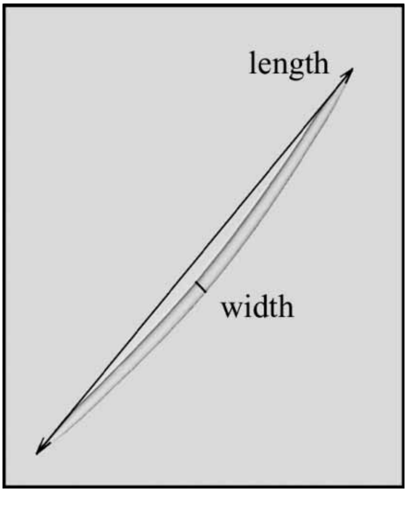

Fig. 1. Measuring size of spicules in the Scion Image Application.

the five sites examined in this study, fifty measurements of each morphometric parameter were randomly selected from up to three specimens. A total of 1000 measurements were finally performed.

Data Analysis. Mean value, minimum (Min), maximum (Max), and standard error (±SE) were calculated for each variable from all study sites. The suitability of the data for parametric analysis was evaluated prior to examination of individual variables (morphological data) for differences among populations by one-way analysis of variance (ANOVA). When populations were significantly different, multiple comparison post-hoc tests (Tukey's HSD) were performed to see which populations differed from one another. A discriminant function analysis (DFA) was used to determine the distinctiveness of the populations. The significance level for statistics was set at a = 0.05. An UPGMA cluster analysis on Euclidean distance was undertaken to evaluate the similarity among sponge populations from different localities. All statistical analyses of the measurement data were performed using StatSoft Statistica 6.0 (STATSOFT, 2002) (FOWLER *et al.*, 1999; ZAR, 1996).

Besides dimensions of spicules, general shape and colour of the sponges along with size and distribution of the spines on spicules were noted as well.

Study area

The research area (Fig. 2 and Fig. 3) encompasses water caves of (1) the small karst poljes with mostly small intermittent creeks located in the middle of Velika Kapela or south-western side of Mt Mala Kapela, (2) Ogulin-Plaški karst plateau on the north-eastern side of Mts Velika Kapela and Mala Kapela, and (3) the source area of the large rivers the Gojačka Dobra and Primišljanska Mrežnica, north east of the Ogulin-Plaški karst plateau.

The study sites include caves and pits with diverse hydrological statuses: springs (Vrelo izvor, Špilja pod Mačkovom dragom, Izvori Košarice, Špilja kod Turkovića, Izvor Vitunjčice, Izvor špilja Gojak, Izvor Zagorske Mrežnice, Izvor Bistraca, Rudnica špilja VI, Tounjčica špilja, izvor Sinjac, špilja Sopot, Izvor Dretulje, Izvor Studenac, Izvor Vidovića studenac, Komarčeva špilja, Veliko vrelo, Periodički izvor u Krakaru, Milino vrelo, Crno vrelo, Špilja iznad Crnog vrela, Markarova špilja, Crnačka špilja), sinkholes (Špiljski sustav Đula-Medvedica, ponor na Crnačkom polju), caves with underground flows (Špilja Pećinik, Mikašinovića špilja, jama Mandelaja, Vidovića špilja) and an estavelle (Luška špilja).

In order to understand the distribution pattern of *Eunapius subterraneus*, it is necessary to describe the hydrological characteristics of its area of distribution. The area where *Eunapius subterraneus* has been found is located north-east of the line dividing the Adriatic and the Black Sea drainage basin and belongs to the Black Sea catchment area, more precisely to the Kupa River catchment area. Although it is a relatively small area, its hydrological properties are very complex.

The letter »**A**« in Fig. 3. denotes the large mostly limestone (permeable) region of Velika and Mala Kapela Mountains, generally characterized by deep karst with lack of surface water flows. Precipitation sinks rapidly through crevices into subsurface flows (BAHUN, 1968). In some small isolated karst poljes only, primarily springs

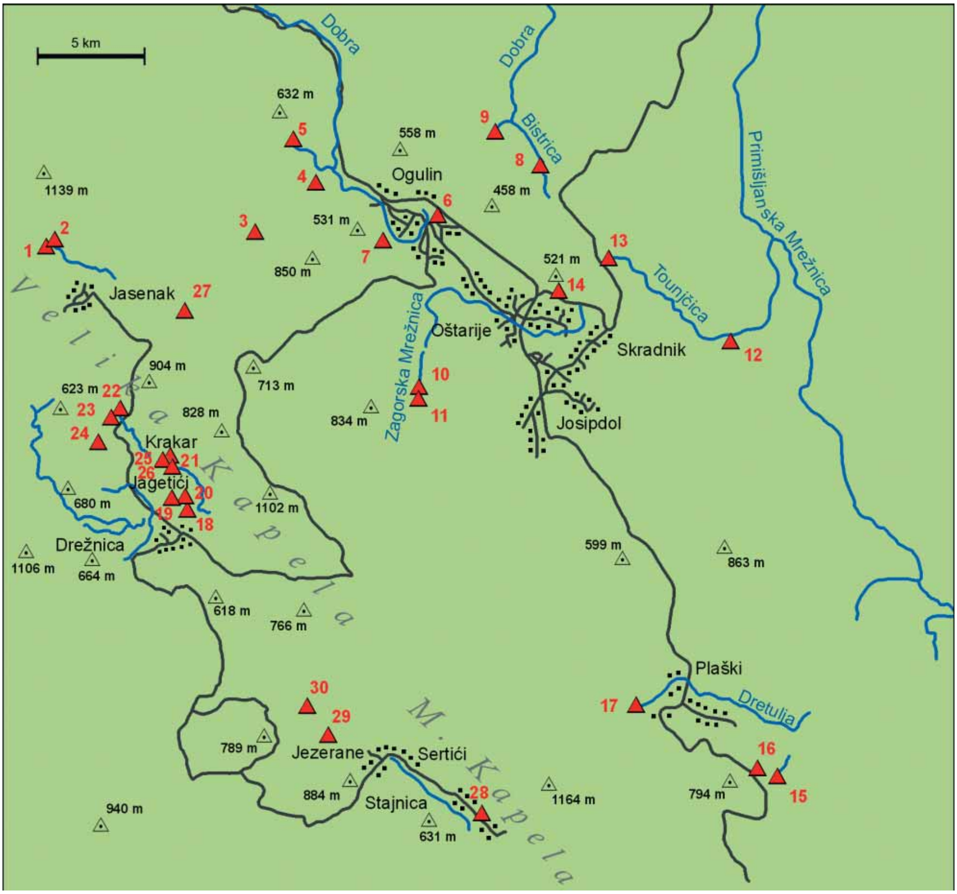

Fig. 2. The study area. 1. Vrelo izvor, 2. Špilja pod Mačkovom dragom, 3. Izvori Košarice, 4. Špilja kod Turkovića, 5. Izvor Vitunjčice, 6. Špiljski sustav Đula-Medvedica, 7. Špilja Pećinik, 8. Mikašinovića špilja, 9. Izvor špilja Gojak, 10. Izvor Zagorske Mrežnice, 11. Izvor Bistraca, 12. Rudnica špilja VI, 13. Tounjčica špilja, 14. Jama Mandelaja, 15. Izvor Sinjac, 16. Špilja Sopot, 17. Izvor Dretulje, 18. Izvor Studenac, 19. Vidovića špilja, 20. Izvor Vidovića studenac, 21. Komarčeva špilja, 22. Veliko vrelo, 23. Periodički izvor u Krakaru, 24. Milino vrelo, 25. Crno vrelo, 26. Špilja iznad Crnog vrela, 27. Luška špilja, 28. Markarova špilja, 29. Crnačka špilja, 30. Ponor u Crnačkom polju

with short intermittent or permanent creeks are situated. For example Crnačka cave is located on the border of the small periodically flooded Crnačko polje.

Many secondary springs appear at the contact line with the mostly impermeable region »**B**«, which forms permanent surface flows. Region »**B**« is a low karst region with three main sinking rivers passing through it, the Ogulinska Dobra River, the Zagorska Mrežnica River and Dretulja River. These surface flows sink again along the contact line with a second, permeable, limestone region »**C**«.

A:
1. Dabar
2. Brinje
3. Stajničko polje
4. Crnačko polje
5. Drežničko polje
6. Drežnički Lug
7. Krakar
8. Jasenak
9. Musulinski potok

B:
1. Lička Jesenica
2. Begovac
3. Sinjac
4. Dretulja
5. Vrnjika
6. Josipdolski potok
7. Rupečica
8. Zagorska Mrežnica
9. Zagorska peć
10. Špiljski sustav Đula-Medvedica
11. Pećinik
12. Vitunjčica

C:
1. Mandelaja
2. Ambarac ponor

D:
1. Slunjčica
2. Primišljanska Mrežnica
3. Rudnica
4. Tounjčica
5. Globornica
6. Bistrica
7. Mikašinovića pećina
8. Izvor špilja Gojak
9. Ribnjak

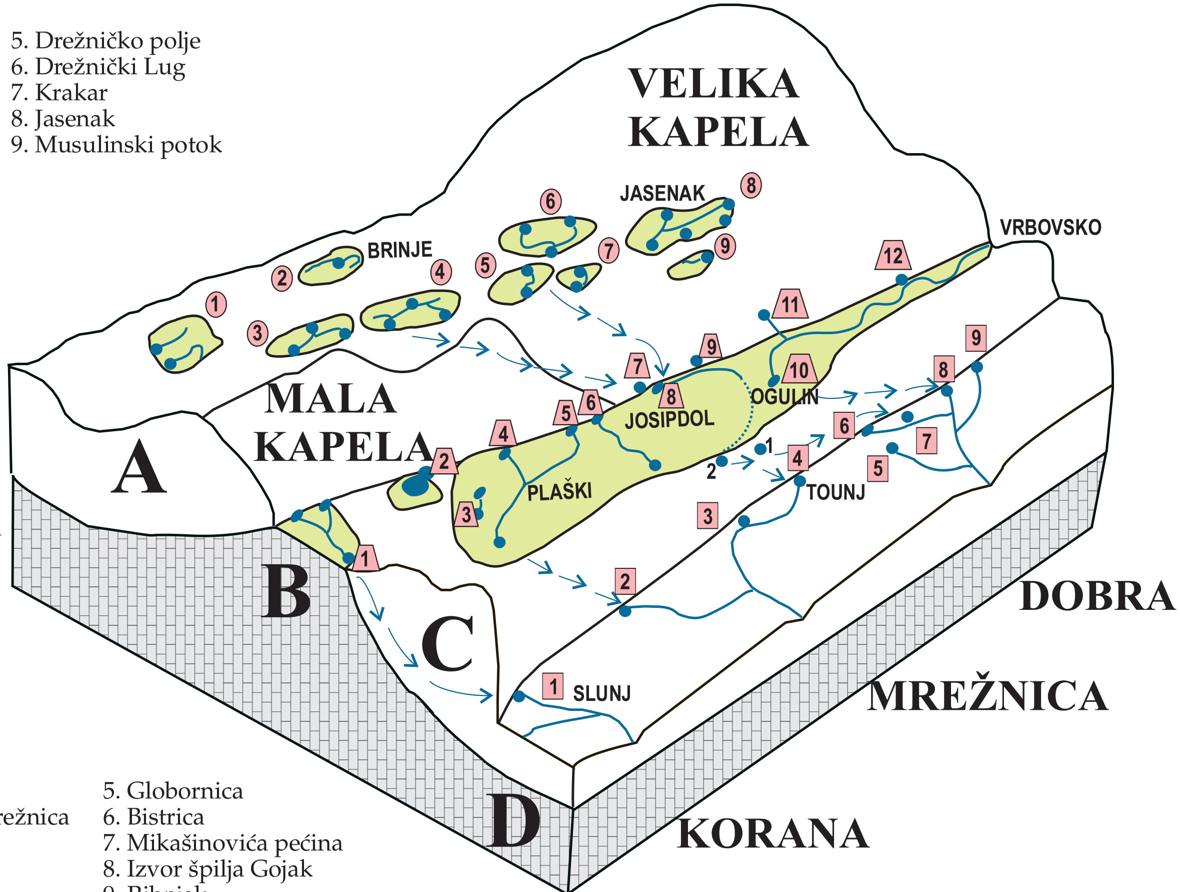

Fig. 3. Hydrological diagram of the study area (Redrawn by N. Tvrtković after BAHUN, 1968).

At the line connecting the sources of the Ribnjak stream, Gojačka Dobra river, Bistrac stream (all Dobra river sources), Kukača, Tounjčica stream, Rudnica, a very short stream, Suvača and Primišljanska Mrežnica river (all Mrežnica river sources), and Slunjčica stream (Korana river affluent) the lower mostly impermeable region (shallow karst with dolomite bedrock) marked »**D**« begins (BAHUN, 1968).

RESULTS AND DISCUSSION

Specimens for preliminary morphological investigation were collected from six localities.

Body external morphology

Sponges at all localities were white, soft and of fragile consistency. Nevertheless, there were some differences in the general shape. Specimens from the caves Tounjčica špilja, Mikašinovića špilja, Špilja u kamenolomu Tounj, Rudnica špilja VI, Mandelaja, and Crnačka špilja range in shape from egg-shaped to cylindrical, their sizes vary from 1 to 8 cm, and they have irregularly wrinkled surfaces (Fig. 4). Specimens from the cave Špiljski sustav Đula–Medvedica and some specimens from Izvor špilja Gojak have a broad, bark-like base adhering to the rocky substrate and a central cone-shaped outgrowth with an osculum on top. The surface of the body is

Fig. 4. *Eunapius subterraneus* Sket & Velikonja 1984, morphotype with wrinkled surface in the cave Tounjčica špilja (Photo: I. Čukušić).

Fig. 5. *Eunapius subterraneus* Sket & Velikonja 1984, morphotype with smooth surface in the cave Izvor špilja Gojak (Photo: I. Čukušić).

smooth (Fig. 5). These two different morphotypes, sharing other diagnostic traits, are presently ascribed to the same subspecies (*E. s. subterraneus*). Many different factors such as environmental constraints and life cycle phases influence the growth form of sponges (MANCONI & PRONZATO, 1991; KAANDORP, 1999). In favour of the notion that local ecological conditions cause the differences in sponge shapes is the fact that both morphological types were found in different passages of the cave Izvor špilja Gojak.

Gemmules

In the sample from the cave Mikašinovića špilja gemmules were not found so our data about gemmular shape originate from other five localities. From all samples gemmules are yellowish-brown, spherical to semi-spherical. They are located at the base of the sponge and attached to the surface of the rock.

Spicules

Gemmuloscleres

Gemmulosclere length varies from 76.7 to 265.3 µm, and the width ranges from 3.8 to 14.8 µm. There are no significant differences in dimensions of gemmuloscleres among different localities (Figs. 6 and 7), which is in concordance with literature data. Gemmuloscleres are slightly, sometimes irregularly, curved oxeas or strongyles. Small numbers of spines are evenly distributed except in the sample from the cave Rudnica špilja VI where they are bigger and denser at the tips.

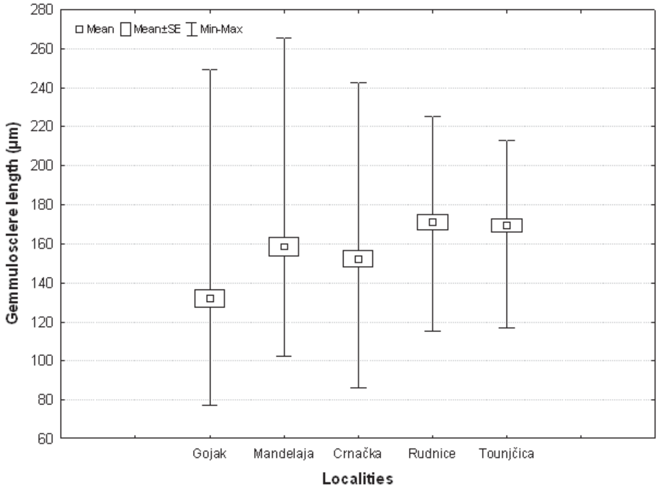

Fig. 6. Gemmulosclere length in different populations of *Eunapius subterraneus* (N=50 gemmuloscleres from each locality).

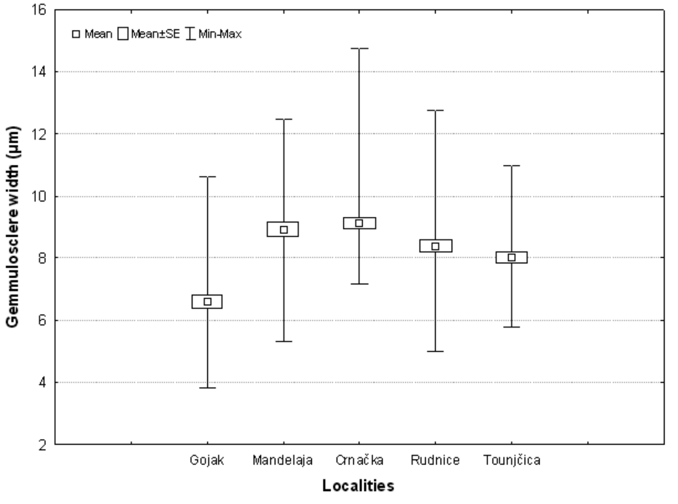

Fig. 7. Gemmulosclere width in different populations of *Eunapius subterraneus* (N=50 gemmuloscleres from each locality).

Megascleres

At the newly-discovered localities, as in the three previously known, megascleres are oxeas, slightly curved with evenly distributed small spines, which can become slightly denser towards the tips. Their length varies from 200 to 628 µm, and their width ranges from 5 to 15 µm. Statistical analysis of megasclere length and

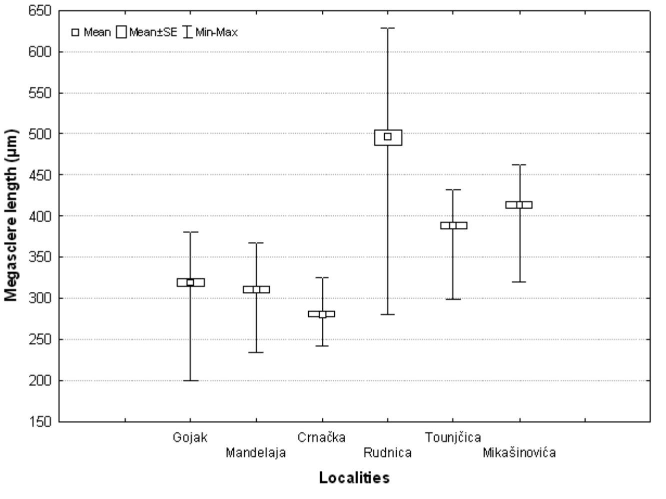

Fig. 8. Megasclere length of specimens from seven different localities (N=50 megascleres from each locality).

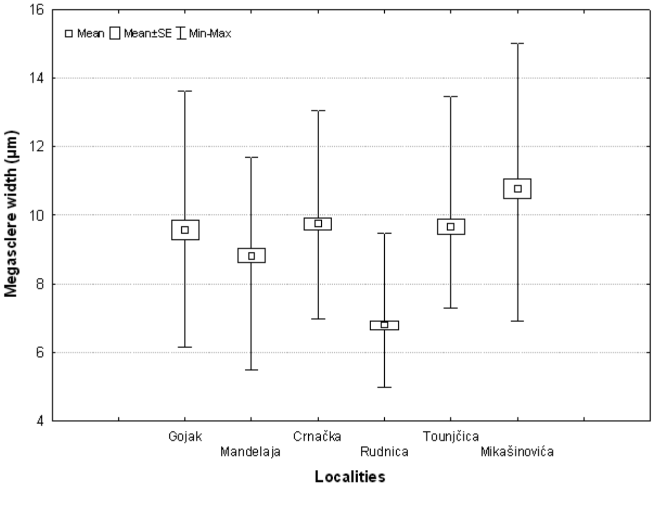

Fig. 9. Megasclere width of specimens from seven different localities (N=50 megascleres from each locality).

width showed significant differences in the sample from the cave Rudnica špilja VI (Figs. 8 and 9). This is in accord with the findings of SKET & VELIKONJA (1986), who reported that megascleres are thinner and longer in samples from Rudnica špilja VI, describing it as a different subspecies. Apart from the difference in size, spines on spicules in the samples from the cave Rudnica špilja VI are bigger, unevenly distributed and always denser towards the tips.

Tab. 1. Results of one-way ANOVA on morphometric variables of gemmuloscleres and megascleres measured on sponge *Eunapius subterraneus* Sket & Velikonja, 1984. Significant *F*-statistics are indicated by ** ($p < 0.001$). Significant comparisons determined by *p*-values < 0.001 (Tukey's HSD) (CR – Crnačka; GO – Gojak; MA – Mandelaja; RU – Rudnica; TO – Tounj).

Morphometric variables	Degrees of freedom	F- statistic	p-values	Significant comparisons
Gemmulosclere length	4 245	0.819	0.513	none
Gemmulosclere width	4 245	1.488	0.206	none
Megasclere length	4 245	17.253**	<0.0001	CR-RU CR-TO GO-CR GO-RU GO-TO MA-RU MA-TO RU-TO
Megasclere width	4 245	9.749**	<0.0001	CR-RU GO-RU MA-RU TO-RU

Our measurements of megasclere width differ significantly from the values reported in the literature. Average values in the literature range from 11.3 to 13.2 µm (SKET & VELIKONJA, 1986), while our average measured width varies between 6.8 and 10.8 µm. It is interesting to note that at the locality cave Rudnica špilja VI the highest megasclere width was lower than the minimal width from the literature. These discrepancies deserve further investigation.

Analysis of morphological data for all the sponge populations revealed significant differences in two of four morphological parameters. There was a significant difference in the megasclere length and width between study sites (one-way ANOVA) (Tab. 1).

Pairwise comparisons revealed significant differences between most populations in megasclere length. The exception was the comparison between Izvor špilja Gojak and Mandelaja (Tukey's HSD, $p = 0.775$), reflecting the great similarity of these populations in megasclere length. Comparisons between Izvor špilja Gojak and Mandelaja (Tukey's HSD, $p = 0.073$), Izvor špilja Gojak and Crnačka špilja (Tukey's HSD, $p = 0.977$), Izvor špilja Gojak and Tounjčica (Tukey's HSD, $p = 0.997$), and Crnačka špilja and Tounjčica (Tukey's HSD, $p = 0.999$) reflected the great similarity of these populations in megascere width. Tukey tests showed that population from Rudnica špilja VI showed a significant difference from all other populations in megasclere width (Tukey's HSD, $p < 0.001$). Comparison between Crnačka špilja and Mandelaja (Tukey's HSD, p = 0.002) reflected low level of differences in megasclere length. The same level of differences was detected between Crnačka špilja and Mandelaja (Tukey's HSD, p = 0.013) as well as Mandelaja and Tounjčica špilja (Tukey's HSD, p = 0.029) in megasclere width (Tab. 1).

Discriminant function analysis of four morphometric variables of the species *E. subterraneus* resulted in four discriminant functions, which explained 100% of the accumulated population variance. The first canonical discriminant function of the discriminant analysis explained 93% of the total variance, which separated the population of the cave Rudnica špilja VI from the other populations. The partial Wilks' lambda values indicate that variable LMS – length of megascleres (F = 12.35, P < 0.001) contributes most to overall discrimination. The projection of the first two discriminant functions is shown in Fig. 10. Although discriminant function analysis was not able clearly to separate all the studied populations (due to the overlapping ranges among groups), it contributed to clarify their relationships. Therefore, four groups could be distinguished: clearly separated are one group formed by the samples of populations from the caves Crnačka špilja and Mandelaja, and the second group which consists of the population from the cave Rudnica špilja VI. The third group from the cave Tounjčica špilja overlaps with a sample from Izvor špilja Gojak which is included in the fourth group (Fig. 10).

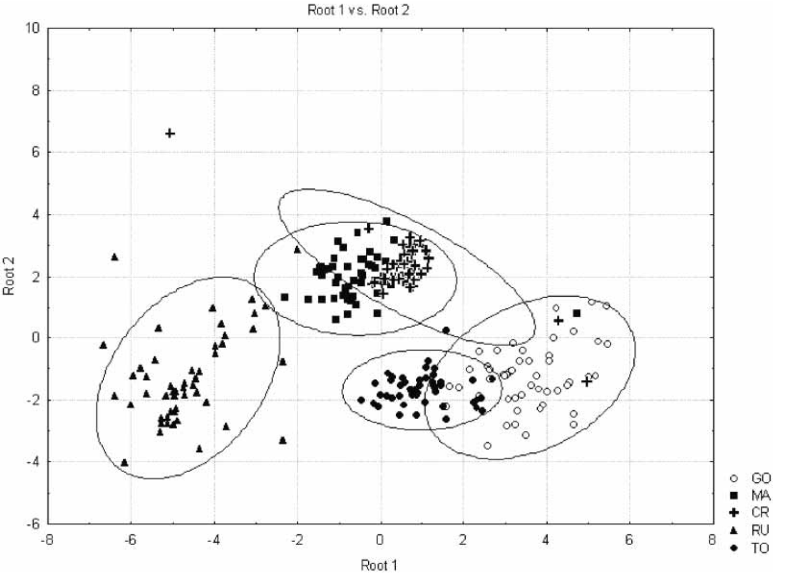

Fig. 10. Scatter plot of the canonical scores from the discriminant analysis. An ellipse surrounds 95% of the measurements within a population. Specimens from five caves (GO – Izvor špilja Gojak; MA – Mandelaja; CR – Crnačka špilja; RU – Rudnica špilja VI; TO – Tounjčica špilja) plotted in canonical variate space with four variables in the model (length and width of megascleres and gemmuloscleres): Wilks' l = 0.026, F (16.74) = 106.101, P < 0.0001.

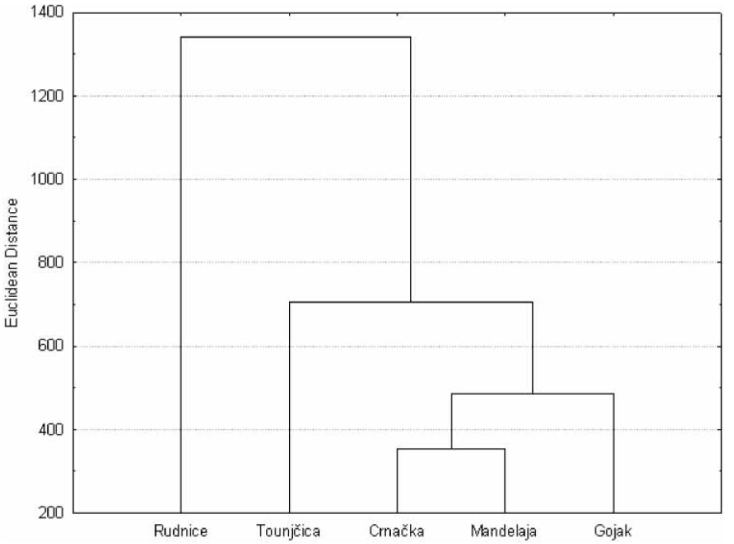

Fig. 11. Dendrogram grouping for populations of *Eunapius subterraneus* from five caves.

The dendrogram (Fig. 11) resulting from the cluster analysis of all morphometric variables of the species *E. subterraneus* revealed two groups of populations. First is formed by the population from the cave Rudnica špilja VI. The larger group, including four populations, is divided into three subgroups:, one from Tounjčica špilja, second from Izvor špilja Gojak, and the last formed by the populations from the caves Crnačka špilja, and Mandelaja. The fact that the cluster analysis grouped together the populations from Crnačka špilja and Mandelaja, indicates that the morphological similarity is in concordance with the hydrological connectivity of the area (BAHUN, 1968).

Future morphometric analysis will be focused on intrapopulation and intraspecific variability, which should clarify taxonomic status of each analysed population. Our results are only preliminary indications, because small samples and only some of the morphological features were used.

Habitat

Our results suggest that *Eunapius subterraneus* inhabits phreatic water. It prefers subterranean channels and passages where hydrodynamism is low and temperature varies from 7 to 11°C. It was found at all explored depths (0–23 m). Sponges were numerous in all localities and they were regularly associated with the cave polychaete *Marifugia cavatica* Absolon & Hrabe 1913. These sponges inhabit total darkness. The species *E. subterraneus* was found in the twilight zone on one occasion only, at the entrance of the cave Izvor špilja Gojak. This record does not change the nature of this species as a true stygobiont, it just indicates that light is not a hindering factor to the species' distribution.

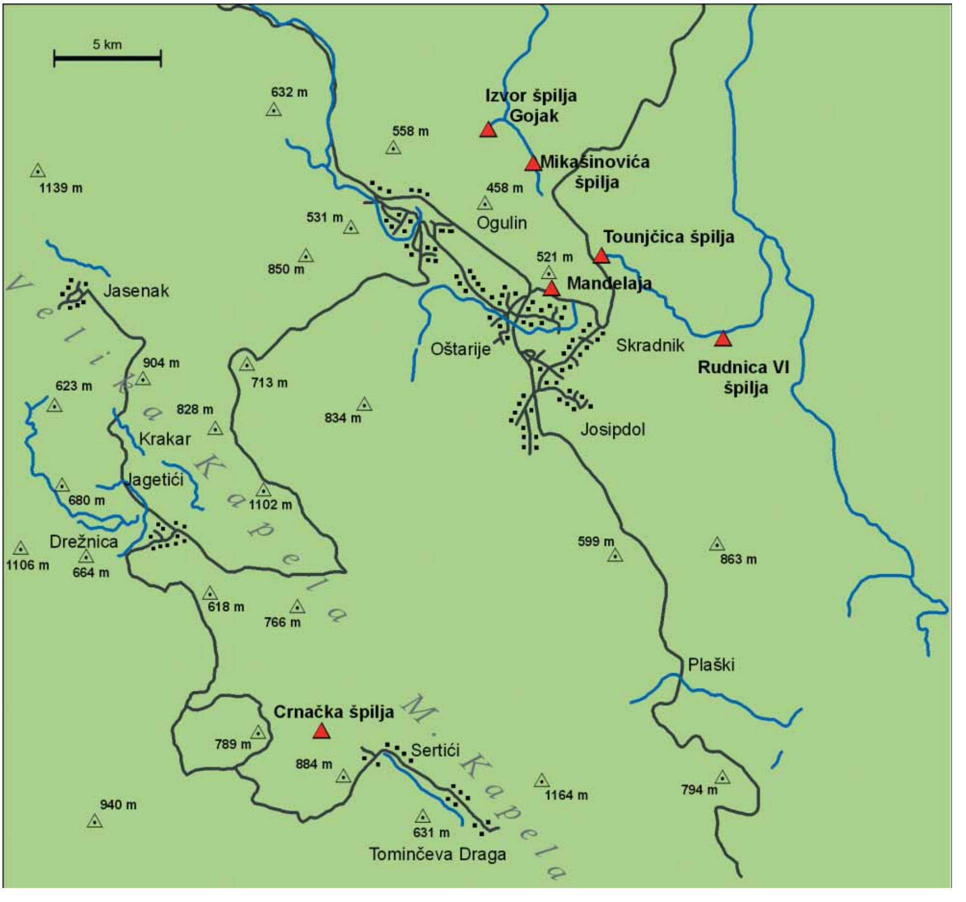

Fig. 12. Distribution of sponges in caves; 1. Izvor špilja Gojak, 2. Mikašinovića špilja, 3. Tounjčica špilja, 4. Mandelaja, 5. Crnačka špilja, 6. Rudnica špilja VI, 7. Špiljski sustav Đula–Medvedica, 8. Špilja u kamenolomu Tounj.

Distribution patterns

The species *E. subterraneus* was confirmed in the caves Tounjčica špilja, Mikašinovića špilja and Rudnica špilja VI (as in SKET & VELIKONJA, 1984), and found for the first time in Mandelaja pit, Crnačka špilja and Izvor špilja Gojak (three new localities) (Fig. 12).

The newly found localities of *Eunapius subterraneus* contribute significantly to the understanding of its living area. The previously known three localities were less then 12 km apart, situated in the source area of large rivers Gojačka Dobra and Primišljanska Mrežnica, north east of the Ogulin-Plaški karst plateau (D region in Fig. 3). Our research showed a wider living area for the species, which is now understood to extend to the Ogulin-plaški karst plateau and to Velika Kapela Mountain (A and C region in Fig. 3), i.e. within a 28 km diameter.

The distribution of subspecies relates to the hydrological features in the research area (Fig. 2). The subspecies *E. s. subterraneus* inhabits underground waters of the rivers Dobra and Zagorska Mrežnica. This is due to the fact that these two systems exchange water in times of high water, meaning that all their localities are more or less well interconnected by a system of subterranean passages (BAHUN, 1968; JALŽIĆ, 2004; JALŽIĆ & BOŽIČEVIĆ, 1970–1971; KUHTA *et al.*, 2001; LACKOVIĆ, 1993). This also relates to the only locality outside the Ogulin-Plaški plateau, the cave Crnačka špilja located on the opposite side of Mt Velika Kapela, which has a subterranean connection with the river Zagorska Mrežnica (BIONDIĆ, 1986). On the other hand, no such connection was documented for the cave Rudnica špilja VI (BAHUN, 1968). Therefore, it is no surprise that Rudnica špilja VI is the only locality in our research where the separate subspecies *E. s. mollisparspanis* was found.

There are reasons to believe that *Eunapius subterraneus* also inhabits two remaining localities, from which material was not collected. It has been demonstrated by tracing that the cave Špiljski sustav Đula-Medvedica is directly linked to the cave Izvor špilja Gojak (KUHTA *et al.*, 2001), while the cave Špilja u kamenolomu Tounj contains water from river Zagorska Mrežnica (LACKOVIĆ, 1993). Therefore, hydrological relations indicate that the populations present in these two caves also belong to the species *Eunapius subterraneus*. Nevertheless, until material is examined, such a hypothesis must not be taken for granted, the more so, since the hydrological relations of the area are not fully understood.

Endangerment and conservation of the species *Eunapius subterraneus*

Although there is a great probability of finding sponge populations in the caves Špiljski sustav Đula-Medvedica and Špilja u kamenolomu Tounj, the fact that in these localities populations were not found is most alarming, for both of the caves are listed as among the ten most endangered karst phenomena in Croatia (PAAR, 2005). Both caves are under great pressure due to river direction changes. The cave Špilja u kamenolomu Tounj is also influenced by the physical destruction, and the cave Špiljski sustav Đula-Medvedica by continuous pollution, sewage and waste dumping (PAAR, 2005a). These facts indicate the necessity to establish whether the populations in these two caves still exist and, if they do, to evaluate the current state of the habitat as well as of the populations. It is vital to devise an urgent conservation strategy and prevent further damage to the species and its habitat.

Along with the directly endangered populations in these two caves, other populations are also exposed to a series of threats. Besides the common problems of karstic water systems, which include a wide distribution of various pollutants, a great problem consists of the major impact of hydrological engineering. The Gojak Hydroelectric Power Plant, built forty years ago, Sabljaci Reservoir and Bukovnik Dam have significantly changed the hydrological characteristics of the whole area. Underground water levels decreased (PASARIĆ, 1961; JALŽIĆ & BOŽIČEVIĆ, 1970–1971), some underground systems dried out (the sinkhole Đulin ponor) and the flows of the Dobra and Zagorska Mrežnica rivers were altered. Unfortunately, effects on sponge populations remain unknown since the species was not discovered before the main changes. This is a good example illustrating a general problem of biospeleology in Croatia: »inventories of subterranean fauna may be so inadequate that many species may go extinct before being discovered« (SCHNEIDER & CULVER, 2004). What kind of and how great an influence will be exerted by the construction of the Lešće Hydroelectric Power Plant in the downstream part of the river Dobra is a matter of discussion.

The species *Eunapius subterraneus* is registered in the Red List of threatened plants and animals of Croatia in the IUCN category EN (endangered) meaning that there is a very high risk of extinction (TVRTKOVIĆ *et al.*, 2004). Although the whole Croatian underground fauna is legally protected, it is absolutely exposed to uncontrolled devastation. Active conservation is still vague and unsupported by any real government strategy, and has been based to date only on the activities of researchers.

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