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New data on distribution of *Hydrurus foetidus* (Villars) Trevisan in freshwater habitats on Vranica Mountain (Bosnia and Herzegovina)

Original Article

Ermin Mašić
University of Sarajevo, Faculty of Science, Department of Biology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
erminmasic@hotmail.com (corresponding author)

Senka Barudanović
University of Sarajevo, Faculty of Science, Department of Biology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
sebarudanovic@gmail.com

Sabina Žero
University of Sarajevo, Faculty of Pharmacy, Department of Natural Sciences in Pharmacy, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
sabina_zero@hotmail.com

Emina Ramić
University of Sarajevo, Faculty of Science, Department of Biology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
emina.ramich@gmail.com

Armin Macanović
University of Sarajevo, Faculty of Science, Department of Biology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
arminecology@gmail.com

Senaid Fejzić
Secondary technical school „Kemal Kapetanovic“ Kakanj, Šehida 32, 72240 Kakanj, Bosnia and Herzegovina
senaidfejzic@yahoo.com

Abstract:

Freshwater habitats such as springs, streams and lakes at mountain area represent extremely fragile ecosystems. These types of habitats occur in the wide area of Vranica Mountain. During our research (August and September 2018) of freshwater habitats in this area, rare golden macroalgae *Hydrurus foetidus* (Villars) Trevisan was found. It has been already detected in Bosnia and Herzegovina, but in this study new localities are presented. Water temperature, pH, dissolved oxygen, specific electrical conductivity, turbidity and total dissolved solids were measured on sampling sites. In addition to the analysis of ecological characteristics of habitats, diatom assemblages were analysed. In this study a total of 48 diatom taxa belonging to 25 genera were identified. The results of diatom indices confirmed the good water quality of the studied sites on which *H. foetidus* was found. This study represents a contribution to algological research in Bosnia and Herzegovina.

Key words:
bioindicators, conservation ecology, macroalgae, springs, streams, water quality

Apstract:

Novi podaci o rasprostranjenosti *Hydrurus foetidus* (Villars) Trevisan u slatkovodnim staništima na planini Vranici (Bosna i Hercegovina)

Slatkovodna staništa kao što su izvori, potoci, reke i jezera na planinskim područjima predstavljaju izuzetno osetljive ekosisteme. Ovi tipovi staništa su zastupljeni na širem području planine Vranice. Tokom istraživanja (Avgust i Septembar, 2018) slatkovodnih tipova staništa na ovom području, utvrđena je retka zlatna makroalga *Hydrurus foetidus* (Villars) Trevisan. Vrsta je već konstatovana u Bosni i Hercegovini, ali u ovom istraživanju predstavljeni su novi lokaliteti. Temperatura vode, pH, rastvoreni kiseonik, elektroprovodljivost, mutnoća i ukupna količina rastvorenih organskih materija su izmereni na mestu uzorkovanja. Pored analize ekoloških karakteristika staništa, analiziran je i sastav diatomeja. Tokom ove studije ukupno je identifikovano 48 dijatomeja iz 25 rodova. Rezultati dijatomskih indeksa potvrdili su dobar kvalitet vode na lokalitetima gdje je utvrđen *H. foetidus*. Ova studija predstavlja značajan doprinos algološkim istraživanjima Bosne i Hercegovine.

Ključne reči:
bioindikatori, konzervacijska ekologija, makroalge, izvori, potoci, kvalitet vode

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Introduction

The species *Hydrurus foetidus* (Villars) Trevisan is a macroscopic golden alga with mucilaginous, branched and bushy to feathery, dark brown thallus up to 30 cm long. The thallus and other characteristics are well described in various publications (John et al., 2003; Wehr et al., 2015).

This species inhabit mountain streams during early spring and lowland rivers at latitudes where seasonal conditions are appropriate for this cold-water species (Klaveness et al., 2011; Klaveness, 2017).

According to Guiry & Guiry (2019), this alga is widely distributed in Europe and it's presence was reported in Austria (Pfister, 1992), Britain (Kristiansen, 2002; Whitton et al., 2003; John et al., 2011), Croatia (Stanković & Leitner, 2016; Koletić et al., 2017), Georgia (Barinova & Kukhaleishvili, 2017), Germany (Mauch & Schmedtje, 2003),

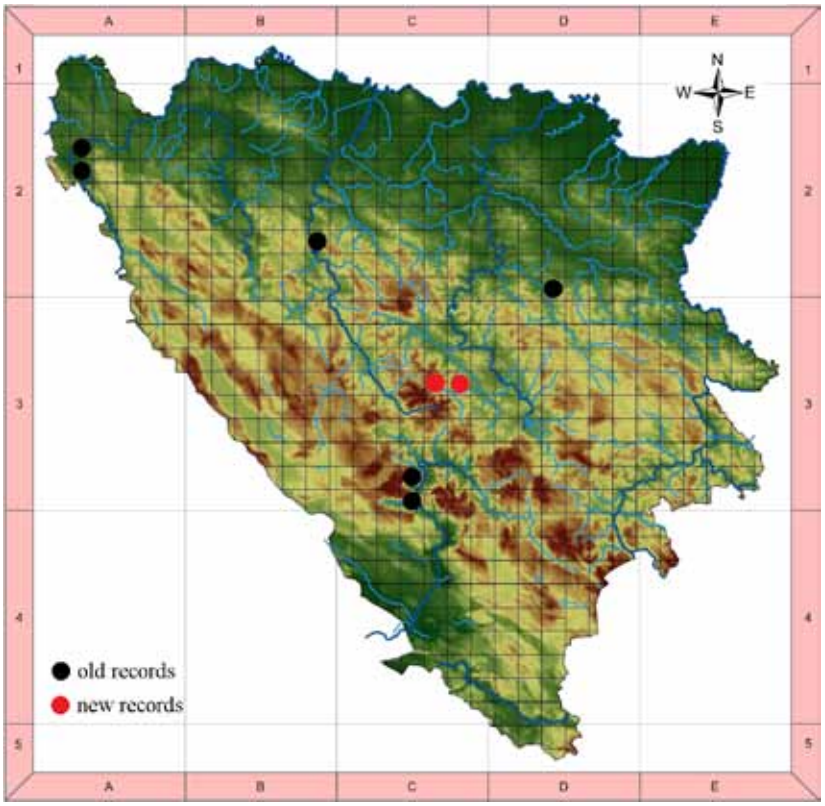

Fig. 1. UTM map of distribution of *Hydrurus foetidus* in Bosnia and Herzegovina

Norway (Klaveness et al., 2011; Klaveness & Lindstrøm, 2011; Klavness, 2012), Romania (Caraus, 2017), Russia (Europe) (Voloshko, 2007; Patova & Demina, 2007; Patova et al., 2014), Serbia (Krizmanić et al., 2008), Slovakia (Hindák & Hindáková, 2016) and Turkey (Europe) (Aysel, 2005).

On the territory of Bosnia and Herzegovina *H. foetidus* was reported only from a few localities (**Fig. 1**), and these data are found in various publications (Protić, 1904; Blagojević, 1966; Kosorić, 1977; Aganović, 1981; Ratković, 1985; Redžić, 1988; Hafner, 1991; Redžić, 1991).

Until now, several algological studies were performed on Vranica Mountain concerning different algal groups and habitats (Protić, 1926; Kapetanović & Hafner, 2007; Barudanović et al., 2017) but did not demonstrate the occurrence of *H. foetidus* in any studied sites.

The main aim of this study was to present new localities where *H. foetidus* occurs considering the ecological characteristics of habitat in which the species was found.

Material and methods

Study area

Vranica Mountain has very heterogeneous geology and petrography. The area is rich in numerous springs, brooks and small rivers, which are active throughout the year. Unfortunately, in recent years due to growing tourism activity anthropogenic impact has increased. These effects are manifested mostly through the construction and expansion of road communication, drainage of wet habitats, springs capturing and the construction of new accommodation facilities (Redžić, 2007).

Sampling sites are listed in **Tab. 1.**

Sampling and algae analysis

Physical and chemical parameters of water were measured directly on sampling sites. Water temperature (°C), pH, dissolved oxygen (mgL^{-1}) and specific electric conductivity (μScm^{-1}) were measured with portable multimeter Orion Star A329, while turbidity (NTU) was measured with Portable turbidimeter AQ3010 and total dissolved solids (ppm) with PCE-CM 41.

Live algological material was collected from six microlocalities in 2018, during summer and autumn seasons, photographed and transported to the Laboratory of algae and fungi at Faculty of Science, University of Sarajevo. Phytobenthos was collected

Table 1. The main physical characteristics of the sampling sites (SP – spring; ST – stream)

Site	Habitat types	Date	Coordinate [N]	Coordinate [E]	Altitude [m]	Anthropogenic disturbance
1.	SP1	11/08/2018	43.95353	17.75757	1682	Moderate
2.	ST1	11/08/2018	43.95353	17.75757	1682	Moderate
3.	ST2	11/08/2018	43.95440	17.75760	1677	Moderate
4.	ST3	25/08/2018	43.95731	17.74518	1727	Moderate
5.	ST4	20/10/2018	43.95563	17.75786	1465	Moderate
6.	ST5	20/10/2018	43.95544	17.75767	1652	Moderate

Fig. 2. Two types of habitats where *Hydrurus foetidus* was recorded: mountain spring (left) and mountain stream (right)

from small stones by scraping or by squeezing of mosses growing on rocks and stones. The collected material was fixed with a 4% formalin. Small portion of *H. foetidus* was collected and observed under the microscope from temporary slides under 40 x magnification. The identification of *H. foetidus* was supported by the following references: Starmach (1985), John et al. (2003) and Wehr et al. (2015).

Laboratory processing of diatoms was carried out applying methods used by Hustedt (1930). Light microscope observation was conducted using Best Scope 2020 microscope. Species composition and the quantitative relationship of diatoms are estimated from the permanent slides under 1000 x magnification.

The identification of diatoms was done according to Lange-Bertalot & Metzeltin (1996) and Cantonati et al. (2017).

The nomenclature of identified algae was adjusted according to the internet base of Guiry & Guiry, 2019.

At least 300 valves in each slide were counted for all samples. Species abundance of diatoms are estimated on a five-degree scale as follows: 1-rare (single valve or frustule), 2-sparse (up to 10% of the sample), 3-frequent (11-15% of the sample), 4-very frequent (51-75% of the sample), 5-common (in more than 75% of the sample) (**Tab. 3**).

Omnidia software (Lecointe et al., 1993) version 6.0.8, was used to calculate diatom indices, including ecological and taxonomic data. Water quality of the localities where *H. foetidus* was assessed based on the following diatom indices: IPS (Coste, 1982), SLAD (Sládeček, 1986), TID (Rott et al., 1999) and SID - ROTT (Rott et al., 1997). Range of diatom indices varied from 1 to 20 and corresponding to the water quality as follows: bad (1-4), poor (5-8), moderate (9-12), good (13-16) and very good (17-20) (Solak et al., 2020).

Results

Description of sampling sites

The species of *H. foetidus* (Chrysophyceae) was found near a reocren spring of mountain stream and on five localities downstream. It lives as a benthic species attached to the mesolithic and megalithic stones on the bottom of the water body an is often associated with mosses. Although it tolerates occasional water fluctuations, in most cases it has been found in running water, but also in places where the water is a little deeper and slower. The altitude of the sites where the *H. foetidus* was recorded, ranged from 1465 to 1727 meters.

The spring and mountain stream are covered with dense macrophytes and helophytes vegetation.

Two types of habitats where *H. foetidus* is recorded are showed on **Fig. 2**, while in **Fig. 3** and **Fig. 4** the macroscopic and microscopic appearance of the thallus are shown.

Physical and chemical parameters of water

A comparison of the physical and chemical parameters of water in the mountain spring and stream revealed the following: the water temperature on sampling sites during the investigated period varied from 6.2 to 19.9 °C. The lowest pH of the water was 7.92 while the highest pH value was 8.50. The highest concentration of dissolved oxygen in water was measured at the site 2 (10.82 mgL^{-1}), while the smallest value was measured at the site 4 (7.56 mgL^{-1}). Value of specific conductivity varied from 162 to 330 μScm^{-1}. The lowest value of turbidity was measured at the site 4 (0.45 NTU), while at the site 6 the highest value of turbidity was measured (2.81 NTU).

Fig. 3. Thallus of *H. foetidus* (Villars) Trevisan

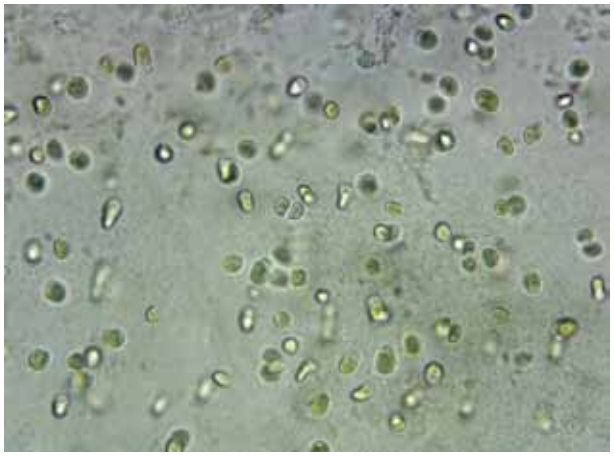

Fig. 4. Microscopic appearance of the thallus of *H. foetidus* (Villars) Trevisan (40 x)

Values of physical and chemical parameters are presented in **Tab. 2.**

Characteristics of habitats of *Hydrurus foetidus* and diversity of diatoms

Hydrurus foetidus has been identified at six sites located in the area of Vranica Mountain. It inhabits mainly spring and fast and clean mountain streams. Together with mosses and other groups of algae, it grows on mesolithic and megalithic bases, and represents a significant product component of these freshwater habitats. The presence of this species indicates good water quality and good ecological status of freshwater ecosystems. In addition to *H. foetidus*, diatoms are second the most prominent group of algae which have been found in this mountain freshwater habitats. A total of 48 diatom taxa belonging to 25 genera were determined in the collected material (**Tab. 3**). The number of recorded species of diatoms in the study sites ranged from 11 to 22. Genera with the highest number of species were *Pinnularia* (5), *Gomphonema* (5), *Diatoma* (4) and *Cocconeis* (4). Altogether 47 diatom taxa mostly belonging to pennate diatoms were found. The centric species, *Aulacoseira crenulata* (Ehrenberg) Thwaites, appeared in only one sample. The most common species were *Odontidium mesodon* (Ehrenberg) Kützing, *Cocconeis lineata* Ehrenberg, *Meridion circulare* (Gréville) C. Agardh, *Cocconeis placentula* Ehrenberg, *Gomphonema minusculum* Krasske and *Achnanthidium minutissimum* (Kützing) Czarnecki.

Water qualities of investigated sites according to diatom indices

Values of IPS diatom index varied from 15.0 to 18.3. According to the IPS diatom index, all studied sites have very good water quality. Saprobic index at four sites indicates good, while on the other studied sites it indicates very good water quality. Trophic index at all investigated sites indicates moderate water quality. Saprobic index according to Rott et al. (1997) ranged from 16.4 to 17.1. High values of diatom indices correspond to a very good water quality of investigated freshwater habitats on Vranica Mountain (**Tab. 3**).

Discussion

Hydrurus foetidus was found in the mountain spring (1682 m a.s.l.) and in the mountain streams with

Table 2. Value of the water temperature, pH, dissolved oxygen, specific electric conductivity, turbidity and TDS [T_1 – water temperature, DO – dissolved oxygen, SC – specific electric conductivity, T_2 – turbidity, TDS – total dissolved substance].

Site	Group	T_1 [°C]	pH	DO [mgL^{-1}]	SC [µScm^{-1}]	T_2 [NTU]	TDS [ppm]
1.	SP1	6.8	7.92	10.35	330	0.80	**N/a**
2.	ST1	6.2	7.97	10.85	162	1.12	**N/a**
3.	ST2	7.4	8.23	10.25	208	0.73	**N/a**
4.	ST3	19.9	8.31	7.56	171	0.45	121
5.	ST4	8.1	8.47	10.51	**N/a**	0.91	267
6.	ST5	7.4	8.50	10.29	219	2.81	161

*N/a-not available

Table 3. Records of *Hydrurus foetidus* and diatom taxa at sampling sites

No.	*TAXA / Site*	*1*	*2*	*3*	*4*	*5*	*6*
H	***Hydrurus foetidus* (Villars) Trevisan**	+	+	+	+	+	+
1.	*Odontidium mesodon* (Ehrenberg) Kützing	4	6	4	1	1	3
2.	*Cocconeis lineata* Ehrenberg	1	2	5	2	3	4
3.	*Meridion circulare* (Gréville) C. Agardh	1	2	1	2	1	1
4.	*Cocconeis placentula* Ehrenberg	1	3	2	1	.	3
5.	*Gomphonema minusculum* Krasske	1	1	1	1	1	.
6.	*Achnanthidium minutissimum* (Kützing) Czarnecki	6	1	.	1	1	1
7.	*Encyonema minutum* (Hilse) D.G. Mann	4	.	1	1	1	.
8.	*Tetracyclus rupestris* (Braun) Grunow in Van Heurck	.	1	1	1	1	.
9.	*Diatoma ehrenbergii* Kützing	.	.	1	4	4	4
10.	*Ulnaria ulna* (Nitzsch) Compère	.	.	1	6	1	1
11.	*Encyonema silesiacum* (Bleisch) D.G. Mann	.	1	1	1	.	.
12.	*Hannea arcus* (Ehrenberg) R.M.Patrick in Patrick & Reimer	.	.	5	.	4	3
13.	*Encyonema ventricosum* (C.Agardh) Grunow in A. Schmidt et al.	1	1	.	.	.	.
14.	*Pinnularia borealis* Ehrenberg	1	1	.	.	.	.
15.	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot	1	1	.	.	.	.
16.	*Gomphonema parvulum* (Kützing) Kützing	.	1	2	.	.	.
17.	*Denticula tenuis* Kützing	.	1	1	.	.	.
18.	*Caloneis alpestris* (Grunow) Cleve	.	1	.	1	.	.
19.	*Diploneis krammeri* Lange-Bertalot et Reichardt	.	1	.	1	.	.
20.	*Epithemia turgida* (Ehrenberg) Kützing	.	1	.	1	.	.
21.	*Gomphonema acuminatum* Ehrenberg	.	.	1	.	1	.
22.	*Cocconeis pediculus* Ehrenberg	1	.	.	.	.	.
23.	*Cymbopleura naviculiformis* (Auerswald) Krammer	1	.	.	.	.	.
24.	*Gomphonema productum* (Grunow) Lange-Bertalot et Reichardt in L-B	1	.	.	.	.	.
25.	*Navicula lanceolata* (C.Agardh) Ehrenberg	1	.	.	.	.	.
26.	*Nitzschia fonticola* Grunow in Cleve & Möller	1	.	.	.	.	.
27.	*Pinnularia viridis* (Nitzsch) Ehrenberg	1	.	.	.	.	.
28.	*Diatoma vulgaris* Bory	.	1	.	.	.	.
29.	*Diploneis ovalis* (Hilse) Cleve	.	1	.	.	.	.
30.	*Eunotia sp. 1*	.	1	.	.	.	.
31.	*Diatoma elongatum* (Lyngbye) Agardh	.	1	.	.	.	.
32.	*Pinnularia rupestris* Hantzsch in Rabenhorst	.	1	.	.	.	.
33.	*Amphora copulata* (Kützing) Schoemann et Archibald	.	1	.	.	.	.
34.	*Aulacoseira crenulata* (Ehrenberg) Thwaites	.	.	1	.	.	.
35.	*Cymbella aspera* (Ehrenberg) H. Peragallo	.	.	1	.	.	.
36.	*Navicula tripunctata* (O.F. Müller) Bory	.	.	1	.	.	.
37.	*Cymbella neolanceolata* W.Silva	.	.	.	1	.	.
38.	*Cymbopleura amphicephala* (Nägeli) Krammer	.	.	.	1	.	.
39.	*Diatoma moniliformis* (Kützing) Williams	.	.	.	1	.	.
40.	*Gomphonema subclavatum* (Grunow) Grunow	.	.	.	1	.	.
41.	*Pinnularia neomajor* Krammer	.	.	.	1	.	.
42.	*Staurosirella leptostauron* (Ehrenberg) D.M. Williams et Round	.	.	.	1	.	.
43.	*Staurosirella pinnata* (Ehrenberg) D.M. Willams et Round	.	.	.	1	.	.
44.	*Surirella spiralis* Kützing	.	.	.	1	.	.
45.	*Nitzschia acidoclinata* Lange-Bertalot	.	.	.	.	1	.
46.	*Pinnularia subrupestris* Krammer	.	.	.	.	1	.
47.	*Cocconeis pseudolineata* (Geitler) Lange-Bertalot	.	.	.	.	.	1
48.	*Cymbopleura austriaca* (Grunow) Krammer	.	.	.	.	.	1
A	***Total number of taxa (N)***	**16**	**22**	**17**	**22**	**13**	**10**
B	***IPS/20***	18.3	18.3	17.7	15.0	17.0	17.0
C	***Sla/20***	16.4	17.1	17.7	14.7	17.2	16.5
D	***Rott TI/20 (TID)***	11.6	8.5	11.5	6.5	11.6	12.0
E	***Rott TI/20 (SID)***	16.5	16.9	16.5	16.7	16.4	17.1

altitude ranged between 1465 and 1727 meters a.s.l. In all studied sites, the species was well developed.

According to literature data (Klaveness, 2017, 2019) *H. foetidus* appears under different ecological conditions. This species is a rheophile, preferring swiftly flowing water. It is also a psychrophile and dependent on low temperature. The species *Hydrurus foetidus* is visibly a large freshwater alga found in cold rivers. Generally, the species may be found during late winter months, sometimes forming dense cover on pebbles and rocks at the river bottom and less prominent in the spray zone. The species is usually well developed during the colder months of the year but disappears in summer with the increasing flow (Klaveness, 2019).

According to Klaveness (2017) this species is an indicator of clean water and good ecological status, but there is a various observation that some population tolerate mesosaprobic or oligo-mesosaprobic condition.

In some occasions, *H. foetidus* thrives well under seasonal climatic conditions, where there is snow in the winter, cold meltwater and moderate summer temperatures (Klaveness, 2019).

By comparing our results, with the results of other literature data, a certain similarity of ecological characteristics of habitas can be obtained.

The results of our study are correlated with other research conducted in the Balkan Peninsula dealing with ecology of *H. foetidus* (Blagojević & Hafner, 1979; Redžić, 1988; Krizmanić et a., 2008; Stanković & Leitner, 2016) especially in terms of habitats characteristics and physical and chemical parameters of water.

On the site in the river Neretva (Redžić, 1988) water temperature ranged from to 11.5 – 14.9 °C, pH value varied between 7.5 – 8.3 and dissolved oxygen ranged between 10.6 – 12.3 mgL^{-1}.

The distribution and ecological characteristics of *H. foetidus* in Serbia are given by the authors Krizmanić et al. (2008). At two investigated sites, water temperature ranged from 4.6 to 9.6 °C, while the oxygen concentration ranged from 11.0 to 15.1 mgl^{-1}. By summarizing a large number of data, but also on the basis of the study carried out, the authors concluded that the measured physical and chemical parameters of water confirm the rheophilic nature of this species.

Stanković & Leitner (2016) recorded *H. foetidus* in Croatia for the first-time during the environmental study of the Bijela rijeka located within the Plitvice Lakes National Park. The water temperature on the studied site was 9.0 °C, pH value 8.3 while the specific electrical conductivity was 441 μScm^{-1}. The concentration of oxygen was 11.02 mgL^{-1}, COD 1.32 mgO_2L^{-1}, and the alkalinity had the values of 259.5 $mgCaCO_3L^{-1}$. In terms of measured nutrients, ammonium was not found in the studied sites, the nitrates had low values (0.003 $mgNL^{-1}$), while the nitrates had somewhat higher values (0.759 $mgNL^{-1}$). On the studied site orthophosphates were also measured, and their value was 0.025 $mgPL^{-1}$. The average water velocity was 0.60 ms^{-1} and the water depth ranged from 20 to 15 cm.

During investigation of algal assemblages in springs of the Konjuh Mountain golden algae *Phaeodermatium rivulare* Hansgirg (Chrysophyceae) was identified (Kamberović et al., 2019).

Unfortunately, species which are very common in alpine springs, such as *H. foetidus* was not recorded in this study. The main reason for absence of this species was due to the lower water velocity and different geological substratum (Kamberović et al., 2019).

In addition to *H. foetidus* which indicates clean water and good ecological status of freshwater habitats according to literature data (Klaveness, 2019), ascertained diatoms indicate the same or similar environmental conditions.

The results of diatom indices confirmed the good water quality of the studied sites on which *H. foetidus* was found.

Along with *H. foetidus,* the authors Stanković & Leitner (2016) found certain diatoms and blue-green algae in the Bijela rijeka which is located in the Plitvice Lakes National Park. In addition to *H. foetidus* same authors identified two macroalgae, namely *Zygnema* sp. and *Vaucheria* sp.

During complex ecological studies on algal assemblages in the Krivaja river (Bosnia and Herzegovina) authors Blagojević & Hafner (1979) noted the presence of *H. foetidus*. In all investigated samples 40 taxa are common and the most prominent are *Chamaesiphon incrustans*, *Nostoc sphaericum*, *Phormidium favosum*, *Phormidium autumnale*, *Vaucheria* sp., *Diatom ehrenbergii*, *Cocconeis pediculus*, *Achnanthidium minutissimum*, *Navicula tripunctata* and *Encyonema ventricosa*.

Despite previously conducted research on Vranica Mountain, the presence of rare and endangered species *H. foetidus* has not been noted. The main reason lies in the fact that the weather conditions that prevail in the area of the Vranica Mountain are very severe, and that earlier research was carried out during favourable climatic conditions and at a time when the species was no longer present.

The species *H. foetidus* inhabits freshwater habitats in high altitudes that are under weak or moderate anthropogenic impacts. As it indicates the good ecological status of mountain springs and streams it can be used in bioindication of oligotrophy and good ecological status of freshwater habitat types

in the mountain area. In this respect, the habitats that provide conditions for the development of this alga have great conservation importance.

According to the Red list of Slovakia, the species *H. foetidus* is classified into endangered categories (EN), while the category of vulnerability is not included in the IUCN Red list (Feráková et al., 2001).

Hydrurus foetidus develops thick mat on solid river bottom, therefore, it is a valuable food source for emerging waterborne insect larvae (Milner et al., 2001, 2009; Zah et al., 2001 in Klaveness, 2017). *Hydrurus foetidus* appears to be of specific importance for the early emerging aquatic larvae of chironomids (Klaveness, 2019).

In a study conducted by the authors Moog & Janecek (1991) in the rivers of the Central Alps, it was determined that the distribution of biomass macrozoobenthos varies significantly if different substrates are compared, and that a positive correlation is determined by the biomass of the benthic macroalgae *H. foetidus*, and negative correlation is determined by the flow of water.

Conclusions

The presented study suggests that the species *H. foetidus* prefers freshwater habitats located at higher altitudes. It usually occurs during the cold months, but if the conditions permit, it can occur even during the warm months when the thallus starts to disintegrate slightly. The physical and chemical characteristics measured on the investigated sites corresponded to the literature data.

Habitats providing conditions for the development of *H. foetidus* on Vranica Mountain have a good water quality.

In our study distributional data are presented, and also environmental characteristics of habitats in which *H. foetidus* usually occures.

In order to expand knowledge about the ecological characteristics of macroalgae in the future, it is necessary to conduct more detailed research not only in the area of Vranica Mountain but also on the other mountains of Bosnia and Herzegovina. In addition to measuring the basic physical and chemical parameters of water, it is necessary to take into account other parameters, such as nutrients and heavy metals.

This study contributes to algological research in Bosnia and Herzegovina and also give good basis for future investigation of macroalgae in this area.

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