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THE FIRST RECORD OF *HYDRURUS FOETIDUS* (VILLARS) TREVISAN (OCHROPHYTA: CHRYSOPHYCEAE) IN CROATIA WITH ECOLOGICAL NOTES

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During research into phytobenthos, macrophytes and macroinvertebrates in Bijela rijeka River in the Plitvice Lakes National Park in 2015, *Hydrurus foetidus* (Villars) Trevisan was recorded for the first time in Croatia. *H. foetidus* is a macroalga belonging to the group of golden algae or chrysophytes (Ochrophyta: Chrysophyceae). It is a stenotherm species and an exclusive inhabitant of cold mountain streams, preferring a fast-flow environment and riffles. The identification of *H. foetidus* in Bijela rijeka River indicates high water quality despite the highly modified river morphology and severe anthropogenic impacts received in Plitvički Ljeskovac village.

Key words: Bijela rijeka River, National Park Plitvice Lakes, *Hydrurus foetidus*, small mountain river, karstic river, new record.

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Tijekom istraživanja fitobentosa, makrofita i makrozoobentosa u Bijeloj rijeci u NP Plitvička jezera u 2015. godini, po prvi je puta u Hrvatskoj utvrđena vrsta *Hydrurus foetidus* (Villars) Trevisan. *H. foetidus* je makroskopski vidljiva alga iz skupine zlatnožutih algi (Ochrophyta: Chrysophyceae). To je stenotermna vrsta, isključivi stanovnik hladnih gorskih tekućica koja preferira plitke dijelove s brzom vodenom strujom. Nalaz alge *H. foetidus* ukazuje na visoku kvalitetu vode u Bijeloj rijeci, unatoč njenoj morfološkoj degradiranosti i antropogenom utjecaju naselja Plitvički Ljeskovac.

Ključne riječi: Bijela rijeka, Nacionalni park Plitvička jezera, *Hydrurus foetidus*, mala planinska rijeka, krška rijeka, novi nalaz.

INTRODUCTION

Sampling of biological elements in rivers and lakes through the national water quality monitoring program (Hrvatske vode, 2015) and specific scientific research efforts that cover the ecological and taxonomic aspects of different aquatic organisms (Ivković *et al.*,

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2007; Stanković *et al*., 2012, Žutinić *et al*., 2014), often miss out small mountain rivers, or investigate only their springs and spring areas that are interesting as Dinaric biodiversity hot-spots (Ivković & Plant, 2015). Therefore, the identification of new species making a contribution to Croatian biodiversity is focused on other types of freshwater habitats (Stanković & Ternjej, 2007). Bijela rijeka River, one of the main water suppliers of the most famous Croatian Plitvice Lakes National Park was investigated in detail in the past (Kostić-Brnek & Brnek-Kostić, 1965; Matoničkin & Pavletić, 1967), but in recent years, only its spring area has been studied (Ivković *et al*., 2010; Mičetić Stanković *et al*., 2015). In 2015, research into water quality based on biological quality elements in Bijela rijeka River was conducted, covering the spring area and the main water course including one of five small artificial lakes with occasional connections to the river (Stanković & Leitner, 2015). One of the interesting results was the first observation of the chrysophyte *Hydrurus foetidus* (Villars) Trevisan in Croatia. The presence of *H. foetidus* is an exceptional example of coldwater stenotherm species occurring in the Bijela rijeka River (Wehr *et al*., 2015). This finding contributes to the biodiversity of Croatian algal flora and to our better knowledge of the present state of ecological conditions in this small, karstic mountain river. Together with the first identification of the taxon in Croatia, we provide a habitat description including basic hydromorphological, physical and chemical water characteristics, as well as a detailed algal taxonomic list on the observed taxa on this specific site in the Bijela rijeka River.

MATERIALS AND METHODS

Study area

Bijela rijeka River is a small, karstic mountain river with a total catchment size of 11.93 km^2 (Stanković & Leitner, 2015). It is part of the Black Sea watershed positioned

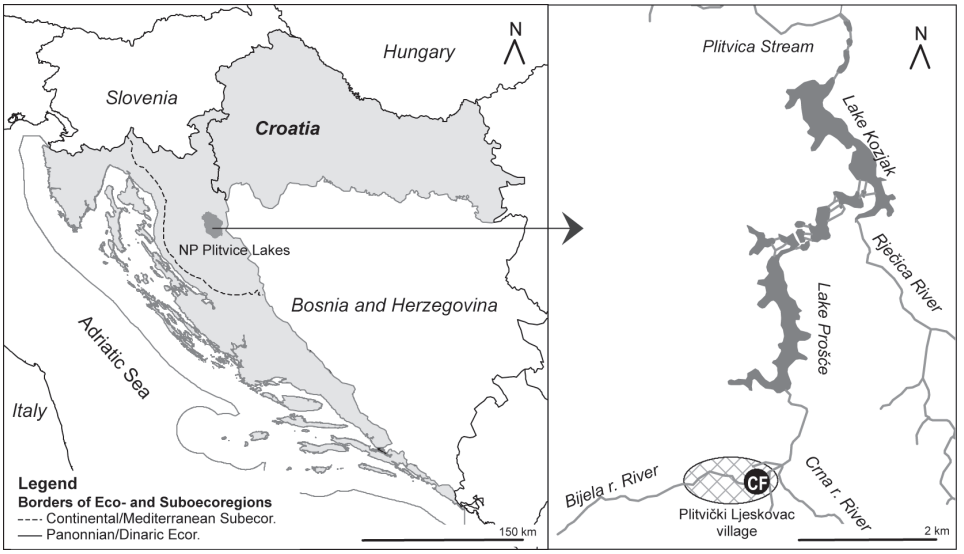

Fig. 1. Map indicating the exact location of the first observation of *Hydrurus foetidus* in Croatia (HF).

in the Continental Subecoregion of the Dinaric Ecoregion of Croatia (Hrvatske vode, 2015). Bijela rijeka River, together with Crna rijeka River, belongs to the spring area of Plitvice Lakes protected by UNESCO as a world natural heritage site since 1979 (Stilinović & Božičević, 1998). These two rivers are the main water source for the famous 16 cascade lakes in the Plitvice Lakes National Park. Bijela rijeka River originates from a small rheocrene spring at 720 m a.s.l. and after a course of 4 km through the forest edges and Plitvički Ljeskovac village, it merges with Crna rijeka River and creates the short Matica River, which flows further into the lakes.

Sampling and analysis of algae

Sampling and analysis of algae was done according to the Croatian Methodology of sampling, laboratory analysis and determination of ecological quality ratio of biological elements (National Gazette, 73/13, National Gazette, 78/15, National Gazette, 151/14) based on CEN standard HRN EN 15708:2010. Multi habitat sampling of phytobenthos in Bijela rijeka River was conducted in May 2015 during investigation of biological elements for indication of the ecological status of the river (Stanković & Leitner, 2015). Algae were collected in two ways: small algae growing on rocks were scraped with a toothbrush, while macroalgae were handpicked or taken by tweezers. In total, four samples were taken, stored in plastic bottles and preserved with 4% formaldehyde for further analysis. One sample was scraped from rocks and the other three samples were handpicked macroalgae: *Zygnema* sp., *Vauscheria* sp. and *H. foetidus*. Based on estimated abundance in river and abundance under the microscope, the final abundance of each taxon was estimated (HRN EN 15708:2010).

For microscopic analysis 400-1000x magnification was used. *H. foetidus* was identified according to Starmach (1985). Other algae were identified at the lowest taxonomic level possible, using up to date identification manuals (Cox, 1996; Eloranta *et al*., 2011; Ettl *et al*., 2009; John *et al*., 2002; Komárek & Anagnostidis, 2000, 2005; Komárek & Büdel, 2013; Krammer & Lange-Bertalot, 2008, 2010, 2011).

Analysis of physical and chemical properties of water

The analysis of the physical and chemical properties of water was done within the water quality monitoring of the Dr. Ivo Pevalek Science Research Centre, Plitvice Lakes National Park. Water depth and water velocity were measured at 20 points throughout the sampling section with Flo-Mate Marsh McBirney 2000.

RESULTS

Hydrurus foetidus (Villars) Trevisan was identified for the first time in Croatia in Bijela rijeka River on the sampling site in Plitvički Ljeskovac village (N44°50'30.9", E15°35'47.5"). It was macroscopically visible, dark brown, ~10 cm in length with slippery feel to it, attached to a small twig (Fig. 2a). After microscopical analysis, one characteristic chromatophore was visible on the top of the ovoid cells grouped in formations resembling branches (Fig. 2b-d).

Description of the sampling site

Both river banks of the Bijela rijeka River are under strong anthropogenic influence, especially the left bank that extends next to the margin of an asphalt road stabilized with

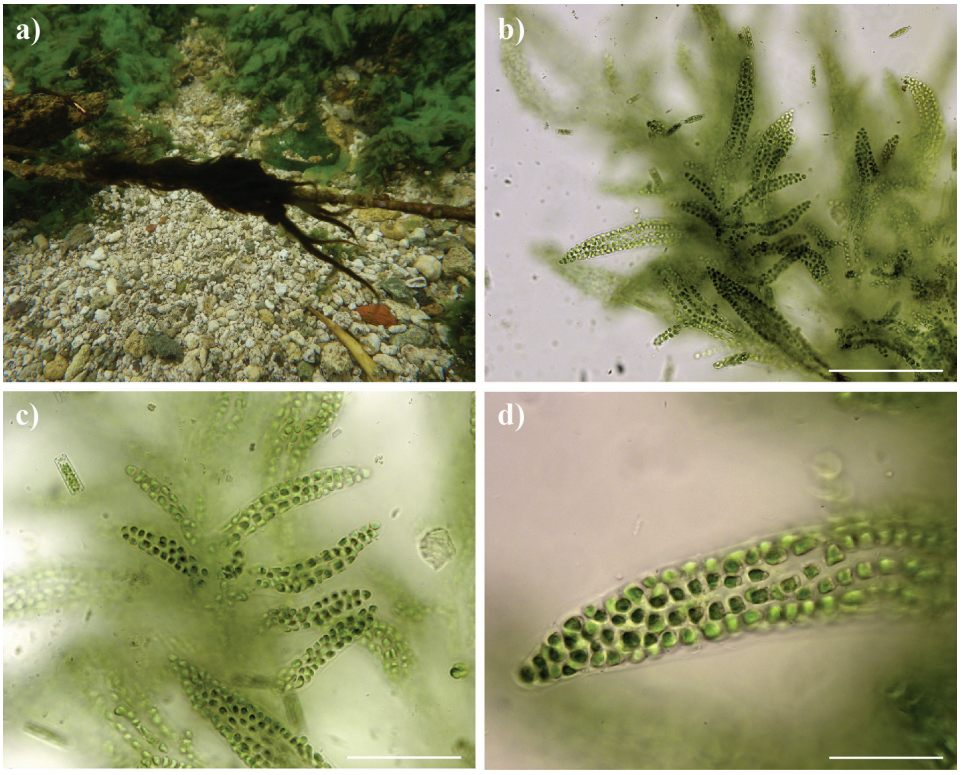

Fig. 2. a) Habitus of *Hydrurus foetidus* (dark brown) with *Zygnema* sp. in the background (green); b) *Hydrurus foetidus* on 100x magnification (BF, scale = 200 μm); c) *Hydrurus foetidus* on 200x magnification (BF, scale = 100 μm); d) *Hydrurus foetidus* on 400x magnification (BF, scale = 50 μm).

Fig. 3. Sampling site of the first observation of *Hydrurus foetidus*: Bijela rijeka River in Plitvički Ljeskovac village, Croatia.

Tab. 1. List of algae found in Bijela rijeka River at a sampling site in Plitvički Ljeskovac village coexisting together with *Hydrurus foetidus*.

Taxa List	Relative abundance
Cyanobacteria	
Chamaesiphon incrustans Grunow	2
Chroococcus turgidus (Kützing) Nägeli	1
Hyella fontana Huber & Jadin	1
Merismopedia glauca (Ehrenberg) Kützing	1
Microcoleus autumnalis (Gomont) Strunecky, Komárek & J.R.Johansen	3
Oscillatoria sp.	2
Oscillatoria tenuis C.Agardh ex Gomont	2
Phormidium sp.	2
Pseudanabaena sp.	1
Tapinothrix janthina (Bornet & Flahault) Bohunická & J.R.Johansen	3
Charophyta	
Zygnema sp.	4
Ochrophyta: Bacillariophyceae	
Amphora sp.	1
Achnanthidium spp.	4
Cocconeis placentula Ehrenberg	2
Cyclotella meneghiniana Kützing	1
Cymbella spp.	2
Diatoma mesodon (Ehrenberg) Kützing	1
Diatoma tenuis C.Agardh	1
Diatoma vulgaris var. *capitulata* Grunow	3
Diploneis sp.	1
Gomphonema spp.	1
Meridion circulare (Greville) C.Agardh	1
Navicula lanceolata Ehrenberg	1
Navicula spp.	2
Navicula tripunctata (O.F.Müller) Bory	1
Nitzschia spp.	1
Ulnaria ulna (Nitzsch) P.Compère	3
Ochrphyta: Chrysophyceae	
Hydrurus foetidus (Villars) Trevisan	1
Rhodophyta	
Audouinella hermannii (Roth) Duby	1
Xantophyta	
Vaucheria sp.	1

rocky blocks (Fig. 3). Dominant substrate of the river bed is microlithal and mesolithal, overgrown by mosses and the macroalga *Zygnema* sp. attached to mosses. The river's width is 5-7 m. The sampling site is just upstream of the confluence of Ljeskovac Stream.

Physical and chemical properties of water

Water temperature was 9.0°C, pH was 8.3 and conductivity was 441 μScm^{-1}. Oxygen concentration was 11.02 mgL^{-1}, COD was 1.32 mgO_2L^{-1} while alkalinity was 259.5 $mgCaCO_3L^{-1}$. With regard to nutrients, ammonia was not detected in the water, nitrites were 0.003 $mgNL^{-1}$, nitrates 0.759 $mgNL^{-1}$ and orthophosphates were 0.025 $mgPL^{-1}$.

Water velocity in Bijela rijeka River in Plitvički Ljeskovac village was 0.16 – 1.01 ms^{-1} where the average water velocity was 0.60 ms^{-1}. In the sampling stretch, the depth of Bijela rijeka River ranged between 20 and 25 cm, with an average depth of 23 cm.

Taxonomic composition of benthic algae

Hydrurus foetidus was found growing only on one spot in Bijela rijeka River, therefore its relative abundance was low (Tab. 1). The dominant alga was *Zygnema* sp., which overgrew mosses in the stream, covering 50% of the river bed. The other 50% of the river bed represented by microlithal and mesolithal was covered with a thick layer of algae, mostly composed of diatoms (*Achnanthidium* sp., *Cocconeis placentula*, *Diatoma vulgaris* var. *ehrenbergii*, *Ulnaria ulna*, etc.) and cyanobacteria (*Chamaesiphon incrustans, Microcoleus autumnalis, Tapinothrix janthina*, etc.). In addition to *H. foetidus* and *Zygnema* sp., a third macroalga was found in Bijela rijeka River in Plitvički Ljeskovac village and was identified as *Vaucheria* sp. It was found at only few spots on the river bed, covering small surface and therefore assessed as having low relative abundance.

DISCUSSION

Recent phycological studies in Croatia have mainly focused on lake and river phytoplankton (Gligora *et al.*, 2007; Gligora Udovič *et al.*, 2015; Stanković *et al.*, 2012). Benthic algae or phytobenthos are rarely in the focus of interest, although it is investigated in the Pannonian ecoregion, more specifically in the Danubian floodplain (Žuna Pfeiffer *et al.*, 2015). Benthic algae have also been investigated in experiments on tufa barriers and artificial substrates in karstic lakes (Kralj *et al.*, 2006; Plenković-Moraj *et al.*, 2002), but never in small mountainous rivers. Our first observation of the chrysophyte macroalga *H. foetidus* drew our attention to the fact that common algae like *H. foetidus* in cold mountain rivers (Krizmanić *et al.*, 2008; Wehr *et al.*, 2015) are not extensively enough studied.

Wehr *et al.* (2015) state that is *H. foetidus* is not only a cold stenotherm species, but it is also restricted to riffles with strong current velocity, and has a higher metabolic demand for nutrients. Despite the sufficient nutrient concentration and the high water velocity shown in our study potentially making Bijela rijeka River a perfect habitat for *H. foetidus*, it was not the dominant taxon. Instead, *Zygnema* sp. Was characteristically dominant, although this taxon has habitat preferences that are opposite (Wehr *et al.*, 2015). One possible explanation could be that *H. foetidus* disappears above 10°C (Starmach, 1985), whereas our observation was slightly below this limit.

Our first observation of *H. foetidus* in Croatia draws attention to the importance of future phycological studies of mountain streams. Those studies might clarify the sea-

sonal dependency of the benthic algae of Bijela ijeka River, contributing, furthermore, to a better understanding of the role of algal biodiversity and its functioning in pristine Croatian mountainous habitats.

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SAŽETAK

Prvi nalaz alge *Hydrurus foetidus* (Villars) Trevisan (Ochrophyta: Chrysophyceae) u Hrvatskoj i njezine osnovne ekološke značajke

I. Stanković & P. Leitner

Hydrurus foetidus (Villars) Trevisan je makroskopski vidljiva alga iz skupine zlatnožutih algi (Ochrophyta: Chrysophyceae), koja je po prvi puta utvrđena u Hrvatskoj tijekom istraživanja fitobentosa, makrofita i makrozoobentosa u 2015. godini u Bijeloj rijeci u NP Plitvička jezera. Alga *H. foetidus* je stenotermna vrsta i isključivi stanovnik hladnih gorskih tekućica, koja preferira plitke dijelove s brzom vodenom strujom. S obzirom na ekološke karakteristike ove makroalge, može se reći kako je Bijela rijeka, kao hladna mala gorska tekućica savršeno stanište za nju. Alga *H. foetidus* je u Bijeloj rijeci utvrđena u naselju Plitvički Ljeskovac, gdje je maksimalna dubina vode samo 25 cm, a izmjerena brzina strujanja vode je dosezala 1.01 m/s. Nalaz ove alge u Bijeloj rijeci ukazuje na visoku kvalitetu vode, unatoč njenoj hidromorfološkoj degradiranosti i antropogenom utjecaju naselja Plitvički Ljeskovac. Značaj ovog istraživanja nije samo u otkrivanju nove vrste makroalge i doprinos poznavanju bioraznolikosti u Hrvatskoj, već je ono rezultat jednog od prvih današnjih sistematičnih istraživanja gorskih tekućica.