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Brown and red shades in the riverbed of the Nera River (Serbia) – update on the distribution and ecology of the association *Hildenbrandio rivularis–Heribaudielletum fluviatilis* Fritsch 1929 corr. Täuscher 2020 in Southeastern Europe

by

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Abstract

Heribaudiella fluviatilis (Areschoug) Svedelius is one of the rare representatives of freshwater brown algae, and its range is restricted to the Northern Hemisphere. *Hildenbrandia rivularis* (Liebmann) J. Agardh is still one of the endangered red algae species in Europe, although its indicator value and distribution have recently been questioned. The association *Hildenbrandio rivularis–Heribaudielletum fluviatilis* Fritsch 1929 corr. Täuscher 2020 occurs mainly in Northern and Central Europe, while its occurrence and ecology in Southern Europe are poorly researched. The lower reaches of the Nera River near the small town of Bela Crkva, Serbia, were searched for macroalgae in August 2021 and June 2022, when stones with macroalgal crusts were collected. Environmental parameters were measured *in situ* and water samples were collected for further analysis. A sample of the epilithic diatom community was collected to calculate diatom indices. We present the occurrence of the association *Hildenbrandio rivularis–Heribaudielletum fluviatilis* in the lower course of the Nera River in Serbia, relating the ecology of the species and habitat to previous findings from the Southeast European region. We discuss potential indicator values of macroalgae associations, rather than of a single species.

Key words: *Heribaudiella fluviatilis*, *Hildenbrandia rivularis*, indicators, conservation

1. Introduction

Brown algae (class Phaeophyceae) are mainly known as a very diverse and widespread group in marine environments, while their occurrence in fresh waters is less frequently observed. *Heribaudiella fluviatilis* (Areschoug) Svedelius is one of the few representatives of freshwater brown algae and is considered rare throughout the Northern Hemisphere, where it is currently observed (Koletić et al. 2018 and references therein).

In the Balkans (i.e. southeastern Europe), *H. fluviatilis* was previously observed in Greece, Slovenia, Bulgaria, and Croatia (Stoyneva et al. 2003 and references therein; Koletić et al. 2018 and references therein), and only recently was it reported for the first time in Serbia (Sabovljević et al. 2023). In Sabovljević et al. (2023), authors Rakonjac and Simić reported findings of *H. fluviatilis* in eastern Serbia: at one site in the Rakitska River in the Balkan Mountains (Stara Planina) in 2019 and at two sites in the Temštica River in the Balkan Mountains (Stara Planina) in 2020 and 2021. At all sites, *H. fluviatilis* was accompanied by red algae. Populations of *Paralemanea annulata* (Kützing) M.L. Vis & R.G. Sheath were detected at all localities, and at two localities (Rakitska River and Temštica River downstream) *H. fluviatilis* was found in a community with *Hildenbrandia rivularis* (Liebmann) J. Agardh, meaning that the association *Hildenbrandio rivularis–Heribaudielletum fluviatilis* Fritsch 1929 corr. Täuscher 2020 (hereafter referred to as the *Hildenbrandio–Heribaudielletum* community) was recorded. *Hildenbrandia rivularis* is a crust-forming red alga that is currently considered a strictly protected species under Serbian law (Official Gazette of the Republic of Serbia 5/2010, 47/2011, 32/2016 and 98/2016, 2016). Until 2017, *H. rivulars* was found in only three localities in Serbia (Simić 2008; Blagojević et al. 2017), but recently Rakonjac and Simić reported it in 30 new localities (Rakonjac, Simić 2023), while Šovran and Knežević (Tomović et al. 2023) reported 12 more (Appendix, map of the current published records of *H. rivularis* and *H. fluviatilis* in Serbia).

Recent records show that *H. fluviatilis* and the *Hildenbrandio–Heribaudielletum* community in the Balkans are mainly associated with mountain regions, i.e. the upper and middle reaches of mountain rivers belonging to the Black Sea basin, and only one record comes from a lowland river (Cetina River, Croatia) belonging to the Adriatic basin. The most recently reported habitats of *H. fluviatilis* without concomitant findings of *H. rivularis* in the Balkans are the Una River in Croatia (Koletić et al. 2018) and the Temštica River (upper reaches) in Serbia (Sabovljević et al. 2023).

The distribution and ecology of *H. fluviatilis* and the *Hildenbrandio-Heribaudielletum* community in southeastern Europe are still poorly understood, likely as a consequence of insufficient research and oversight in field studies (Koletić et al. 2018). Updating the current knowledge of ecology and biogeography of the *Hildenbrandio-Heribaudielletum* community is particularly important in the context of a recently reported phenomenon of *H. rivularis* expansion from mountains and highlands to lowlands and the questioned indicator value of this crustaceous red alga (Jakubas-Krzak et al. 2023). Jakubas-Krzak et al. (2023) reported a significant increase in the abundance of *H. rivularis* populations in the lowland areas of Poland, parallel with their disappearance from mountains and highlands, which were known as typical habitats of the species. Furthermore, the species has recently been reported mainly from eu- and hypertrophic waters, in contrast to its previous association with oligotrophic habitats. The expansive nature and ubiquitous character of *H. rivularis* is highlighted along with the hypothesis that climate change, i.e. warming and eutrophication–reoligotrophication are the processes underlying the observed expansion of *H. rivularis* in Europe in recent decades (Jakubas-Krzak et al. 2023). However, *H. rivularis* is still considered one of the most threatened red algae species throughout Europe (Jakubas-Krzak et al. 2023). New data on the ecology of the *Hildenbrandio-Heribaudielletum* community may contribute to recognizing the currently unclear consistency pattern of the co-occurrence of these species.

The purpose of this paper is to present the occurrence of the *Hildenbrandio-Heribaudielletum* community in the lower course of the Nera River in Serbia, within the protected Outstanding Natural Landscape Area "Karaš-Nera", at the very edge of the Pannonian Plain (Banat). In its upper course in Romania, the Nera River is a typical mountain river that forms a beautiful gorge on its way through the Cheile Nerei–Beuşniţa National Park, but when it leaves the Banat Mountains and flows down into the Banat Plain (Bela Crkva depression), it becomes slower and wider, but never shows the actual characteristics of a lowland river. Shortly after entering Serbia near the village of Kusić (about 15 km), it joins the Danube as a left tributary. The Nera basin is torrential in nature and heterogeneous in geological composition and cuts into its own and then into alluvial sediments of the Danube downstream from the village of Kusić (Bogdanović, Marković 2005).

2. Materials and methods

Stones with macroscopic algal crusts were collected in mid-August 2021 and again in June 2022 in the lower reaches of the Nera River, by the small town of Bela Crkva, Serbia (44°52′21.8″N; 21°25′34.5″E; Fig. 1a–b). The citizens of Bela Crkva use this locality as a resort, particularly during the summer months. At this locality, the riverbed is covered with stones and gravel, and in summer the river is shallow (0.5 m on average, but in a few depressions > 1 m). The right bank is overgrown with shrubby and woody riparian vegetation, while the left bank is covered with sand and is used as a beach. However, the river course at this site is oriented in an east–west direction, thus exposed to the sun almost equally along both banks.

At the time of stone collection in June 2022, water parameters (pH, conductivity, total dissolved substances, oxygen concentration and saturation) were measured using the following field instruments: Eutech/Oakton® Instruments and a YSI ProODO optical dissolved oxygen meter. A water sample was also collected for further analysis according to the standard analytical procedures (APHA 1995) at the Institute of Public Health of Serbia "Dr Milan Jovanović Batut". The river bottom was explored by snorkeling 50 m upstream, and the DAFOR scale, i.e. dominant (D), abundant (A), frequent (F), occasional (O), rare (R), based on visual observation was applied to estimate macrophytobenthos (algae and aquatic plants) cover.

Three to five random stones (3 to 5 cm in diameter) collected from the littoral zone were scraped in the

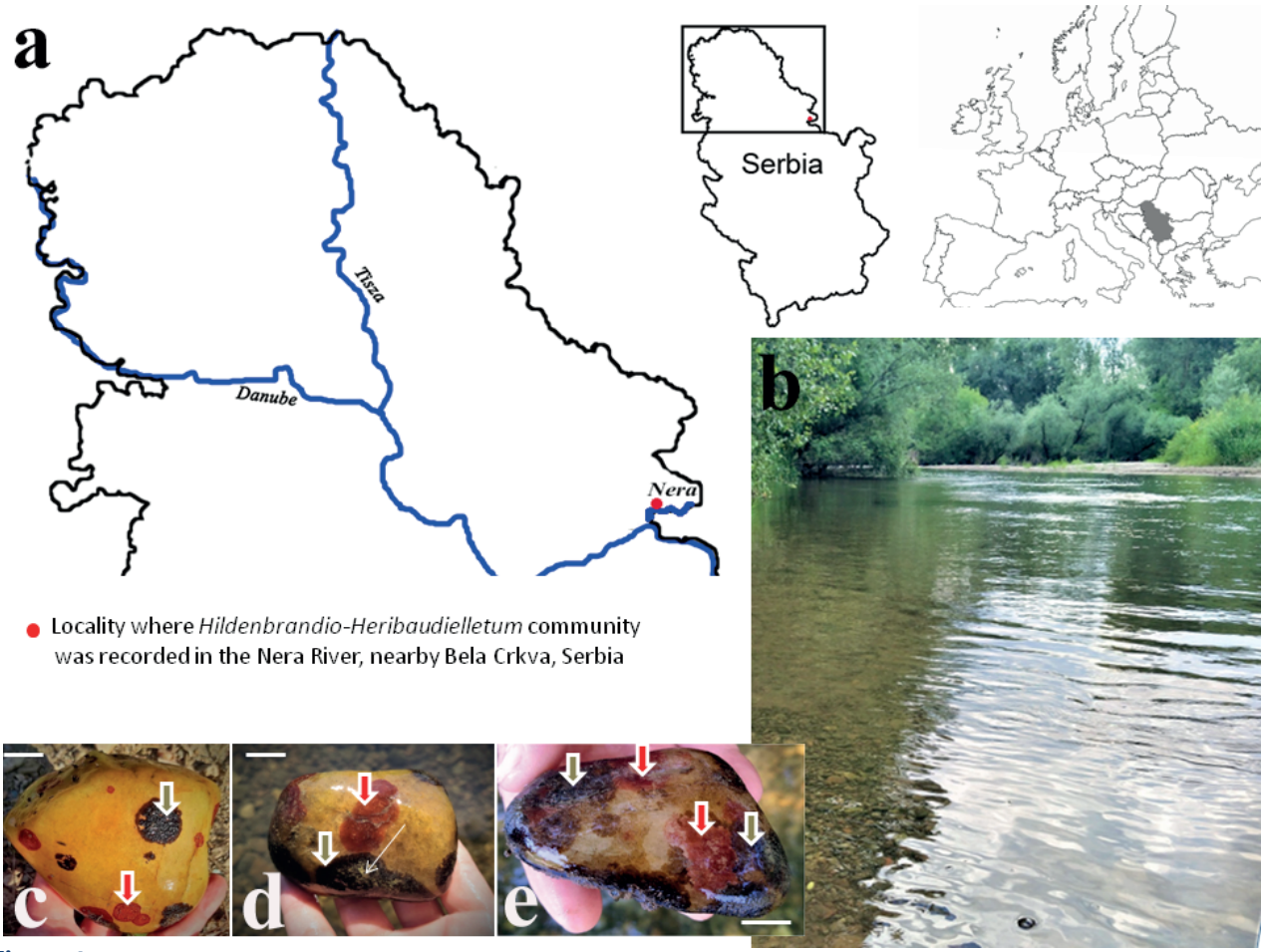

Figure 1

Locality of the *Hildenbrandio-Heribaudielletum* community – lower reaches of the Nera River, near Bela Crkva. (a) Geographical location of the locality in Europe and Serbia. (b) Field photograph of the locality. (c, d and e) Stones with macroscopic algal crusts, colonies of different shapes (brown arrows – *Heribaudiella fluviatilis* crusts; red arrows – *Hildenbrandia rivularis* crusts; white arrow – "white puffs", i.e. "Chantransia" phase); scale size 2 cm. Photo credits: Ivana Trbojević and Smiljana Jovanović.

field and one composite sample of the epilithic diatom community was obtained. The sample was preserved with formaldehyde (4% final concentration), and in the laboratory all the collected material was treated using the hot acid method (Taylor et al. 2005) and mounted on Naphrax® medium to prepare permanent diatom slides. Taxonomic analyses and quantification of diatoms (at least 400 valves were counted) were carried out using a Carl Zeiss AxioImager M1 microscope and an AxioCam MRc5 digital camera with AxioVision 4.9 software. Taxonomic identification was performed according to the standard literature (Lange-Bertalot et al. 2017) and taxonomic names were verified following Guiry & Guiry (2024). Diatom indices were calculated using OMNIDIA 6 software (Lecointe et al. 1993), and diatom indices IPS and CEE were considered for the water quality assessment of the investigated part of the Nera River, in accordance with the legislation of the Republic of Serbia and the national water body classification (Official Gazette of the Republic of Serbia 96/2010; Official Gazette of the Republic of Serbia 74/2011).

In the laboratory of the Department of Algology and Mycology at the Institute of Botany and Botanical Garden "Jevremovac" at the Faculty of Biology of the University of Belgrade, stones with macroscopic algal crusts were first inspected using a Nikon SMZ 745T stereomicroscope equipped with a Dual Sight 1000 camera, and then crusts were scraped with a razor blade, and slides were prepared and examined using the same equipment as for diatom analysis. Identification of the material was based on literature sources (Eloranta et al. 2011; Koletić et al. 2018). Part of the material was preserved with 4% formaldehyde and deposited in the Department of Algology and Mycology – algae wet collection (BEOU) No. 6749.

3. Results and discussion

The *Hildenbrandio–Heribaudielletum* community was found on stones collected from the Nera River on 16 August 2021 and again on 5 June 2022 (Fig. 1c–e). The *Hildenbrandio–Heribaudielletum* community at this site can be described as abundant (A) and primarily associated with a river bank with faster river flow (> 1 m s^{-1}), deeper water (≥ 0.5 m), and a substrate of boulder-sized stones (radius ≥ 3 cm). Riparian vegetation was developed along this river bank, but the riverbed was mostly not shaded. In its microhabitat, the *Hildenbrandio–Heribaudielletum* community was the only community – no other macrophytes were detected, while a few small *Myriophyllum spicatum* L. colonies were present in the deeper parts near the river bank (R). The opposite river bank was shallower and the bed was covered with pebble/cobble-sized stony substrate and sand. The *Hildenbrandio–Heribaudielletum* community was absent in this area, but sporadic tufts of *Cladophora* sp. (R) were observed on cobbles. Koletić et al. (2018) identified a similar spatial distribution of *Heribaudiella fluviatilis* at the localities of its occurrence in Croatia, associated with sites of faster water flow and similar habitat conditions (predominantly unshaded sites and absence of other aquatic vegetation). It can be suggested that appropriate stone size, light availability and fast flow are anticipating elements for the *Heribaudiella–Hildenbrandia* community. In our study, thalli of both species adhered firmly to the stone substrate and were observed macroscopically in the form of individual, well-defined, regular disk-shaped (Fig. 1c) or irregularly shaped colonies (Fig. 1d) that overgrew each other and formed coalesced algal crusts covering almost the entire surface of the stones (Fig. 1c and Fig. 2a). *Heribaudiella fluviatilis* was recognized by its dark brown coloration, whereas colonies of *H. rivularis* were distinctly red. Specific encrusted "white puffs" on the thalli of *H. fluviatilis*, previously noted by Koletić et al. (2018) and described as the "Chantransia" phase, were also noted in our study (Fig. 1d) and could be described microscopically in the same way.

Microscopically, *H. fluviatilis* thalli exhibited a layered morphology and consisted of a basal pseudoparenchyma formed by densely arranged, frequently and irregularly branched filaments, containing numerous discoidal plastids (Fig. 2d). Cell dimensions matched well with the spans of length and width reported by Koletić et al. (2018) and Sabovljević et al. (2023), supporting further morphometric studies toward a possible regional morphotype description, as suggested by Koletić et al. (2018). Vertical series of filaments (rarely dichotomously branched) and unilocular sporangia were also observed. *Hildenbrandia rivularis* thalli were microscopically distinguished by a bright red colored colony crust, characterized by irregularly shaped cells with thick cell walls (Fig. 2c). The structure of *Hildenbrandia rivularis* thalli corresponded well to descriptions in the literature. It consisted of a thin basal layer formed by densely arranged branched filaments firmly adhering to the substrate (Fig. 2b) and a pseudoparenchymatous layer formed by erect filaments (Eloranta et al. 2011; Jakubas-Krzak et al. 2023).

The locality in the Nera River, where stones with macroscopic algal aggregations were collected, was either partially shaded or fully exposed to sunlight. Water quality parameters measured in the field on 5 June 2022 were as follows: temperature 23°C, pH 8, oxygen concentration and saturation 7.6

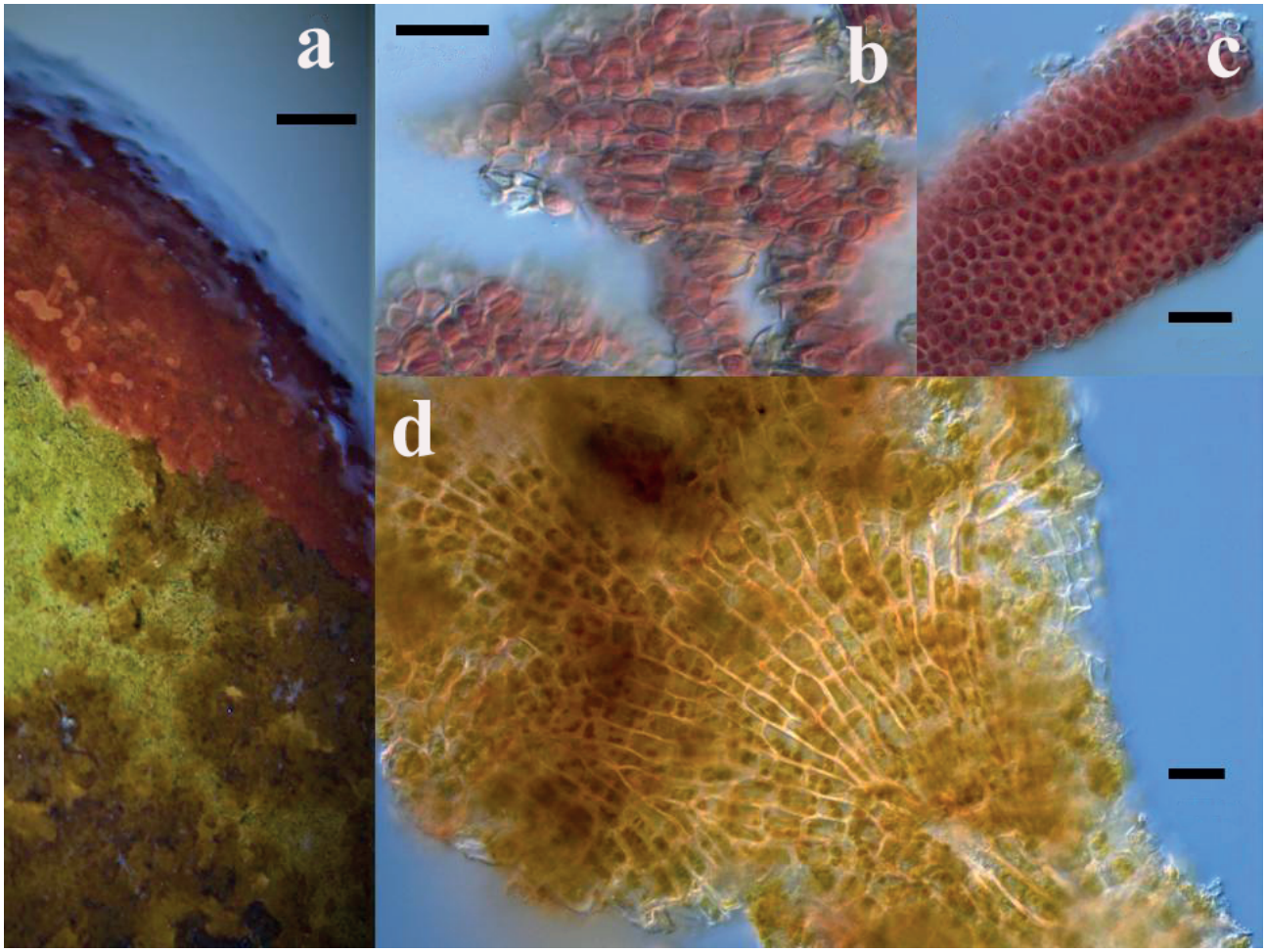

Figure 2

(a) *Hildenbrandio-Heribaudielletum* community – amalgamated algal crust (scale size 1000 µm). (b and c) Microscopic details of Hildenbrandia rivularis thalli (scale size 20 µm). (d) Microscopic details of *Heribaudiella fluviatilis* thalli (scale size 20 µm). Photo credits: Jelena Krizmanić.

mg l^{-1} and 89%, conductivity 288 µS cm^{-1} and total dissolved substances 203 mg l^{-1}. The water in the Nera River locality was much warmer (about 10°C) compared to other sites where the *Hildenbrandio–Heribaudielletum* community was recorded in Serbia (Sabovljević et al. 2023), but close to the Kupa River (middle course, Vukova Gorica), from where Koletić et al. (2018) reported an abundant *Hildenbrandio–Heribaudielletum* community, and where the maximum water temperature was 25.5°C. The results of laboratory analysis of the water showed a total nitrogen concentration (TN) of 2.13 mg l^{-1}, total phosphorus (TP) 0.05 mg l^{-1}, dH°8, calcium (Ca^{2+}) 52 mg l^{-1} and magnesium (Mg^{2+}) 6.1 mg l^{-1}. Considering the chemical and physical elements, the investigated part of the Nera River belonged to class II of ecological status, i.e. the ecological status was assessed as good (Official Gazette of the Republic of Serbia 96/2010; Official Gazette of the Republic of Serbia 74/2011). Not far downstream from the place where the *Hildenbrandio–Heribaudielletum* community was found in our study, wastewater from Bela Crkva flows untreated into the Nera River. Upstream from the study site, the Nera River is under pressure of agriculture and pastoral farming. Summarizing the latest literature sources on Central European populations, autecological characteristics of *H. rivularis* can be formulated as follows: a shade-loving and thermotolerant species, indicative of hard, alkaline and oxygen-rich waters, meso- to eutrophic (to polytrophic), and beta-mesosaprobic (Jakubas-Krzak et al. 2023; Täuscher, Krumbiegel 2020, and references therein). *Heribaudiella fluviatilis* is still ecologically under-researched, both worldwide and in Europe, but according to available data for Central

European populations, it is described as a beta- to alpha-mesosaprobe, characteristic of meso- to eutrophic fast flowing waters (Täuscher, Krumbiegel 2020, and references therein). Our results contribute to both the autecology of the species and provide relevant updates for the geographical region of Southeastern Europe.

A taxonomically diverse diatom flora was identified in the analyzed sample from the Nera River, which included 36 diatom taxa belonging to 20 genera (Appendix, List of diatom taxa and their % contribution in quantitative analyses). Quantitative analysis showed that *Achnanthidium pyrenaicum* (Hustedt) H.Kobayasi and *Cocconeis euglypta* Ehrenberg were dominant species (79.33% and 9.89%, respectively). The obtained values of the diatom indices, i.e. Pollution Sensitivity Index (IPS) and Groupes Indice CEE (CEE) (18.6 and 17.8, respectively) indicated class I water quality, corresponding to high ecological status of the locality near Bela Crkva, where the *Hildenbrandio–Heribaudielletum* community was found (Official Gazette of the Republic of Serbia 96/2010; Official Gazette of the Republic of Serbia 74/2011). When combining biological, chemical and physical elements, the final ecological status of the investigated part of the Nera River can be described as good.

Jakubas-Krzak et al. (2023) recently questioned the indicator value of *H. rivularis*, reporting on its significant expansion in Poland and arguing for its ability to adapt to anthropogenic pressures. The authors analyzed indicator values of *H. rivularis* based on its occurrence irrespective of co-occurring species (*H. fluviatilis* was one of the most common in riverine high-flow habitats), but emphasized the importance of taxonomic associations and suggested detailed autecological studies. Our study corroborates these conclusions, modestly suggesting that the potential of indicator values of macroalgae associations, rather than of single species, could be considered in future studies. Such a perspective can be justified not only based on our results, but also with reference to other ecological data available for *Hildenbrandio–Heribaudielletum* communities in the Balkans (Koletić et al. 2018; Sabovljević et al. 2023). A broader perspective for this approach relies on the fact that, unlike Central European populations, *H. fluviatilis* is an indicator of medium to low nutrient levels in North America (Wehr 2015; Wehr et al. 2019) and that it is characterized as an indicator of low TP levels (Schneider, Lindstrøm 2011) in Norway.

Täuscher & Krumbiegel (2020) recently provided a comprehensive report on the occurrence of the *Hildenbrandio–Heribaudielletum* community in the small Tangelnscher Bach River (Saxony-Anhalt, Germany), highlighting the importance of their finding for biodiversity conservation, as these taxa are red-listed and indicators of good ecological water quality. Our report also emphasizes the importance of species and habitat conservation, given that the finding was made in a recently protected area – the Outstanding Natural Landscape "Karaš-Nera" (Official Gazette of AP Vojvodina No. 14/2015 and 54/2018).

The fact is that two species, *H. rivularis* and *H. fluviatilis*, also occur independently of each other, reflecting the specificity of their own ecological niches, which overlap considerably. Our study suggests that further collection and analysis of data on this niche overlap could provide valuable ecological information. Our results are an important contribution to the knowledge of the ecology and biogeography of the *Hildenbrandio–Heribaudielletum* community, which reflects the issue of species and habitat conservation in Southeastern Europe.

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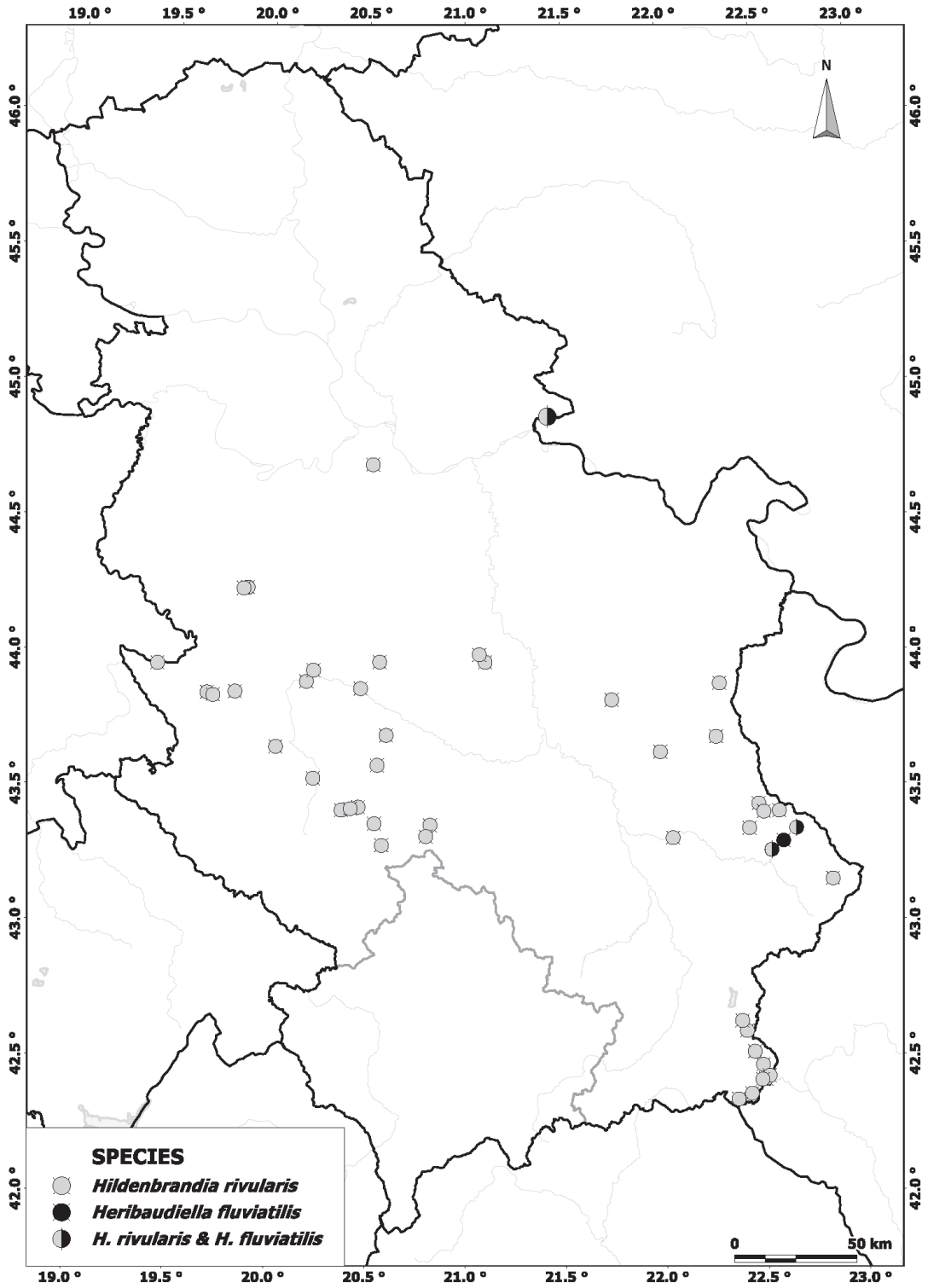
Appendix

Map of the current published records of *Hildenbrandia rivularis* and *Heribaudiuella fluviatilis* in Serbia, including records presented in this article and data provided in Simić (2008), Blagojević et al. (2008), Tomović et al. (2023) and Sabovljević et al. (2023).



List of diatom taxa and their % contribution in quantitative analysis (+ indicates presence in the sample, but not in the quantification when at least 400 valves were counted).

Taxon	%
Achnanthidium minutissimum (Kützing) Czarnecki	0.45
Achnanthidium pyrenaicum (Hustedt) H.Kobayasi	79.33
Amphora pediculus (Kützing) Grunow	2.7
Cocconeis euglypta Ehrenberg	9.89
Cocconeis lineata Ehrenberg	+
Cocconeis pediculus Ehrenberg	+
Cymbella affinis Kützing	+
Cymbella compacta Østrup	+
Diatoma vulgaris Bory	+
Encyonema minutum (Hilse) D.G.Mann	+
Encyonema ventricosum (C.Agardh) Grunow	0.9
Navigeia decussis (Østrup) Bukhtiyarova	+
Gomphonella olivacea (Hornemann) Rabenhorst	0.45
Gomphonema tergestinum (Grunow) Fricke	+
Melosira varians C.Agardh	+
Navicula capitatoradiata H.Germain ex Gasse	0.45
Navicula cryptotenella Lange-Bertalot	0.45
Navicula cryptotenelloides Lange-Bertalot	+
Navicula gregaria Donkin	+
Navicula lanceolata Ehrenberg	+
Navicula metareichardtiana Lange-Bertalot & Kusber	0.45
Navicula tripunctata (O.F.Müller) Bory	1.35
Nitzschia fonticola (Grunow) Grunow	0.9
Nitzschia heufleriana Grunow	0.45
Nitzschia recta Hantzsch ex Rabenhors	0.9
Nitzschia sigmoidea (Nitzsch) W.Smith	+
Odontidium mesodon (Ehrenberg) Kützing	+
Planothidium reichardtii Lange-Bertalot & Werum	+
Reimeria sinuata (W.Gregory) Kociolek & Stoermer	+
Reimeria uniseriata Sala, Guerrero & Ferrario	0.9
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	+
Sellaphora pupula (Kützing) Mereschkovsky	+
Surirella brebissonii var. *kuetzingii* Krammer & Lange-Bertalot	0.45
Ulnaria ulna (Nitzsch) Compère	+