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Uncovering Two Freshwater Brown Algae *Bodanella lauterborni* and *Heribaudiella fluviatilis* in Serbia (Southeast Europe)

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Abstract

Bodanella lauterborni W.M. Zimmermann and *Heribaudiella fluviatilis* (Areschoug) Svedelius are members of brown algae (Phaeophyceae) that exclusively inhabit freshwater habitats. *Heribaudiella fluviatilis* is the most frequently reported freshwater brown alga, widely distributed in the Northern Hemisphere. In contrast, *B. lauterborni*, one of the rarest algae globally, has been reported in only four glacial Alpine lakes and has not been observed in nature for nearly 50 years. Since 2019, the species has been considered locally extinct at its type locality, and its presence in the other three lakes is also questionable. Here, we report the occurrence of *B. lauterborni* in three springs on the Vlasina Plateau (Southeast Serbia), being the first finding of the species in Southeast Europe and the fifth discovery globally in environmental conditions not previously described for the species. We also provide detailed data on the morphology, ecology, and biogeography of *B. lauterborni* and *H. fluviatilis*. Additionally, we report the non-obligate association *Hildenbrandio rivularis-Heribaudielletum fluviatilis* discovered in two rivers. Our findings significantly expand the known ecological and geographical range of phaeophytes, highlighting Southeast Europe as a refugium for freshwater Phaeophyceae biodiversity.

Keywords: Phaeophyceae; spring; river; lake; crystalline shales; distribution; ecology; morphology; *Hildenbrandio rivularis-Heribaudielletum fluviatilis*

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1. Introduction

Brown algae (class Phaeophyceae) are predominantly marine, with only a few lineages colonizing freshwater environments [1,2]. Although this group is prevalent and diverse in marine ecosystems globally, freshwater representatives are the least diverse among all groups of freshwater algae [1]. Despite a relatively long history of surveys on freshwater phaeophytes, their actual diversity, geographical distribution, and ecological requirements remain incompletely known; most taxa are known only from scattered locations in Europe, North America, and Asia, and their classification and phylogeny are still not fully resolved [1,3–10]. Until recently, only seven species from six genera were

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known from freshwater environments: *Bodanella lauterborni* W.M. Zimmermann, *Heribaudiella fluviatilis* (Areschoug) Svedelius, *Sphacelaria fluviatilis* Jao, and *S. lacustris* Schloesser & Blum have been reported exclusively from freshwater environments, while *Ectocarpus subulatus* Kützing, *Pleurocladia lacustris* A. Braun, and *Porterinema fluviatile* (Porter) Waern also occur in brackish or marine ecosystems [2,5,6,8,11]. In 2025, Mellado-Díaz et al. [12] described a novel freshwater member of this algal group, *Fluvioralfsia iberica* Mellado-Díaz & J.L.Moreno, from streams in Spain.

All freshwater representatives are characterized by simple morphology and small size, but all of them can form small filamentous tufts and cushions, as well as pseudoparenchymatous crusts [6]. Although macroscopic aggregations of freshwater brown algae are often visible to the naked eye in the field, they are not easy to identify. Field researchers may mistakenly interpret these macroscopic aggregations as cyanobacteria, diatoms, or even lichens [13]. Consequently, these algae are frequently overlooked or insufficiently studied, and records of their occurrence remain relatively infrequent [6].

Data on morphology and reproduction for most freshwater phaeophytes remain incomplete, and many aspects of their life cycles are still unclear. For example, based on similarities in morphological features between *B. lauterborni* and *H. fluviatilis*, some authors suggest that *B. lauterborni* could represent a morphological variant of *H. fluviatilis* [1,2]. Several attempts have been made to clarify their classification and phylogeny. These studies have indicated that *B. lauterborni* and *H. fluviatilis* are genetically closely related, although their precise phylogenetic placement remained unresolved for a long time [1,3,4,14]. However, de Reviers et al. [15] subsequently placed these two species within the family Lithodermataceae.

Bodanella lauterborni is the only member of the monotypic genus *Bodanella* [5]. This species is one of the lesser-known members of this algal group, described almost a hundred years ago, and has been reported only from Austria. The first record of *B. lauterborni* was from Lake Constance (Austria-Switzerland-Germany) by Lauterborn, while its formal recognition and description as a new species were given by Zimmermann [16]. This species was named after its type locality, Lake Constance (German: Bodensee), and its original discoverer. Following its description, this alga was reported from three additional Austrian lakes: Lunzer Untersee [17,18], Traunsee [18], and Zeller See [19]. All of these lakes are natural, oligotrophic, glacial Alpine lakes situated on the karstic plateau at altitudes ranging from 395 m (Lake Constance) to 750 m (Zeller See) above sea level [20,21]. The last observation of *B. lauterborni* in Lake Constance was reported almost 50 years ago by Müller and Geller [22], and since then, the species has not been recorded in the wild. Following the most recent investigation of Lake Constance during 2017–2019, the species was considered locally extinct at its type locality [23].

According to the available literature, *H. fluviatilis* is the only member of the monotypic genus *Heribaudiella* [5,6]. This benthic alga is the most widely recognized freshwater brown alga, currently known to occur only in the Northern Hemisphere [6]. It has been reported from scattered localities across Europe [1,5–7,9,10,24–30], North America [1,31–33], Russia [34], China [35], and Japan [36,37]. Despite its wide distribution, the number of records remains generally low, and the species is therefore commonly regarded as rare [2]. This alga colonizes stable rocky substrata in streams, rivers, and lakes [2], although it has also been recorded in several karst springs [7,38]. According to the available literature data, *H. fluviatilis* prefers clear waters with high current velocities, low concentrations of inorganic nutrients, stable flow conditions, and more resistant rocks [2].

Since data on the geographical distribution of freshwater brown algae are generally scarce and all freshwater brown algae are considered rare [2,5], each new study contributes to filling important knowledge gaps regarding their morphology, reproduction, ecology,

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and biogeography. Recent studies, however, suggest that this algal group may not be as rare as previously assumed but rather overlooked and under-sampled [7–10,12,33,39].

This paper aims to report the first discovery of *B. lauterborni* populations from three springs in Serbia, representing the fifth record worldwide. In addition, we provide a detailed description of the morphological features of *B. lauterborni* and *H. fluviatilis* as helpful guidelines for further identification, along with comprehensive characterization of the environmental conditions in which these two species occur. Furthermore, the non-obligate community *Hildenbrandio rivularis-Heribaudielletum fluviatilis* Fritsch 1929 corr. Täuscher 2020 (hereafter referred to as the *Hildenbrandio-Heribaudielletum* community), as well as the associations of *B. lauterborni* and *H. fluviatilis* with other benthic macroalgae, are also described.

2. Materials and Methods

Field surveys were performed from April 2017 to June 2024 and included 454 sampling locations across more than 200 Serbian aquatic ecosystems, primarily as part of macro-algological studies.

2.1. Sampling

Thalli of *B. lauterborni* were initially collected in July 2019 at the St. Nicholas spring (S1) located on the Vlasina Plateau, Southeastern Serbia (Table 1, Figures 1 and 2A,B), from a wooden trough through which water is diverted for drinking. Living material was scraped from the wooden surface. After the initial discovery of this population, samples from this location were collected and examined in more detail in September 2020, October 2021, and July 2022, and several other nearby springs with similar characteristics were also carefully investigated. Additional material of *Bodanella* was collected in September 2020, October 2021, and July 2022 from an unnamed spring located 100 m from the St. Nicholas spring (S2) and also from the surface of a wooden trough (Table 1, Figure 2C,D). In July 2022, *B. lauterborni* was detected at another unnamed spring located 1 km from the St. Nicholas Spring (S3) (Table 1, Figure 2E,F).

Table 1. The morphometric, physical, and chemical parameters of water from the *Bodanella lauterborni* localities in Serbia.

Locality	**S1**				**S2**			**S3**
Geographical coordinates	N 42.72704				N 42.72806			N 42.732910
	E 22.318				E 22.31723			E 22.316267
Altitude (m)	1416				1415			1409
Date	**VII**	**IX**	**X**	**VII**	**IX**	**X**	**VII**	**VII**
	2019	**2020**	**2021**	**2022**	**2020**	**2021**	**2022**	**2022**
Geological base	crystalline shales				crystalline shales			crystalline shales
Woody trough/spring width (m)	0.15				0.2			0.5
Maximum water depth (cm)	2				2			3
Type of substrate	wooden trough				wooden trough			stone, mud
Temperature (°C)	7.1	5.8	6.0	7.3	6.1	6.5	10.6	10.3
pH	7.38	7.28	7.31	7.33	7.37	7.39	7.35	7.59

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Table 1. *Cont.*

Locality	S1				S2			S3
Conductivity ($\mu S\ cm^{-1}$)	/	<20	<20	<20	<20	<20	<20	60
Water hardness ($mg\ CaCO_3\ L^{-1}$)	/	<10	<10	<10	<10	<10	<10	20
Dissolved oxygen ($mg\ O_2\ L^{-1}$)	9.8	7.2	6.8	9.5	10.3	9.1	9.1	9.61
Oxygen saturation (% O_2)	101.2	100.4	100.8	102.1	102.7	102.4	100.9	99.8
Ammonium ($mg\ N\ L^{-1}$)	/	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Nitrates ($mg\ N\ L^{-1}$)	/	<1	<1	<1	<1	<1	<1	<1
Orthophosphates ($mg\ P\ L^{-1}$)	/	0.19	0.21	0.18	0.14	0.16	0.12	<0.05
Insolation	++	++	++	++	+++	+++	+++	+
Surrounding vegetation	beech forest				meadow			deep, dark beech forest
Co-occurring algae/ coverage (%)	/	diatoms/ 20	/	/	/	/	/	/

Note: S1—St. Nicholas Spring; S2—unnamed spring located 100 m far away from the St. Nicholas spring; S3—unnamed spring located 1 km far away from the St. Nicholas spring; + full shade, ++ partial shade, +++ full sunlight.

Figure 1. Geographical locations of freshwater Phaeophyceae localities in Southeast Europe and Austria. Note: ● *Bodanella lauterborni*: S1—St. Nicholas spring, S2—unnamed spring 100 m from the St. Nicholas spring, S3—unnamed spring 1 km from the St. Nicholas spring, C—Constance Lake, Z—Zeller See, T—Traunsee, L—Lunzer Untersee; ● *Heribaudiella fluviatilis*: T1—Temštica River (upper stream), U—Una River; ● *Hildenbrandio-Heribaudielletum* community: K1—Kupa River (spring), K2—Kupa River (middle course), CE—Cetina River, R—Rakitska River, T2—Temštica River (downstream), N—Nera River; I—Iskur River, D1, D2—Dragalevska River, ZH—Zhitolyub spring, V—Vedena rivulet; ● *Porterinema fluviatile*: M—Mlava Spring.

Figure 2. Localities and macroscopic view of *Bodanella lauterborni*. (**A**) S1 spring (St. Nicholas spring); (**B**) brownish-black cushions of *B. lauterborni* on a woody trough in S1 spring (arrow); (**C**) S2 spring (unnamed spring located 100 m from the St. Nicholas spring); (**D**) brownish-black cushions of *B. lauterborni* on a woody trough in S2 spring (arrow); (**E**) S3 spring (unnamed spring located 1 km from the St. Nicholas spring); (**F**) brownish-black cushions of *B. lauterborni* on a stone in S3 spring (arrow).

Thalli of *H. fluviatilis* were collected from three locations in two rivers located on Stara Planina Mt., Eastern Serbia (Figure 1). The thalli were initially collected in August 2019 in the Rakitska River (R) (Table 2, Figures 1 and 3A), by cutting them from dry stones. After species identification, samples from this location were collected and examined in more detail in September 2020 and 2021, and additional streams and rivers in the surrounding area were studied more thoroughly. Further material attributed to *Heribaudiella* was collected in October 2020 and 2021 from the Temštica River upper stream (T1) (Table 2, Figures 1 and 3B) and downstream (T2) (Table 2, Figures 1 and 3C).

Table 2. The morphometric, physical, and chemical parameters of water from the *Heribaudiella fluviatilis* localities in Serbia.

Locality		**R**		**T1**	**T2**
Geographical coordinates		N 43.34322		N 43.26319	N 43.29689
		E 22.67787		E 22.55005	E 22.61065
Altitude (m)		734		510	393
Date	**VIII**	**X**	**X**	**X**	**X**
	2019	**2020**	**2021**	**2021**	**2020**
Geological base		red sandstone		red sandstone	red sandstone, limestone
River width (m)		3.5		7	9
Maximum water depth (cm)	10	10	10	10	10
Type of substrate		rock/stone		rock/stone	stone
Current velocity (m s^{-1})	1.6	1.8	1.8	1.7	1.5
Temperature (°C)	13.2	9.3	5.7	6.7	8.7
pH	7.28	7.15	7.23	7.63	7.55
Conductivity (µS cm^{-1})	70	40	90	80	200
Water hardness (mg $CaCO_3$ L^{-1})	30	20	40	40	100
Dissolved oxygen (mg O_2 L^{-1})	9.94	9.98	9.95	10.3	9.61
Oxygen saturation (% O_2)	101.3	101.8	101.1	105.9	97.3
Ammonium (mg NH_4 L^{-1})	0.02	<0.02	<0.02	<0.02	0.02
Nitrates (mg NO_3 L^{-1})	<1	<1	<1	<1	<1
Orthophosphates (mg PO_4 L^{-1})	0.08	0.14	0.11	0.14	0.16
Insolation	++	++	++	++	+++
Surrounding vegetation		shrubbery; meadow		shrubbery; meadow	meadow
Coverage (%)	15	10	10	5	15
Co-occurring macroalgae/coverage (%)	*Paralemanea annulata*/10 *Riverina rivularis*/5 *Gongrosira* sp./30	*Riverina rivularis*/5 *Gongrosira* sp./20	*Paralemanea annulata*/10 *Riverina rivularis*/5 *Cladophora* sp./10 *Gongrosira* sp./30	*Paralemanea annulata*/20	*Nostoc* sp./10 *Paralemanea annulata*/10 *Riverina rivularis*/5 *Cladophora* sp./10 *Gongrosira* sp./10

Note: R—Rakitska River; T1—Temštica River (upper stream); T2—Temštica River (downstream); ++ partial shade, +++ full sunlight.

Figure 3. Localities of *Heribaudiella fluviatilis* in Serbia. (**A**) Rakitska River; (**B**) Temštica River (upper stream); (**C**) Temštica River (downstream).

In addition to *B. lauterborni* and *H. fluviatilis*, thalli of other benthic macroalgae were also collected from the investigated ecosystems by scraping them from woody or stony substrata.

The collected material was immediately preserved with 4% formaldehyde for morphological analyses. All collected samples are deposited in the wet collection of the Centre for Conservation of Biodiversity and Fishing in inland waters—Aquarium at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac.

Macroscopic benthic algae coverage (%) was assessed by visual analysis, according to Rodriguez et al. [40].

2.2. Microscopic Observation and Species Identification

In the laboratory, the collected material was examined under a Motic BA310 microscope (Motic, Hong Kong, China), with magnification up to 1000×, photographed using a BRESSER (9 MP) camera (Bresser GmbH, Rhede, Germany) and MicroCamLab version 6.1.4.0 software package. Species identification followed the taxonomic keys and descriptions according to Eloranta et al. [5], John et al. [27], Jüttner et al. [41] (2015), Wehr et al. [2], Necchi and Vis [42], and Vis and Necchi [43].

2.3. Environmental Conditions

At each investigated locality, the geological foundation, type of substratum, current velocity (m s^{-1}), surrounding vegetation, and insolation (+ full shade, ++ partial shade, +++ full sunlight) were determined. Physical and chemical properties of the water were measured in situ according to the American Public Health Association [44]. In situ measurements included physical parameters—temperature (°C) and conductivity (μS cm^{-1}), and chemical parameters—pH, water hardness (mg $CaCO_3$ L^{-1}), dissolved oxygen (mg O_2 L^{-1}), and oxygen saturation (% O_2). Temperature, conductivity, pH, and water hardness were determined using the HI 98129 Portable pH/EC/TDS/°C Combo Pocket instrument (HANNA Instruments, Woonsocket, RI, USA), while dissolved oxygen and oxygen saturation were determined using the Mettler Toledo^{TM} Portable pH/Conductivity/Dissolved Oxygen/Ion Meter (Mettler Toledo, Gieβen, Germany). Ex situ measurements of environmental parameters were conducted to determine the concentrations of ammonium ions (mg NH_4 L^{-1}), nitrates (mg NO_3 L^{-1}), and orthophosphates (mg PO_4 L^{-1}). For that purpose, we used the AQUALITIC AL400 Photometer (Tintometer GmbH, Dortmund, Germany).

2.4. Statistical Analyses

Principal component analysis (PCA) was performed to identify the environmental factors that are responsible for species occurrence in Serbian aquatic ecosystems, using the

Statistica 13.0 software package [45] (StatSoft Inc., Tulsa, OK, USA) and Microsoft Excel 365 (version 16.0, Microsoft, Redmond, WA, USA) [46]. The statistical analyses were conducted for the investigated localities where all environmental parameters were obtained (n = 137). Only the species relevant to this study are presented in the ordination diagram. Pearson correlation coefficients between the analyzed environmental variables and the first six principal components were calculated.

The species occurrence map was created using QGIS version 3.38.2 software [47].

3. Results

3.1. Morphology and Reproduction

Macroscopic aggregations of *B. lauterborni* were found in the form of brownish-black cushions firmly attached to wooden (Figure 2B,D) or stony surfaces (Figure 2F). Microscopically, the thalli were composed of only creeping, uniseriate, richly and irregularly branched filaments (Figure 4A–F). The filaments were made up of thick-walled, elongated, and inflated cells (18–37 µm long × 12–20 µm wide) with numerous small discoid parietal plastids (2–5 µm in diameter) (Figure 4C–E). Most of the cells comprising the apical filaments contained only the cytoplasm and vacuoles (Figure 4F). Neither unilocular nor plurilocular sporangia were observed. The morphological features of *B. lauterborni* are summarized in Table 3.

Table 3. Comparative review of the morphological and reproductive features of *Bodanella lauterborni* and *Heribaudiella fluviatilis*.

Species		*Bodanella lauterborni*		*Heribaudiella fluviatilis*	
Data Source		**This Study**	**Literature**	**This Study**	**Literature**
Thalli		brownish-black cushions	brown cushions [5,6]	thick dark crusts; brown crusts	olive-brown or dark brown crusts [5–7]
Growth habit		uniseriate creeping filaments	uniseriate creeping filaments [5,6]	uniseriate creeping and upright filaments densely arranged into several layers of cells	uniseriate filaments; basal creeping system crowded; tightly packed upright filaments [5–7]
Branching pattern		common, irregular	common, irregular [5,6]	frequent, irregular or dichotomous	frequent, irregular or dichotomous [5–7]
Vegetative cells		elongated, inflated	inflated, quadrate, angular, ovoid, or bent [5,6]	elongated	rectangular, elongated [5–7]
		12–20 µm wide	10–16 µm wide [5,6]	9–20 µm wide	6–15 µm wide [5–7]
		18–37 µm long	10–25 µm long [5,6]	20–37 µm long	5–32 µm long [5–7]
Plastids		numerous, discoid, parietal	numerous, discoid, parietal [5,6]	numerous, discoid	numerous, oval or discoid [5–7]
Sporangia		not observed	unilocular: ovoid or globose, observed only in culture [5,6]	unilocular: terminal, ellipsoid	unilocular: terminal, inflated, ovoid, or clavate [5–7]
			plurilocular: unknown [5,6]	plurilocular: not observed	plurilocular: rare [5–7]
Zoospores		not observed	pyriform, with laterally inserted flagella [5,6]	not observed	pyriform or irregular in shape [5–7]

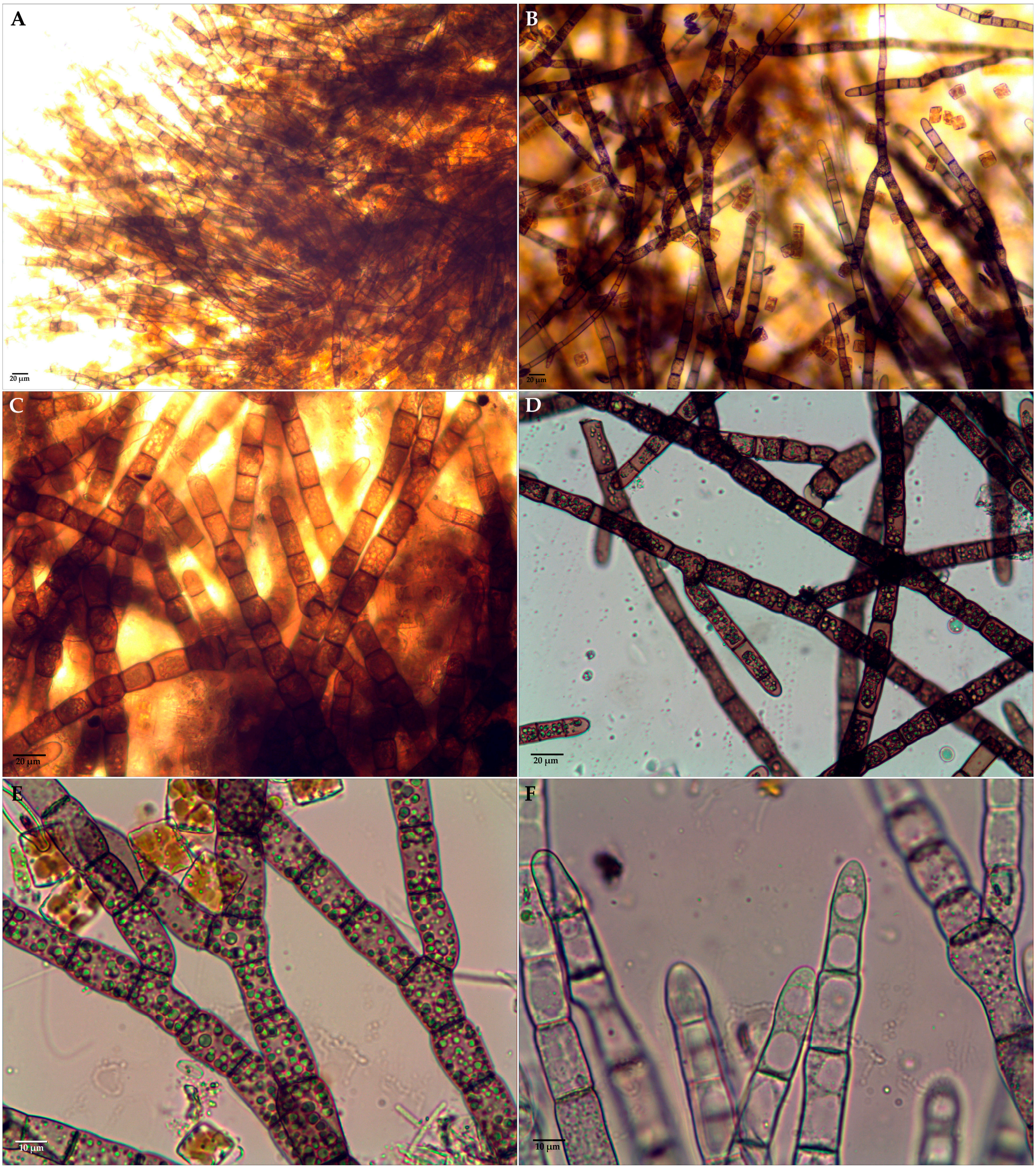

Figure 4. Morphological features of *Bodanella lauterborni*. (**A**,**B**) Microscopic view of the thalli; (**C**–**E**) irregularly branched filaments composed of thick-walled elongated and inflated cells with numerous plastids; (**F**) empty parts in older cells that compose the apical filaments. Scale bars: (**A**–**D**) 20 µm; (**E**,**F**) 10 µm.

During the initial collection in 2019, thalli of *H. fluviatilis* were found as dried dark thick crusts in the Rakitska River (Figure 5A). In 2020 and 2021, thalli were found in the form of small disc-shaped brown crusts, 0.5–5 cm in diameter, unevenly scattered on the rocky surface (Figure 5B). The entire underside of the thalli adhered to the substratum. Individual colonies coalescing and covering the rocky surface were also noticeable. A detailed description of the morphological and reproductive features of *H. fluviatilis* has been published by Rakonjac and Simić in Sabovljević et al. [9] and is summarized in Table 3 and presented in Figure 6.

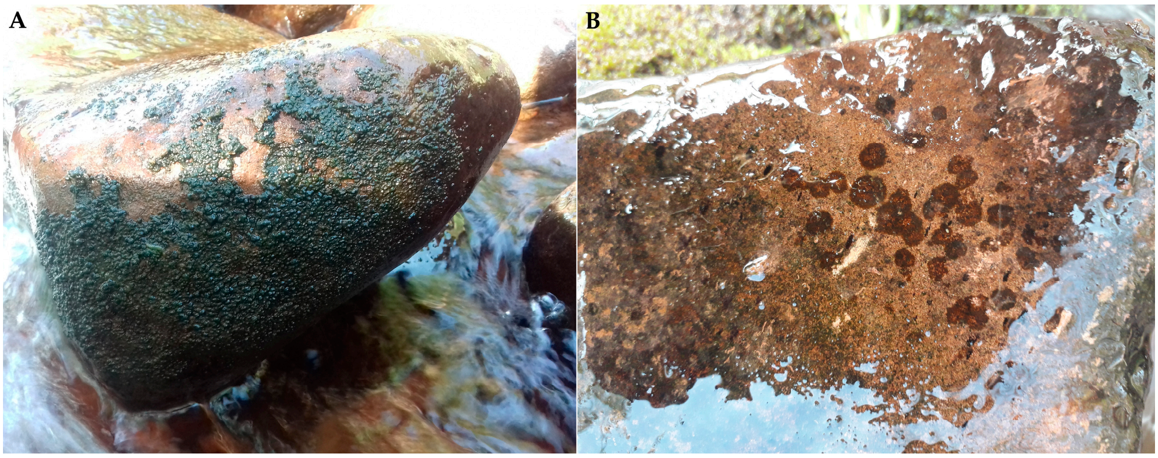

Figure 5. Habitus of *Heribaudiella fluviatilis* thalli. (**A**) Dried macroscopic crusts on the stone from sampling at the Rakitska River in 2019; (**B**) characteristic brown macroscopic crusts.

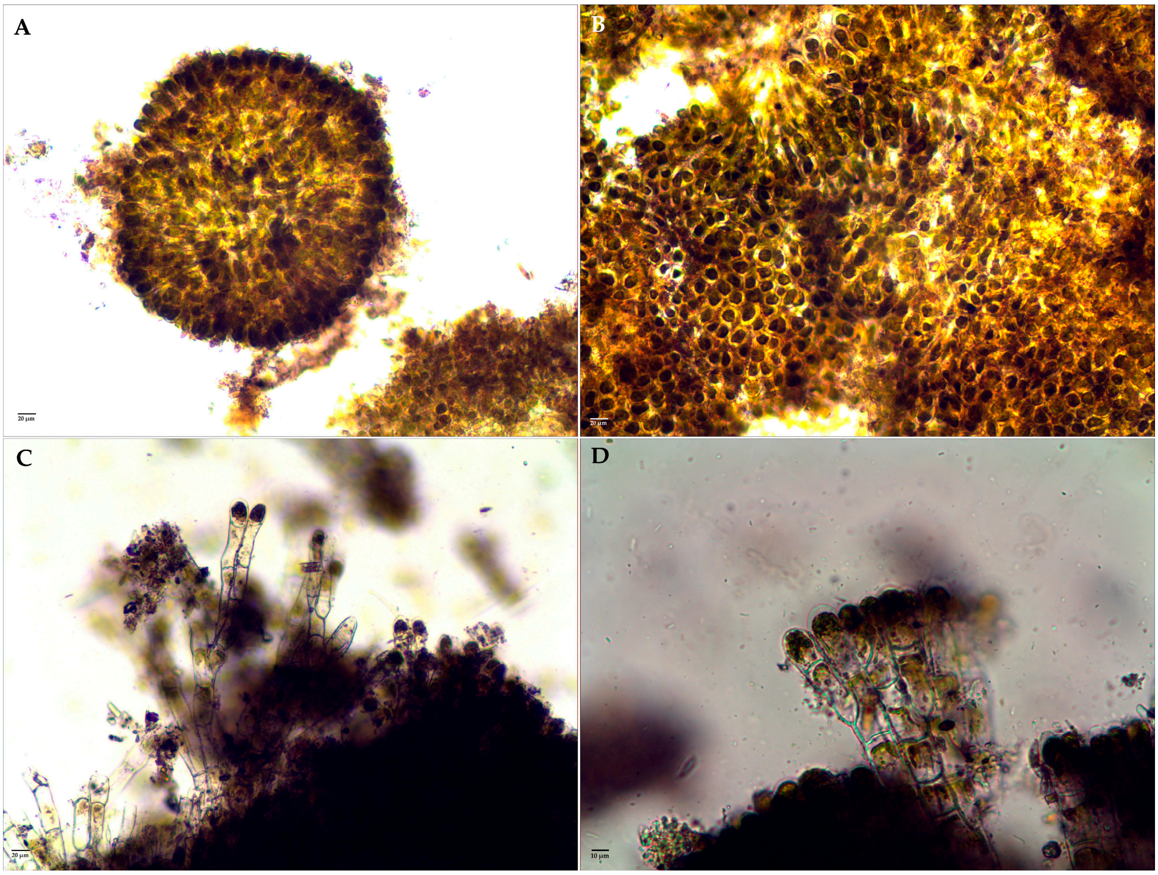

Figure 6. Morphological features of *Heribaudiella fluviatilis*. (**A**,**B**) Prostrate filaments forming a crust; (**C**,**D**) vertical filaments with terminal unilocular sporangia. Scale bars: (**A**–**C**) 20 µm; (**D**) 10 µm.

3.2. Distribution and Ecology

Bodanella lauterborni was recorded at springs S1 and S2, covering more than 50% of the wooden trough surface at S1 and 10% at S2. In S1, thalli of *B. lauterborni* were found coalescing on the wooden surface. At S3, thalli of *B. lauterborni* were found in trace amounts on small stones but were also found in mud samples. The physical and chemical properties of *B. lauterborni* habitats have been summarized in Table 1.

No other macroscopic algal aggregations were found in association with *B. lauterborni*. However, in September 2020, *B. lauterborni* was found forming slightly gelatinous cushions together with the diatoms *Odontidium hyemale* (Roth) Kützing and *O. mesodon* (Kützing) Kützing at S1 (Figure 4E).

The physical and chemical properties of habitats where *H. fluviatilis* occurred are summarized in Table 2. Along with this encrusted brown alga, populations of *Paralemanea annulata* (Kützing) M.L.Vis & R.G.Sheath were detected at all localities. At the Rakitska and Temštica (downstream) rivers, along with *H. fluviatilis* and *P. annulata*, populations of green algae *Cladophora* sp. and *Gongrosira* sp. were also found. At these locations, we documented the presence of the *Hildenbrandio-Heribaudielletum* community (Figure 7), formed by the brown encrusting *H. fluviatilis* and the red encrusting *Riverina rivularis* (Liebmann) C.W.Vieira & G.W.Saunders (Syn. *Hildenbrania rivularis*), which covered a small area on several stones. At the Rakitska River, *R. rivularis* covered *H. fluviatilis*, while at the Temštica River, *H. fluviatilis* covered *R. rivularis*. At both locations, *H. fluviatilis* covered a larger surface than *R. rivularis* (Table 2).

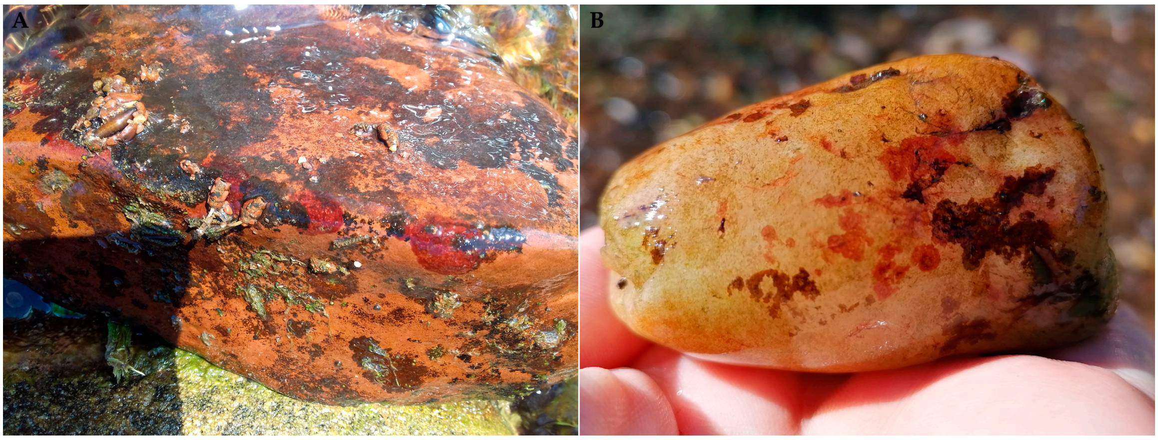

Figure 7. *Hildenbrandio rivularis-Heribaudielletum fluviatilis* community. (**A**) Temštica River; (**B**) Rakitska River.

3.3. Statistical Analysis

The PCA model explained 74.26% of the total data variability, reducing the 15 observed variables to six principal components that capture most of the variation in the dataset. The largest contributions come from PC1 (19.58%) and PC2 (16.12%), while the remaining components contribute less.

The results of the PCA indicate that the concentrations of ammonium, nitrate, and orthophosphate ($r = -0.168$, $r = -0.113$, $r = -0.144$, respectively) contribute negligibly to the formation of the first and second components, suggesting that, within this model, they do not significantly influence the presence and distribution of red and brown algal taxa.

The greatest contributions to the formation of PC1 are from altitude, electrical conductivity, and water hardness. A strong positive correlation was observed for altitude ($r = 0.707$), while strong negative correlations were recorded for electrical conductivity ($r = -0.888$) and water hardness ($r = -0.885$). The second principal component (PC2) is mainly influenced by river width ($r = -0.520$), pH ($r = -0.544$), oxygen saturation ($r = -0.655$), and dissolved oxygen concentration ($r = -0.509$), all of which show moderate negative correlations.

The results of the PCA indicate that *B. lauterborni* shows a preference for high altitudes, fast-flowing waters, low water temperatures, and shallow, slightly alkaline waters. Additionally, low values of electrical conductivity and water hardness significantly influence its distribution (Figure 8).

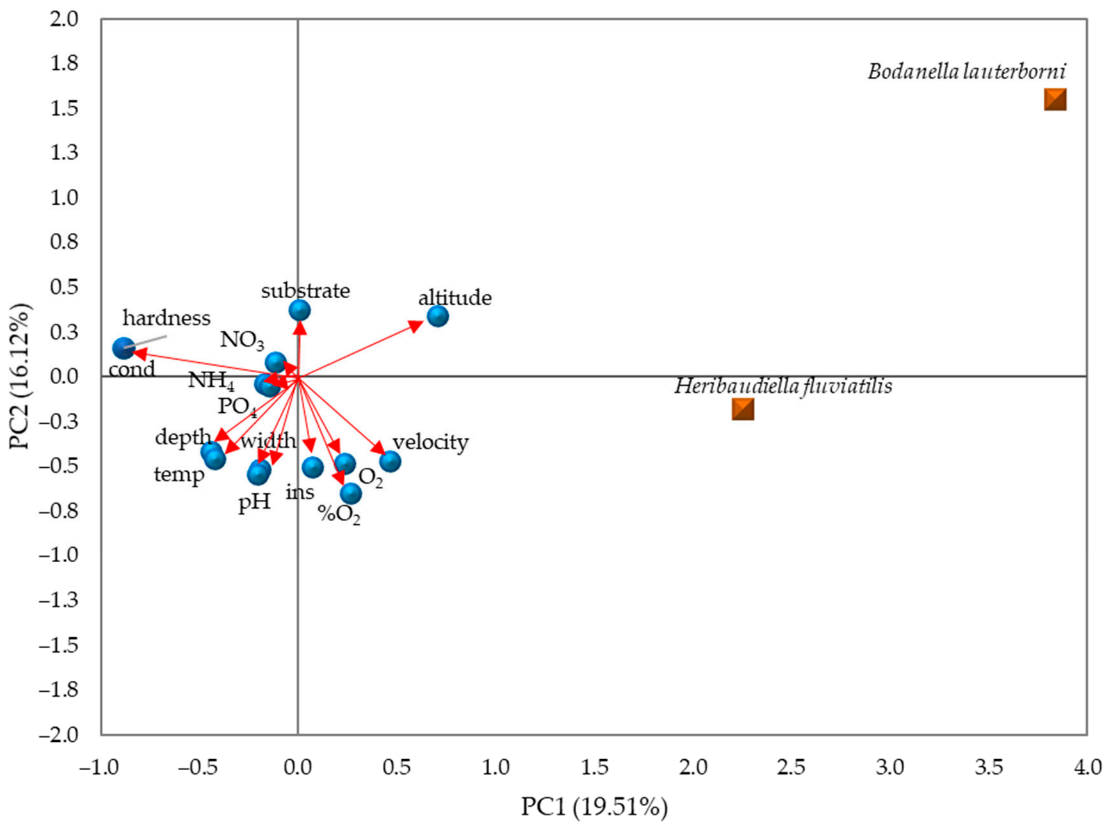

Figure 8. Principal component analysis (PCA) of morphometric, physical, and chemical parameters of the environment and *Bodanella lauterborni* and *Heribaudiella fluviatilis*. Note: cond—conductivity; width—ecosystem width; depth—maximum depth; temp—water temperature; O_2—dissolved oxygen concentration; %O_2—dissolved oxygen saturation; ins—habitat insolation.

In contrast, the analysis suggests that *H. fluviatilis* prefers low water temperatures and low levels of electrical conductivity and water hardness, as well as higher oxygen concentrations and saturation levels. This species also tends to thrive in areas with fast-flowing waters, greater habitat insolation, and shallow depths (Figure 8).

4. Discussion

Every discovery of freshwater brown algae raises new questions regarding their presumed rarity, ecology, and biogeographical patterns. Are they truly rare, or is their rarity the result of under-sampling, overlooking them during field surveys, their seasonal ephemeral dynamic, or simply insufficient knowledge of their appearance in nature among researchers?

In the past, studies of macroalgae in Serbia mainly focused on filamentous and gelatinous morphological forms [48–57]. During 2017–2024, we conducted an extensive investigation of all morphological forms of macroalgae [8,9,58–62]. Likewise, after the first discovery of a freshwater brown alga, *Porterinema fluviatile*, in Serbia [8], we paid more attention to this algal group. Nevertheless, our first findings of *B. lauterborni* and *H. fluviatilis* were entirely coincidental. During the initial sampling, the thalli of these two species resembled cyanobacterial macroscopic aggregations.

Bodanella lauterborni, considered one of the rarest algae in the world, is known only from deep lakes (>15 m), where it grows on rocky substrata [2,5]. Consequently, our first finding of this alga on a wooden trough used to divert water from the spring for drinking was unexpected. After re-sampling and a detailed examination of the collected material, we identified this alga as *B. lauterborni*. Thalli of *B. lauterborni* have been described to occur as basal or creeping filaments, forming small brown cushions on rocky substrata [2], while Geitler [17] described pseudoparenchymatous discs composed of creeping uniseriate and frequently irregularly branched filaments. Cells have been described as irregularly shaped: ovoid, inflated, quadrate, angular, or rounded [2]. In our study, *B. lauterborni* formed brownish-black cushions firmly attached to the wooden or stony surface, composed of richly irregularly branched creeping filaments. Regarding the filament cells, we found thick-walled, elongated, and inflated that were slightly longer and wider cells than those described in the literature. Terminal, thin, and short hairs, which have been observed only in culture material [22], were not detected in our material. As previously mentioned, the reproductive cycle of this alga is incompletely known. According to available literature, unilocular sporangia and approximately 16 released slow-moving zoospores per sporangium have been observed only under culture conditions [5]. Similarly, we did not observe any reproductive structures in our collected material.

Data on the ecology of *B. lauterborni* are scarce and old. The last observation of the species was almost 50 years ago [22]. According to available literature data, this species is characterized as a lacustrine and deep-water alga, growing epilithic at temperature ranges from 6 to 8 °C [2,5,22]. Since the first discovery of *B. lauterborni* in Lake Constance, environmental conditions in this lake have changed substantially, mainly due to the immigration of the zebra mussel, *Dreissena polymorpha* Pallas, into this lake, but also because of increasing phosphorus concentrations provoking strong phytoplankton production [63]. Eutrophication of this lake was recognized in 1959 and 1964, the International Commission for the Protection of Lake Constance decided to implement an extensive wastewater purification programme with the aim of the lake's re-oligotrophication, which proved highly successful [20,64]. However, the most recent attempt to find *B. lauterborni* in Lake Constance during 2017–2019 was without success [65]. Therefore, almost 100 years after its first discovery, the Red List of Red and Brown algae of Baden-Württemberg [23] stated *B. lauterborni* was locally extinct at its type locality, so the species' possible presence in the other three lakes has become critically important. Unfortunately, some data suggest that environmental conditions in these three lakes have also changed, which can negatively affect the survival of the populations of *B. lauterborni* [65]. According to Schütz et al. [66], cultured material could be used for the reintroduction of the species in Lake Constance. However, the problem with the invasive *D. polymorpha* and, lately, the new invader *D. rostriformis* Andrusov, is almost impossible to resolve, so reintroduction of *B. lauterborni* possibly would not be effective.

At the time of its first discovery by Zimmermann [16] in Constance Lake, *B. lauterborni* was found in association with *Riverina rivularis* (Syn. *Hildenbrandia rivularis*) and *Aegagropila brownii* (Dillwyn) Kützing (Syn. *Aegagropila linnaei* Kützing). In this study, we found it co-occurring with diatoms *Odontidium hyemale* and *O. mesodon*. The lack of associated macroalgae with *B. lauterborni* is likely due to the specific environmental conditions of its habitat. We believe these conditions, while suitable for *B. lauterborni*, likely prevent the establishment of other algae, which explains the absence of the typical macroscopic algae associations seen with *H. fluviatilis* in our study.

Our findings of *B. lauterborni* indicate that this alga has not become extinct in the wild. Spring represents a new type of habitat for *B. lauterborni*. We found this species in water temperatures ranging from 5.8 °C to 10.3 °C, pH ranging from 7.28 to 7.59, conductivity up to 60 μS cm^{-1}, and water hardness up to 20 mg $CaCO_3$ L^{-1}, low concentrations of ammonium and nitrates, and orthophosphate concentrations ranging from 0.14 mg L^{-1} to 0.21 mg L^{-1}, on small stones and wooden substratum in fast-flowing water, in full shadow, partial shadow, or full sunlight. Although freshwater brown algae are mainly reported from streams and rivers, their occurrence in springs is not uncommon. Truly freshwater populations of *Ectocarpus* were reported from three springs in Florida [67], while *Pleurocladia lacustris* was found in karst springs in South Australia [2]. In Southeastern Europe, the occurrence of *Heribaudiella fluviatilis* has been reported from karst springs in Croatia [7] and Bulgaria [38]. Recently, *Porterinema fluviatile* has been described from a pure freshwater karst spring in Serbia [8].

Although we found *B. lauterborni* under environmental conditions not previously reported as suitable for the species, there may be some underlying connections. One possible explanation may lie in the presence of a natural lake in this area in the past [68]. The Vlasina Plateau is characterized by numerous small groundwater springs that presumably supplied the historical natural lake. The presence of *B. lauterborni* in three of several investigated groundwater springs may indicate that the species primarily inhabits groundwater springs. After the natural lake's swelling in the Vlasina Plateau area, previously submerged groundwater springs stayed on dry land, so *B. lauterborni* adapted to these newly formed environmental conditions.

Additionally, the geological base on which the species was previously found and the geological base on which we found it both consist of crystalline shales. During its first discovery in Lake Constance, *B. lauterborni* was found on the Molasse rocks [16]. These rocks are composed of sandstone, shales, and conglomerates formed by molasse sedimentation from the praeocean in front of the rising Alps and Himalayas mountains in the late Paleocene [69,70]. Molassic sediments found in Greece, Bulgaria, and North Macedonia are formed on the metamorphic rocks of the Rhodope massif [71–74]. In Serbia, molassic sediments are also present, although data about their occurrence in Southeastern Serbia are lacking [75]. Nevertheless, the geological base of the Vlasina Plateau, where we recorded *B. lauterborni*, also belongs to the Rhodope massif and is mostly composed of crystalline shales [76]. Therefore, our discovery indicates that this alga should be searched for in lakes as well as in other types of freshwater ecosystems on the substrata of crystalline shales.

Another freshwater brown alga identified in our study, *H. fluviatilis*, is characterized by olive-brown to dark brown crusts with rounded or irregular outlines but with distinct margins according to available literature descriptions [2,5]. The thalli we initially observed in the Rakitska River were in the form of thick dark crusts, covering rocky surfaces 15 cm above the water level, so we assumed that this was some cyanobacterial macroscopic aggregation. This unusual cyanobacterial-like macro-appearance of *H. fluviatilis* may be a consequence of thalli drying out for a relatively long period of time. During subsequent sampling, we recorded typical brown-crusting *H. fluviatilis* thalli with rounded or irregular outlines, but with distinct margins in both the Rakitska and Temštica rivers, whereas the form observed during the initial sampling was not detected again. On some rocks, we also noticed that multiple colonies coalesced to cover the entire rocky surface. Regarding the size of the filament cells, we observed slightly longer and wider cells than those described by Eloranta et al. [5] and Wehr [2].

Most previously published data indicate that *H. fluviatilis* prefers clear streams and lakes and stable rocky substrata. It has been recorded in a broad range of thermal and light conditions, slightly alkaline waters with moderate conductivity, and low concentrations of nutrients [2,7,10]. In our research, *H. fluviatilis* was recorded on rocky substratum in partially shaded or fully sunlit rivers, with a temperature ranging from 5.7 °C to 13.2 °C, in slightly alkaline waters, with low to moderate conductivity and hardness, and with low nutrient concentrations.

Heribaudiella fluviatilis was reported to co-occur with other benthic macroalgae, mostly Rhodophyta representatives, such as the genera *Bangia*, *Riverina* (Syn. *Hildenbrandia*), *Lemanea*, and *Chantransia* [2,7,10], but also cyanobacterial genera *Schizothrix*, *Homoeothrix*, and *Nostoc* and green alga *Chaetophora pisiformis* (Roth) C. Agardh [2]. In the present study, it was most frequently found co-occurring with *Paralemanea annulata* (Rhodophyta), *Gongrosira* sp., and *Cladophora* sp. (Chlorophyta), and at one location with *Nostoc* sp. (Cyanobacteria).

The species had been documented to co-occur with the crusty-forming red alga *Riverina rivularis*, usually overgrowing it [77–80]. This combined distribution, firstly described as the *Hildenbrandia-Lithoderma* community by Fritsch (1929), is currently considered as *Hildenbrandio rivularis-Heribaudielletum fluviatilis* community, which has been frequently documented in Northern and Central Europe [77–84]. In contrast, in South Europe, these two species have typically been reported separately [85–88]. In Southeast Europe, the *Hildenbrandio-Heribaudielletum* community has been documented only in Bulgaria [38], Croatia [7] and recently in Serbia [9,10]. In this study, *R. rivularis* was found overlapping *H. fluviatilis* at the Rakitska River, while at the Temštica River, *H. fluviatilis* overlapped *R. rivularis*. At both locations, these species were also found growing separately, and along the entire river stretch, *H. fluviatilis* has much more abundant growth and development than *R. rivularis*. A recent comprehensive report by Täuscher and Krumbiegel [89] emphasized the significance of these community findings for biodiversity conservation, as these species are red-listed in Germany. *Riverina rivularis* is also strictly protected in Serbia by National legislation [90]. Updating the knowledge of this community may help clarify the inconsistent co-occurrence pattern of *H. fluviatilis* and *R. rivularis*. Furthermore, enhancing our understanding of the ecology and biogeography of the *Hildenbrandio-Heribaudielletum* community is particularly important, given recent observations of *R. rivularis* expansion from mountainous and highland areas into lowland regions [91]. In Serbia, crusty-forming red alga *R. rivularis* has been reported at 48 locations from its first observation in 2008 till nowadays [10,57,60,92,93], whereas the *Hildenbrandio-Heribaudielletum* community was found at only three of these locations [9,10].

Riverina rivularis was also reported in association with *B. lauterborni* in Lake Constance [16]. Subsequent combined records of *R. rivularis* and *H. fluviatilis* have led some researchers to suggest that *B. lauterborni* might be a morphological variant of *H. fluviatilis*, due to their morphology as well as their phylogenetic closeness [1–4]. However, our observations and knowledge on *B. lauterborni* and *H. fluviatilis* do not support this hypothesis. Based on extensive morphological analyses of collected samples of both species during three-year surveys, we found no evidence that *B. lauterborni* is the morphological variant of *H. fluviatilis*; moreover, these two species are morphologically clearly distinct. *Bodanella lauterborni* formed small cushions on woody and stony surfaces, firmly attached to the substrata but composed of loosely packed, richly and irregularly branched filaments, while *H. fluviatilis* formed crusts composed of irregularly branched filaments, densely arranged into several layers of cells. Therefore, our findings suggest that those two species, both macroscopically and microscopically, look quite different, although further genetic analyses will be necessary to clarify their evolutionary relationships.

The discovery of *B. lauterborni* and *H. fluviatilis* in Serbia greatly contributes to the global understanding of their distribution, particularly when compared to existing records in the Global Biodiversity Information Facility (GBIF) database. Currently, GBIF data for *B. lauterborni* are limited, with only four previously documented locations worldwide and three records in culture collections [94]. In contrast, *H. fluviatilis* has a broader documented presence in the GBIF database [95], primarily across Northern and Western Europe. However, records from Southeastern Europe are still fragmented and scarce. The identification of this species at three riverine locations in Serbia helps to fill a significant biogeographical gap in the Balkan Peninsula. Our findings of *B. lauterborni* in three springs and *H. fluviatilis* at three riverine localities in Serbia provide essential new data that will help fill these gaps.

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Once these records are added to the global database, they will offer a much clearer picture of where these rare brown algae can survive and how to protect their habitats.

5. Conclusions

In Serbia, information on freshwater brown algae has expanded substantially in recent years, largely due to intensified phycological surveys. Considered as possibly extinct, the freshwater brown alga *B. lauterborni* was discovered in Serbia. This paper represents a significant contribution to the knowledge of the ecology and distribution of this alga, reported from three groundwater springs on wooden and stony substrata, therefore being found in environmental conditions not previously reported as suitable for the species.

The findings of this study have significant practical implications for both future surveys and conservation efforts. Our results suggest that future searches for this rare freshwater alga should specifically target high-altitude springs with crystalline shale substrates, including artificial wooden troughs, which serve as unique micro-refugia. The discovery of *B. lauterborni* in these springs provides essential data for reassessing its conservation status from "possibly extinct" to "extant in refugia". Our study underscores the need to prioritize the protection of these isolated spring environments to ensure the survival of such specialized and rare species.

In southeast Europe, *H. fluviatilis* has, until recently, been documented only sporadically; however, eight additional records have been reported from Croatia and Serbia. This study provides distributional data and detailed morphological descriptions of *H. fluviatilis* from Serbian rivers, further clarifying its ecological requirements and presence within Southern Europe.

Although our records remain among the few documented occurrences of freshwater phaeophytes in southeastern Europe, we strongly believe that these algae are more widespread than currently recognized but under-sampled and frequently overlooked. Therefore, we recommend comprehensive research focusing on dark crust-forming, tuft, and cushion macroscopic algal aggregations. Additionally, future studies combining molecular data with morphological analyses will be essential for clarifying phylogenetic relationships as well as resolving existing taxonomic uncertainties and identification problems.

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