

Spotting the Spots: the Freshwater Brown Alga *Heribaudiella fluviatilis* (Areschoug) Svedelius within Stream Communities of Southeastern Europe

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Abstract – This paper reports the occurrence of the freshwater brown alga *Heribaudiella fluviatilis* (Areschoug) Svedelius in four new localities in southeastern Europe, as well as the first records for Croatia. Additionally, we report here a new type of substrate for *H. fluviatilis*, on stones in karstic rivers characterized by tufa formations. Despite a generally low number of findings, this and previous studies indicate that the species is not as rare as generally perceived; rather, it is overlooked, under-collected and therefore under-reported. The paper provides detailed diagnostic features of the thalli and habitats where the alga was found, with notes on corresponding vegetation, physical conditions and water chemistry. Additionally, along with the new records of the red alga *Hildenbrandia rivularis* (Liebmann) J. Agardh in Croatia, the associations of *Heribaudiella*-*Hildenbrandia* are reported for streams of Black Sea and Adriatic basins.

Phaeophyta / Rhodophyta / *Heribaudiella* / *Hildenbrandia* / freshwater / habitats / distribution / first record / Croatia

INTRODUCTION

Brown algae (class Phaeophyceae) are usually associated with marine ecosystems. Freshwater members of the class are often overlooked or under-researched, their occurrence being thus infrequently reported. *Heribaudiella fluviatilis* (Areschoug) Svedelius (hereafter: *Heribaudiella*) belongs to a group of roughly seven freshwater species from six genera within the class Phaeophyceae (Silberfeld, 2014; Holmes & Whitton, 1975; Wehr & Stein, 1985). This contrasts with the over 2000 species described in marine environments (Reviere *et al.*, 2007). Because marine habitats are not the exclusive habitats for brown algae, it is necessary to explore this freshwater group more fully.

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Each new study and publication about *Heribaudiella*, often referred to as a rare species, is increasingly replacing the gaps in knowledge with objective facts about the ecology and biology of freshwater Phaeophyceae. Even though it was described in detail by Svedelius in 1930 (Svedelius, 1930), information has been fragmentary, from scattered locations in Europe (Athanasiadis, 1996; Ludwig & Schnittler, 1996; Wehr, 2003; Whitton *et al.*, 2003; McCauley & Wehr, 2007; Eloranta *et al.*, 2011; John *et al.*, 2011; Täuscher, 2011; Täuscher, 2014; Täuscher, 2016) North America (Wehr & Perrone, 2003; McCauley & Wehr, 2007), Japan (Yoshizaki *et al.*, 1984) and China (Jao 1941). The alga is currently known to be distributed only in the northern hemisphere (Guiry *et al.*, 2014), while Wehr and Stein (1985) and Stoyneva *et al.* (2003) provided detailed references for the European and Asian distribution of this species known at the time. Thus far, in southeastern Europe *Heribaudiella* has been reported only from Bulgaria (Stoyneva *et al.*, 2003) and Greece (Anagnostidis, 1968), with no published notes about this species in Croatia or neighbouring countries, apart from one isolated record from Slovenia (Lazar, 1975).

The encrusting, largely inconspicuous, cushion-like brown *Heribaudiella* thalli occur in rivers, and can at times become a major component of the algal flora in small rivers. Also, the alga has been reported in rocky littoral zones of lakes (Kann, 1993; Wehr, 2015). The thalli appear as brown, crusty spots on the surface of stones (Figs 1-2), and can be indistinct in appearance and easily overlooked. This was emphasized by Holmes and co-authors, who noted that it can easily be misidentified when encountered in the field (Holmes *et al.*, 1972; Holmes & Whitton, 1975).

Heribaudiella typically colonizes basalt, basaltic andesite, quartz, greenstone, marble, and granite stones (Holmes & Whitton, 1975, 1977a,b,c; Kann, 1978; Wehr & Stein, 1985; Kusel-Fetzmann, 1996; Wehr & Perrone, 2003). The species tolerates a wide range of ecological conditions, from oligotrophic to eutrophic waters (Israelsson, 1938), neutral to slightly alkaline pH, a wide range of inorganic calcium, phosphorus and nitrogen concentrations and habitats from heavily shaded to fully open (Wehr & Stein, 1985). Nevertheless, the overall data suggest that *Heribaudiella* is most commonly found in systems with intermediate to low nutrient levels with vegetation and algal compositions that share several similarities (Wehr & Stein 1985; Wehr & Perrone 2003).

So far, although a few checklists have been published related to freshwater macroalgae for southeastern Europe (Caraus, 2002; Stoyneva *et al.*, 2003; Temniskova *et al.*, 2008; Kwandrans & Eloranta, 2010; Caraus, 2012; Caraus, 2017), relevant data with descriptions and the number of findings are still very limited (Cvijan *et al.*, 2003). Macro-algological studies in Croatian freshwater ecosystems have been

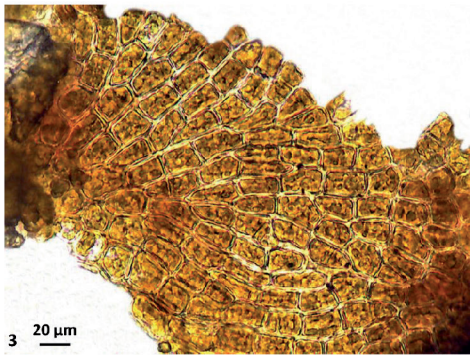
Figs 1-8. 1. Stone from the Kupa-Vukova Gorica with *Hildenbrandia rivularis* thalli (left arrow, large red coat), *Heribaudiella fluviatilis* (right arrow, brown irregular spot) and “Chantransia” phase (middle arrow) as lightly incrustated with calcium carbonate (white puffs). 2. Stone from the Cetina River with *Heribaudiella* thalli (left arrow, brown spot) and “Chantransia” phase overgrowing *Heribaudiella* and incrusting calcium carbonate (right arrow, white puffs). 3. Material from the Una River with surface view of one layer of *Heribaudiella* branching filaments. 4. Material from the Kupa-Kupari with plurilocular sporangia developed on a stalk. 5. Material from the Kupa-Vukova Gorica with unilocular sporangia on the tip of a vertical filament (arrowed cell). 6. Material from Cetina River with surface view of *Heribaudiella* (brown) slightly overlapping and growing close to *Hildebrandia* (red). 7. Material from the Kupa-Vukova Gorica with side view of erect filaments of *Hildebrandia*. 8. Material from the Kupa-Vukova Gorica with details of filaments of the “Chantransia” phase. (Photos by N. Koletić)



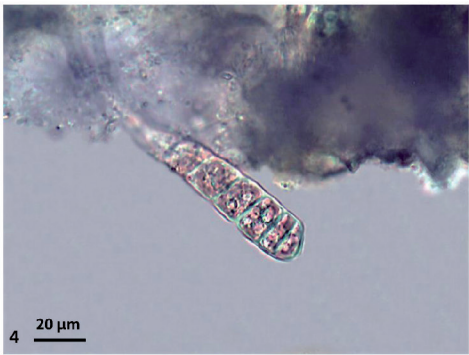
1 1 cm



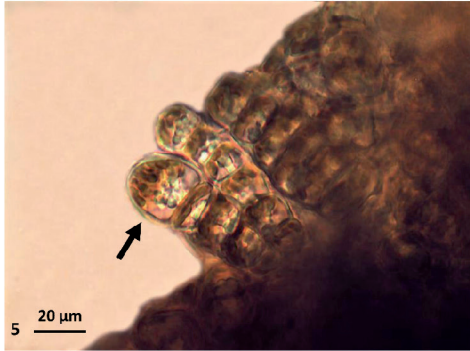
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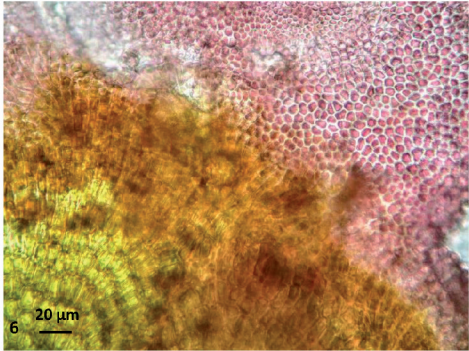
3 20 μm



4 20 μm



5 20 μm



6 20 μm



7 10 μm



8 10 μm

mostly related to members of the Characeae (e.g. Blaženčić & Randelović, 1994; Blaženčić & Blaženčić, 2002; Blaženčić *et al.*, 2006; Alegro *et al.*, 2016a; Alegro *et al.*, 2016b), while other groups are poorly investigated. A few specific papers have dealt with algological flora in fresh waters, emphasizing the limited number of macroalgal species (Golubić, 1957; Marčenko, 1958; Matoničkin & Pavletić, 1961; Pavletić & Matoničkin, 1965; Viličić, 1980; Plenković-Moraj, 1997; Stanković & Leitner, 2015; Žuljević *et al.*, 2016; Koletić *et al.*, 2017). So far there have been no published reports on freshwater brown algae in Croatia.

The aim of our paper is to present the occurrence of *Heribaudiella* in Croatia, and draw attention to its presence being mostly neglected in southeastern Europe. Furthermore, we aimed at providing detailed descriptions of the environment in which it grows, including the microhabitat and the co-occurring algae and at presenting in detail the micro-diagnostic features of this species, as a useful guideline for further identification of this inconspicuous taxon.

MATERIAL AND METHODS

Fieldwork was performed in Croatia on the rivers Kupa, Una and Cetina, during 2009-2017, primarily as a part of an investigation monitoring macrophytic vegetation. The investigated localities on the rivers Kupa and Una are situated in their upstream, karst, mountainous fast-flowing sections. They belong to the Black Sea Basin and are situated in the Continental Subecoregion of the Dinaric Ecoregion of Croatia, while the Cetina River is a slow-flowing river situated in the karst lowland of the Adriatic Sea Basin, classified in the Mediterranean Subecoregion of the Dinaric Ecoregion of Croatia.

Collection of live material was carried out in September 2017 at the confluence of Loskun Stream, a tributary of the Una River (N 44.695833, E 15.956388; 243 m a.s.l.; hereafter: Una River), and in November 2017 in the spring area of the Kupa River near Kupari village (N 45.504959, E 14.700611; 374 m a.s.l.; hereafter: Kupa-Kupari). Algae were collected from riverbeds along with the stones to which they were attached, and the intact material was transported to the laboratory. Live material was scraped off the stone surface with a razor blade, analysed under the microscope with 40-1000 $\times$ magnification, photographed and identified according to Eloranta *et al.* (2011), John *et al.* (2011) and Wehr *et al.* (2015). For the purpose of preservation, collected material was desiccated with silica gel, and additionally some material was preserved with 4% formaldehyde and deposited in herbarium ZA (Thiers, 2017).

After the initial discovery of these populations, samples from previously visited locations were examined in more detail. Additional material attributed to *Heribaudiella* was found in ZA herbarium collection, collected in May 2009 on the lower course of the Cetina River (N 43.439026, E 16.751059; 10 m a.s.l.; hereafter: Cetina River) and in July and December 2017 in the upper course of the Kupa River near Vukova Gorica Village (N 45.456707, E 15.339435; 155 m a.s.l.; hereafter: Kupa-Vukova Gorica). Even though the location on the Cetina River is just 6 km from the confluence with the Adriatic Sea, conditions were not brackish.

A macrophyte vegetation survey of all sites was performed along 100 m of the watercourse. Species coverage and abundance were assessed using the expanded Braun-Blanquet scale (Barkman *et al.*, 1964; Braun-Blanquet, 1964; Dierschke,

1994), where category 2 was subdivided into 2m, 2a and 2b. The nomenclature of the plant species follows Euro+Med Plantbase (2017). The physical and chemical properties of water were recorded monthly by the Croatian State Institution for Water Management “Hrvatske vode” during the period 2013-2017.

RESULTS

Brown crusts of *Heribaudiella* were detected on stones from four sites shown in Fig 9. In Una River, thalli of *Heribaudiella* covered large surfaces of submerged stones at the barriers with small waterfalls. Crusts were approximately 0.5-2 cm in diameter, unevenly scattered on the stone surface. On the stones from the Kupa and Cetina Rivers, the alga was growing less abundantly, but with larger thalli, 3-5 cm in diameter, overlapping with the encrusting red alga *Hildenbrandia rivularis* (Liebmann) J. Agardh (hereafter: *Hildenbrandia*), which covered almost the entire surface of several stones.

The microscopic examination of the *Heribaudiella* thalli has revealed the following micro-structure: the thalli consist of irregularly branched filaments, densely arranged into 5-10 layers of cells, forming a closed crusty structure. The filament cells are 8-15 µm wide and 22-26 µm long, containing on average 8 (10) spherical plastids, evenly distributed along the cell wall. The cells are 2 to 2.5 times longer than they are wide, extending longitudinally towards the direction of the filaments, and reducing towards the edge of the thalli, to attain an ultimate ratio of 1:1. Unilocular and plurilocular sporangia were found at the filament endings, distributed along the edge of the thalli. Unilocular sporangia are approximately 25 µm wide and 25 µm long, containing approximately 8 (10) cylindrical, 10 µm long and 2 µm wide plastids, arranged in the apical part of the cell. Plurilocular sporangia were detected developed on the stalks, growing from the spores released on the edges of the thalli. Their cells are 18 µm wide and 10 µm long, containing approximately 8 (10) spherical plastids 5 µm in diameter, evenly distributed within the cell.

Furthermore, on these sites, abundant tufts firstly identified as *Audouinella pygmaea* (Kützinger) Weber-van Bosse (hereafter: “*Audouinella*”) were detected on the surface of the *Heribaudiella* thalli (Fig 2 & Fig 8). Along with encrusted brown and red algae, a large population of the golden macroalga *Hydrurus foetidus* (Villars) Trevisan was also found, in fast flowing parts at Kupa-Kupari. All sites were characterized by a predominantly coastal vegetation, and vegetation surveys of the sites are given in Table 1. The physical and chemical properties of water are given in Table 2.

Regarding the riverbed, all localities were characterized by mesolitoral (6-20 cm) and megalithal (20-40 cm) rocky substrate and tufa formations, the latter especially common at Kupa-Vukova Gorica and the Una River. During the sampling, water was approximately between 20 and 50 cm deep. Although the forest vegetation was developed alongside the course, the particular sites with algae were unshaded. The water was fast-flowing, especially at the natural barriers with small waterfalls. Algal crusts were typically observed in the microhabitats characterized by the fastest flow and an absence of other aquatic vegetation. Our vegetation surveys have shown that at Kupa-Vukova Gorica, the macrophyte vegetation was growing in the same segment of the riverbed, but in habitats with a slower flow than at the site inhabited

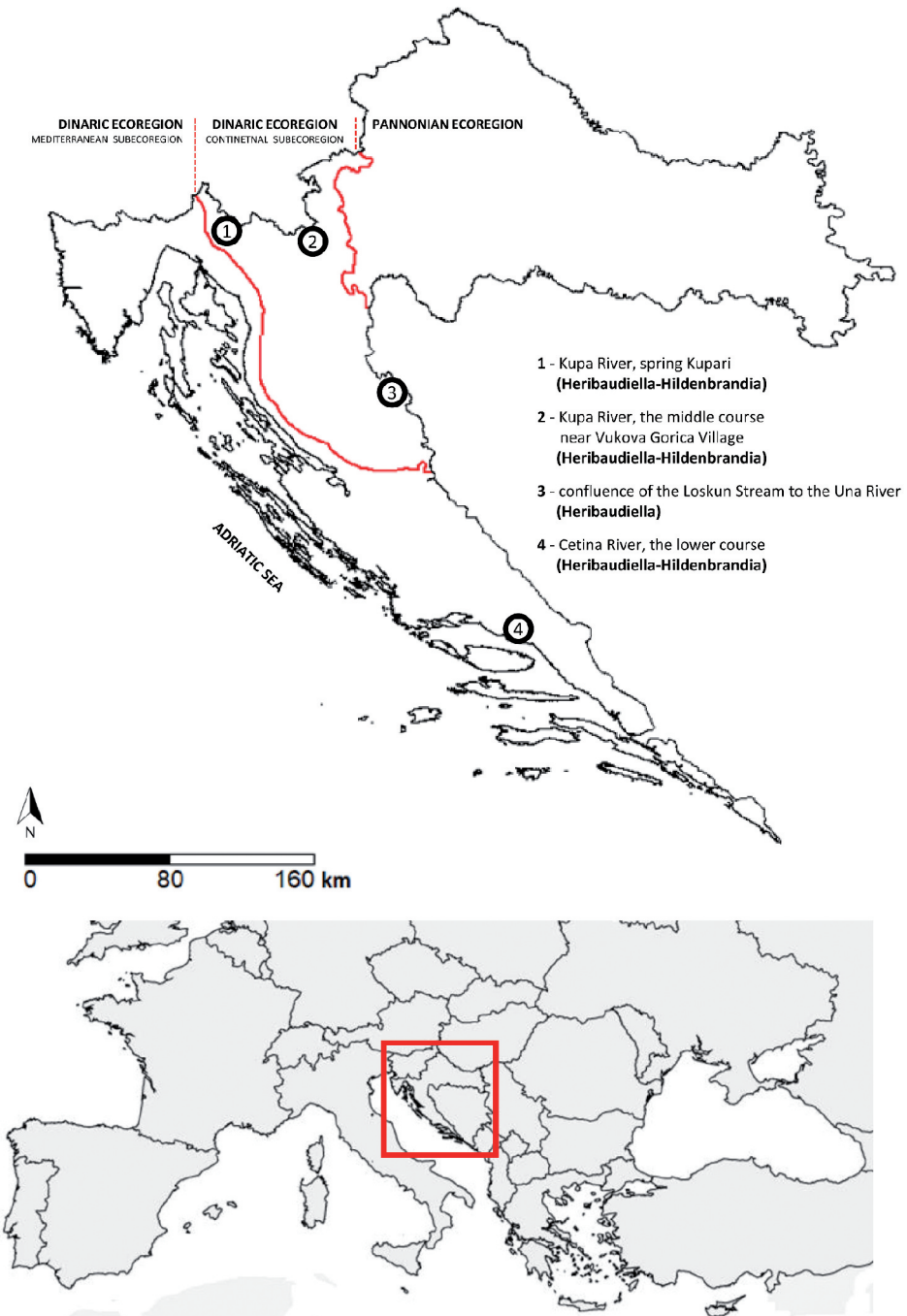


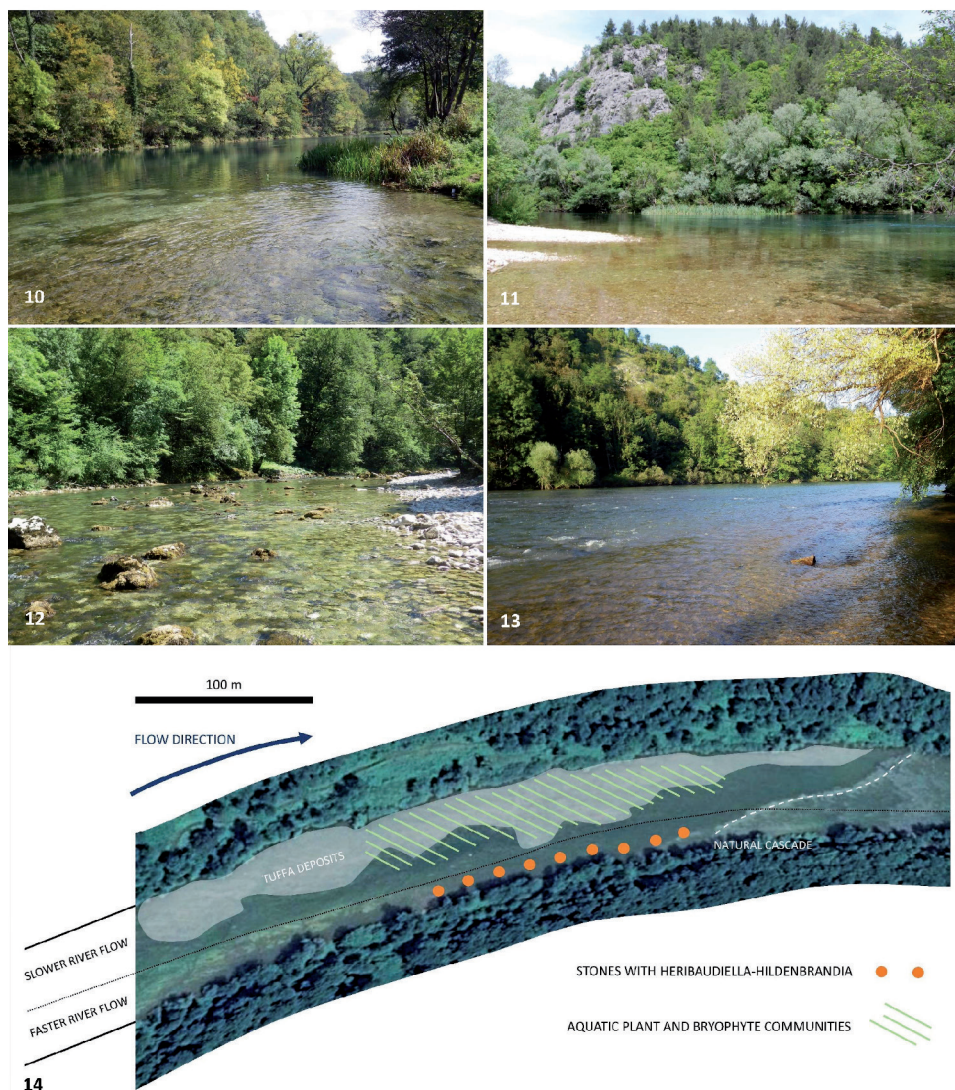
Fig 9. Locations of *H. fluviatilis* in Croatia with specific associations. Locations 1-3 are in the Black Sea Basin, location 4 is in the Adriatic Basin (the scale refers to the map above).

Table 1. Vegetation survey performed using the expanded Braun-Blanquet scale on the *Heribaudiella fluviatilis* localities. Key for abundance ranks: **R** - 1–3 individuals; + - up to 50 individuals, $C < 1,5\%$; **1** - abundant, $1,5\% < C < 3\%$; **2m** - very abundant, $3\% < C < 5\%$; **2a** - $5\% < C < 15\%$; **2b** - $15\% < C < 25\%$; **3** - $25\% < C < 50\%$; **4** - $50\% < C < 75\%$; **5** - $C > 75\%$ (C – cover).

	Kupa River (spring area; Kupari)	Kupa River (middle course; Vukova Gorica)	Una River (Loskun Stream Confluence)	Cetina River (lower course)
algae				
<i>Chara</i> sp.		2b		
<i>Cladophora</i> sp.	1	2m		
<i>Heribaudiella fluviatilis</i> (Aresch.) Sved.	1	2m	2b	2m
<i>Hildenbrandia rivularis</i> (Liebmann) J. Agardh	+	2m		2m
<i>Hydrurus foetidus</i> (Villars) Trevisan	2m			
<i>Nostoc</i> sp.		2m		
<i>Vaucheria</i> sp.	1			
“Chantarsia” phase of red algae		+		+
bryophytes				
<i>Cinclidotus aquaticus</i> (Hedw.) Bruch & Schimp.	2b			+
<i>Cinclidotus fontinaloides</i> (Hedw.) P. Beauv.	2m	1		
<i>Cinclidotus riparius</i> (Host ex Brid.) Arn.	1	1		
<i>Cratoneuron filicinum</i> (Hedw.) Spruce	+			
<i>Fissidens</i> sp.		2m		
<i>Fontinalis antipyretica</i> Hedw.		2m	2b	1
<i>Palustriella falcata</i> (Brid.) Hedenäs	+			
<i>Rhynchostegium riparioides</i> (Hedw.) Cardot	2b	2m	+	2a
vascular plants				
<i>Agrostis stolonifera</i> L.			1	
<i>Berula erecta</i> (Huds.) Coville			3	+
<i>Ceratophyllum demersum</i> L.				R
<i>Mentha aquatica</i> L.			2m	+
<i>Myriophyllum spicatum</i> L.		1		
<i>Myosotis scorpioides</i> L.			1	
<i>Potamogeton lucens</i> L.		1		
<i>Potamogeton nodosus</i> Poir.		2m		
<i>Potamogeton perfoliatus</i> L.		1		
<i>Potamogeton</i> × <i>salicifolius</i> Wolfg.		2m		
<i>Ranunculus trichophyllus</i> Chaix in Vill.		+	+	
<i>Sparganium emersum</i> Rehmman			2a	
<i>Sparganium erectum</i> L.			2a	

Table 2. Physical and chemical properties of water at the *Heribaudiella fluviatilis* localities measured during the period 2013–2017 based on monthly measurements (Data source: Croatian Institute for Water Management “Hrvatske vode”)

PARAMETER	Kupa River (spring area; Kupari)			Kupa River (middle course; Vukova Gorica)			Una River (Loskun Stream Confluence)			Cetina River (lower course)		
	RANGE	MEAN		RANGE	MEAN		RANGE	MEAN		RANGE	MEAN	
Temperature (°C)	2.0-12.0	7.6		3.0-25.5	12.4		10.0-17.0	12.3		10.2-18.1	13.7	
pH	8.0-8.3	8.2		7.7-8.5	8.2		7.5-8.6	8.2		7.8-8.2	8.1	
Conductivity (µS cm ⁻¹)	239-283	266		246-332	306		396-844	501		323-402	358	
Dissolved oxygen (mg O ₂ L ⁻¹)	11.6-13.5	12.4		9.1-14.7	11.4		9.1-11.4	10.6		9.9-11.4	10.7	
Oxygen saturation (%)	91.8-107.8	103.3		93.6-122.0	105.7		94.5-106.200	99.7		99.7-105	102.9	
Carbonates (mg CaCO ₃ L ⁻¹)	119-148	137		129-185	166		128-267	204		161-189	176	
Ammonium (mg N L ⁻¹)	<0.0100-0.0240	0.0136		0.0100-0.0550	0.0232		<0.0020	<0.0020		<0.0100-0.0120	<0.0100	
Nitrates (mg N L ⁻¹)	0.4700-0.8800	0.6214		0.3800-0.8400	0.5575		0.3180-0.6960	0.5133		0.2640-0.3880	0.3206	
Total nitrogen (mg N L ⁻¹)	0.5300-0.9200	0.6786		0.4400-0.9200	0.6717		0.3900-1.3200	0.7740		0.2669-0.3897	0.3418	
Orthophosphates (mg P L ⁻¹)	<0.0050	<0.0050		<0.0050-0.0130	<0.0050		<0.0030-0.0200	0.0075		<0.0060-0.0100	0.0062	
Total phosphorus (mg P L ⁻¹)	<0.0250	<0.0250		<0.0250-0.0330	<0.0250		<0.0030-0.0280	0.0112		<0.0150-0.0150	<0.0150	



Figs 10-14. **10.** Habitats of *H. fluviatilis* on confluence of Loskun stream and the Una River, **11.** Cetina River, lower course, **12.** spring area of the Kupa River near Kupari village and **13.** middle course of the Kupa River near Vukova Gorica village with **14.** graphically-tagged satellite image indicating the spatial distribution of the habitat and its ecological elements (Photo by A. Alegro, V. Šegota & N. Koletić).

by the algae, where macrophyte vegetation was lacking. In addition, in these parts with slower water and macrophyte vegetation tufa deposits were especially abundant, however, not on the stones covered with *Heribaudiella*, *Hildenbrandia* and “*Audouinella*” thalli (Figs 10-13). Habitat conditions of *Heribaudiella* sites are summarized in Fig 14.

DISCUSSION

In spite of the extensive study of macrophyte vegetation performed in the past in Croatia within the context of national water quality monitoring, encrusting forms of algae were not recognized, since the monitoring scheme included only more conspicuous branched and filamentous macroalgae. For this reason, our first finding of *Heribaudiella* in Croatia was more or less coincidental. As in the observations made by Holmes & Whitton (1975), the alga was probably overlooked in many previously visited localities. The new records were the result of observing the distinctive brown spots on stones in the Una River. This presumption was confirmed after subsequent identification of the alga within some of the previously collected samples. While studying the biogeography and ecology of *Heribaudiella* in North America, Wehr & Stein (1985) concluded that the current world distribution of this species is probably incomplete simply because crust-forming algae are less conspicuous and receive less attention than filamentous forms. The same authors report that after a systematic survey of streams and rivers of the United Kingdom, *Heribaudiella* was recorded in as many as 13% of surveyed sites (Wehr & Stein, 1985). All these findings point out to the importance of comprehensive, systematic studies in determining the actual distribution of this alga in various regions.

So far, few records of *Heribaudiella* have been made in southeastern Europe. Anagnostidis (1968) reports two records from Greece, with no further descriptions other than the name of the localities. More recently, five records have been provided for Bulgaria by Stoyneva *et al.* (2003), describing the thalli and habitats of recorded populations. Our findings represent the second record in southeastern Europe within the Black Sea Basin, and the first record related to watercourses in the Adriatic Basin. New findings suggest that the alga has a much wider distribution than currently recorded, and may be even quite common in southeastern Europe, but systematic studies of its distribution are needed to support this presumption. Along with the detailed description of its habitat, micro-diagnostic features of the thalli presented in this study can serve as a useful guideline for further recognition of this alga. Regarding the size of the filament cells, we found slightly wider and shorter cells than the standard values described by Eloranta *et al.* (2011). Furthermore, we recorded a more regular, spherical form of the unilocular sporangia, rather than the cylindrical form described by Eloranta *et al.* (2011). Differences in cell dimensions are sometimes used for describing morphotypes (Hansring, 1905; Skuja, 1925; Waern, 1938, 1949; Israelson, 1938), and further morphometric studies of southeastern European populations might employ this possibility.

Heribaudiella has been documented often to co-occur with the red alga *Hildenbrandia*, usually as *Heribaudiella* overgrowing crusts of *Hildenbrandia* (Budde, 1927; Fritsch, 1929; Geitler, 1932; Kusel-Fetzmann, 1996). The combined distribution of *Hildenbrandia* and *Heribaudiella* in Europe was first described by Budde (1927) and later described in more detail by Fritsch (1929; as the *Hildenbrandia-Lithoderma* association). The combined growth of *Heribaudiella*-*Hildenbrandia* had been recorded mainly from northern and central Europe (Skuja, 1925; Budde, 1927; Fritsch, 1929; Geitler, 1932; Waern, 1938; Kann, 1966; Holmes & Whitton 1977 a,b,c; Kusel-Fetzmann, 1996) while in southern Europe these two species have so far been reported separately (Margalef, 1953; Anagnostidis, 1968; Róbert, 1976; Sabater *et al.*, 1989; Dell'Uomo 2001). Nevertheless, our records from southeastern Europe confirm the findings of Stoyneva *et al.* (2003) that the

Heribaudiella-Hildenbrandia association occurs in more southern parts of Europe as well.

Our findings also represent new records of the red alga *Hildenbrandia* in Croatia. We suggest that *Hildenbrandia* may also be more common in Croatian watercourses than has been recorded thus far (Golubić, 1957; Matoničkin & Pavletić, 1961; 1962; 1967), but no systematic studies have been undertaken to record its presence. Regarding habitat characteristics, *Heribaudiella* seems to prefer faster-flowing parts of rivers and streams, combined with the absence of water macrophyte vegetation. Interestingly, we have found the alga in extremely karstic environments, in which tufa formations were observed, notably in the Kupa and Una rivers. Although the alga was not colonizing the actual tufa, the environment at these two sites was extremely prone to tufa-formations in areas of reduced current; this is a river system not previously reported as a suitable environment for this species.

The record of “*Audouinella*” indicates the presence of several other algae within the recorded community. Namely, the thalli identified as *A. pygmaea* is actually the “Chantransia” (i.e. sporophyte) phase of some Batrachospermales or Thoreaales (Chiasson *et al.*, 2005; Necchi and Oliveira, 2011), mainly of *Batrachospermum* genus. This finding is in line with what was previously known, as the occurrence of the “Chantransia” phase on the thalli of *Heribaudiella* is occasionally recorded in nature (Svedelius, 1930; Pueschel & Stein, 1983; Wehr & Stein, 1985). Although we searched the riverbed along a wider area within the watercourse, we found no evidence of the corresponding gametophyte which would enable the identification. Thus, we could not identify the exact species associated with the “Chantransia” phase, and further studies might be needed to resolve this issue. In locations where “*Audouinella*” was also present with *Heribaudiella* and *Hildenbrandia*, it is interesting to note that calcium carbonate was slightly deposited on the “*Audouinella*” tufts, while the other two species showed no sign of calcium carbonate deposits. This is not surprising, since it is known that precipitation occurs on the wrinkled surfaces where a larger amount of periphyton is deposited; therefore the tufted growth form of “*Audouinella*” may enhance the precipitation of dissolved minerals from water. During microscopic analysis, on the surface of *Heribaudiella-Hildenbrandia* no microscopic periphyton was detected apart from “*Audouinella*” tufts.

Based on the physical and chemical parameters presented in this study and in comparison with past studies (Wehr & Stein, 1985; Wehr, 2015), we generally describe *Heribaudiella* as a eurythermal freshwater species. In Croatia, it was recorded in watercourses of various thermal conditions; from cold mountainous areas of 4°C to warm slower flows with temperatures up to 25°C. On the other hand, the pH spectrum and nutrient levels have shown more narrow ranges, whereas the species was recorded in conditions with pH generally between 7 and 8, and on average low concentrations of nutrients and moderate conductivity, supporting previous records (Wehr & Perrone, 2003). This generally broad range of environmental preferences across its range corroborates our conclusions that the lack of recognition of this alga from Croatian studies for over a century is most probably due to its inconspicuous thallus. Without further examination, this species can easily remain unnoticed, under-collected and under-reported. More likely, *Heribaudiella* is more likely a widespread species, the distribution of which has been insufficiently investigated across its range.

CONCLUSION

The study represents a significant contribution to the knowledge of freshwater algae in Croatia, being the first record of freshwater brown algae in general, and accordingly the first record of *Heribaudiella*. Also, the red alga *Hildebrandia* was reported here with new locations and habitat descriptions. In addition to detailed descriptions of *Heribaudiella* thalli and habitats, the study finds that the alga occurs in extremely karstic habitats, a condition not previously reported for this species. Although our records are among the very few reports of *Heribaudiella* in southeastern Europe, we firmly believe that the alga is generally much more frequent, but systematically under-researched. Therefore we recommend specific, comprehensive studies of its distribution in order to get a better understanding of its wider distribution.

REFERENCES

- ALEGRO A., STANKOVIĆ I., ŠEGOTA V., VAN DE WEYER K. & BLAŽENČIĆ J., 2016a — Macrophytic vegetation in the oligotrophic Mediterranean Lake Vrana (Island of Cres, Northern Adriatic) – New insight after 50 years. *Botanica Serbica* 40(1): 21-28.
- ALEGRO A., ŠEGOTA V., RIMAC A., HRŠAK V., VUKOVIĆ N., KOLETIĆ N. & STANKOVIĆ I., 2016b — Macrophyte vegetation of rivers in Croatia – diversity and ecological indication potential. In: REŠETNIK I. & LJUBEŠIĆ Z. (eds) *Book of abstracts of the 5th Croatian Botanical Symposium with International Participation*. Zagreb: Croatian botanical society, 6 p.
- ANAGNOSTIDIS K., 1968 — Untersuchungen über die Salz- und Süßwasser-Thiobiocönosen (Sulphuretum) Griechenlands. *Wissenschaftliches Jahrbuch der Physiko-Mathematischen Fakultät Aristoteles-Universität Thessaloniki* 1: 406-860.
- ATHANASIADIS A., 1996 — Taxonomisk litteratur och biogeografi av Skandinaviska rödalger och brunalger. *Göteborg: Algologia*, pp. 1-280.
- BLAŽENČIĆ J. & BLAŽENČIĆ Ž., 2002 — Rare and threatened species of charophytes (Charophyta) in Southeast Europe. *Phytologia Balcanica* 8: 315-326.
- BLAŽENČIĆ J. & RANĐELOVIĆ V., 1994 — *Chara visianii* J. Blaženčić et V. Randelović sp. nov. (Characeae) from the river Krka (Croatia). *Cryptogamie, Algologie* 15: 73-79.
- BLAŽENČIĆ J., STEVANOVIĆ B., BLAŽENČIĆ Ž. & STEVANOVIĆ V., 2006 — Distribution and ecology of charophytes recorded in the West and Central Balkans. *Cryptogamie, Algologie* 27: 311-322.
- BARKMAN J.J., DOING H. & SEGAL, S., 1964 — Kritische Bemerkungen und Vorschläge zur quantitativen Vegetationsanalyse. *Acta botanica Neerlandica* 13: 394-419.
- BRAUN-BLANQUET J., 1964 — *Pflanzensoziologie. Grundzüge der Vegetationskunde*. 3. Wien-New York: Auflage Springer Verlag.
- BUDDE H., 1927 — Die Rot- und Braunalgen des Westfälischen Sauerlandes. *Berichte der Deutschen botanischen Gesellschaft* 45: 143-150.
- CARAUS I. 2002 — The algae of Romania. *Studii si cercetari, Universitatea Bacau, Biologie* 7: 1-694.
- CARAUS I., 2012 — *Algae of Romania. A distributional checklist of actual algae*. Version 2.3 third revision. Bacau: University of Bacau.
- CARAUS I., 2017 — *Algae of Romania. A distributional checklist of actual algae*. Version 2.4. *Studii si cercetari, Universitatea Bacau, Biologie* 7 7: 1-1002.
- CHIASSON W.B., SABO N.J. & VIS M.L., 2005 — Affinities of freshwater putative chantransia stages (Rhodophyta) from molecular and morphological data. *Phycologia* 44:163-168.
- CVIJAN M., BLAŽENČIĆ J. & SUBAKOV-SIMIĆ G., 2003 — *Flora algi Srbije 2 – Rhodophyta*. Belgrade: NNK internacional.
- DELL'UOMO A., 2001 — Les algues des milieux lotiques et leur importance dans l'évaluation de la qualité biologique de l'eau. In: *X. Optima Meeting, Palermo, 13-19 Septembre 2001.*, p. 84.
- DIERSCHKE H., 1994 — *Pflanzensoziologie. Grundlagen und Methoden*. Stuttgart: Eugen Ulmer Verlag.

- ELORANTA P., KWANDRANS, J. & KUSEL-FETZMANN, E., 2011 — Rhodophyta and Phaeophyceae. In: *Süßwasserflora von Mitteleuropa*, 7. Heidelberg: Spektrum Akademischer Verlag, p. 155.
- EURO+MED, 2017 — Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Retrieved December 19, 2017 from <http://ww2.bgbm.org/EuroPlusMed/>.
- FRITSCH F.E., 1929 — The encrusting algal communities of certain fast-flowing streams. *New phytologist* 28: 165-196.
- GEITLER L., 1932 — Notizen über *Hildenbrandia rivularis* und *Heribaudiella fluviatilis*. *Archiv für protistenkunde* 76: 581-588.
- GUIRY M.D., GUIRY G.M., MORRISON L., RINDI F., VALENZUELA MIRANDA S., MATHIESON A.C., PARKER B.C., LANGANGEN A., JOHN D.M., BÁRBARA I., CARTER C.F., KUIPERS P. & GARBARY D.J., 2014 — AlgaeBase: An On-line Resource for Algae. *Cryptogamie, Algologie* 35(2): 105-115.
- GOLUBIĆ S., 1957 — Vegetacija alga na slapovima rijeke Krke u Dalmaciji (Die Algenvegetation an Wasserfällen des Flusses Krka in Dalmatien). *Jugoslavenska akademija znanosti i umjetnosti* 312: 207-260.
- HOLMES N.T.H., LOYD E.J.H., POTTS M. & WHITTON B.A., 1972 — Plants of the River Tyne and future water transfer scheme. *Vasculum* 57: 56-78.
- HOLMES N.T.H. & WHITTON B.A., 1975 — Notes on some macroscopic algae new or seldom recorded for Britain: *Nostoc parmelioides*, *Heribaudiella fluviatilis*, *Cladophora aegagropila*, *Monostroma bullosum*, *Rhodoplax schinzii*. *Vasculum* 60: 47-55.
- HOLMES N.T.H. & WHITTON B.A., 1977a — The macrophytic vegetation of the River Tees in 1975: observed and predicted changes. *Freshwater biology* 7: 43-60.
- HOLMES N.T.H. & WHITTON B.A., 1977b — The macrophytic vegetation of the River Swale, Yorkshire. *Freshwater biology* 7: 545-558.
- HOLMES N.T.H. & WHITTON B.A., 1977c — Macrophytes of the River Wear: 1966–1976. *Naturalist (Hull)* 102: 53-73.
- ISRAELSON G., 1938 — Über die Süßwasserphaeophycéen Schwedens. *Botanische noten* 1938: 113-128.
- JAO C.-C., 1941 — Studies on the freshwater algae of China. VII. *Lithoderma zonatum*, a new freshwater member of the Phaeophyceae. *Sinensia* 12: 239-44.
- JOHN D., WHITTON B.A. & BROOK A.J., 2011 — *The freshwater algal flora of the British Isles*, 2nd edn. Cambridge: Cambridge University Press.
- KANN E., 1966 — Der Algenaufwuchs in einigen Bächen Österreichs. *Verhandlungen des internationalen vereins limnologie* 16: 646-654.
- KANN E., 1978 — Sytematik und Ökologie der Algen österreichischer Bergbäche. *Archiv für hydrobiologie* 53: 405-643.
- KANN E., 1993 — Der litorale Algenaufwuchs im See Erken und in seinem Abfluß (Uppland, Schweden). *Algological studies* 69: 91-112.
- KOLETIĆ N., ALEGRO A., SEGOTA V., VUKOVIĆ N., RIMAC A. & VILOVIĆ T., 2017 — New sites of rare cold-water golden algae *Hydrurus foetidus* (Villars) Trevisan (Ochrophyta: Chrysophyceae) in Croatia. *Natura Croatica* 26(2): 305-311.
- KUSEL-FETZMANN E.L., 1996 — New records of freshwater Phaeophyceae from lower Austria. *Nova Hedwigia* 62: 79-89.
- KWANDRANS J. & ELORANTA P., 2010 — Diversity of freshwater red algae in Europe. *Oceanological and hydrobiological studies* 35(1): 161-169.
- LAZAR J., 1975 — *Widespread of Fresh-water Algae in Slovenia*. Ljubljana: Slovenska Akademija Znanosti in Umetnosti. p. 83.
- LUDWIG G. & SCHNITTNER M., 1996 — Rote Liste gefährdeter Pflanzen Deutschlands. *Schriftenreihe für vegetationskunde* 28: 1-744.
- MARČENKO E., 1958 — Prilozi poznavanju vegetacije alga na području slapova Plitvičkih jezera (Beiträge zur Kenntnis der Algenvegetation in den Wasserfällen des Plitvicer Seengebietes). *Jugoslavenska akademija znanosti i umjetnosti* 320: 106-152.
- MATONIČKIN I. & PAVLETIĆ Z. 1961 — Biljni i životinjski svijet na sedrenim slapovima jugoslavenskih krških voda. *Biološki glasnik* 14: 105-128.
- MATONIČKIN I. & PAVLETIĆ Z. 1962 — Karakteristike biocenoza na sedrenim slapovima rijeke Krke u Dalmaciji (Charakteristik der Lebensgemeinschaften auf den Wasserfällen des Jugoslawischen Flusses Krka). Zagreb: Krš Jugoslavije, knjiga 3.
- MATONIČKIN I. & PAVLETIĆ Z., 1967 — Hidrologija potočnog sistema Plitvičkih jezera i njegove ekološko-biocenološke značajke (Hydrologie der Bachsysteme von Plitvička jezera und ihre Ökologische und Biozöologische Charakteristik). Zagreb: Krš Jugoslavije, knjiga 5.

- MARGALEF R., 1953 — Algunos organismos interesantes de las aguas dulces de los Pirineo. *Pirineos* 9: 407-420.
- NECCHI O. Jr. & OLIVEIRA M.C., 2011 — Phylogenetic affinities of “Chantransia” stages in members of the Batrachospermales and Thoreaales (Rhodophyta). *Journal of phycology* 47:680-686.
- McCAULEY L.A.R., WEHR J.D., 2007 — Taxonomic reappraisal of the freshwater brown algae *Bodanella*, *Ectocarpus*, *Heribaudiella*, and *Pleurocladia* (Phaeophyceae) on the basis of *rbcL* sequences and morphological characters. *Phycologia* 46: 429-439.
- PAVLETIĆ Z. & MATONIČKIN I., 1965 — Biološka klasifikacija gornjih tijekova krških rijeka (Biologische Klassifikation oberer Läufe der Kartstgewässer). *Acta botanica Croatica* 24: 151-162.
- PLENKOVIĆ-MORAJ A., 1997 — Croatian Freshwater *Chlorophyceae*. *Natura Croatica* 6: 67-89.
- PUESCHEL C.M. & STEIN J.R., 1983 — Ultrastructure of a freshwater brown alga from western Canada. *Journal of phycology* 19:209-215.
- REVIERS B. DE, ROUSSEAU F. & DRAISMA S.G.A., 2007 — Classification of the Phaeophyceae from the past to the present and current challenges. In: BRODIE J. & LEWIS J. (eds) *Unravelling the Algae: the past, present, and future of algal systematics*. Boca Raton: CRC Press (Taylor and Francis Group), pp. 267-284.
- RÓBERT A., 1976 — Formatiuni acvatice algale. simbioza. Parazitizm. In: PÉTERFI St. & IONESCU Al. (eds) *Tratat de Algologie*. Bucuresti: Editura Academiei Republicii Socialiste Romania, pp. 361-374.
- SABATER S., ABOAL M. & CAMBRA J., 1989 — Nuevas observaciones de Rodoficeas en algas epicontinentales de NE y SE de Espana. *Limnetica* 5: 93-100.
- SILBERFELD T., ROUSSEAU F. & DE REVIERS B., 2014 — An Updated Classification of Brown Algae (Ochrophyta, Phaeophyceae). *Cryptogamie, Algologie* 35(2): 117-156.
- SKUJA H., 1925 — Bemerkungen über die Süßwasserarten der Gattung *Lithoderma* in Lettland. *Hedwigia* 65: 331-340.
- STANKOVIĆ I. & LEITNER P., 2016 — The first record of *Hydrurus foetidus* (Villars) Trevisan (Ochrophyta: Chrysophyceae) in Croatia with ecological notes. *Natura Croatica* 25(2): 223-231.
- STOYNEVA M.P., STANCHEVA R. & GÄRTNER G., 2003 — *Heribaudiella fluviatilis* (Aresch.) Sved. (Phaeophyceae) and the *Hildenbrandia rivularis* (Lieb.) J. Ag. - *Heribaudiella fluviatilis* (Aresch.) Sved. association newly recorded in Bulgaria. *Berichte des naturwissenschaftlichen-medizinischen verein Innsbruck* 90: 61-71.
- SVEDELIUS N., 1930 — Über die sogenanntem Süßwasser-Lithodermen. *Zeitschrift für botanik* 23: 892-918.
- TÄUSCHER L., 2011 — Checklisten und Gefährdungsgrade der Algen des Landes Brandenburg I. Einleitender Überblick, Checklisten und Gefährdungsgrade der Cyanobacteria/Cyanophyta, Rhodophyta und Phaeophyceae/Fucophyceae. *Verhandlungen des botanischen vereins von Berlin und Brandenburg* 144: 177-192.
- TÄUSCHER L., 2014 — Checkliste der Algen (Cyanobacteria et Phycophyta). In: FRANK D. & NEUMANN V. (Eds) *Bestandssituation der Pflanzen und Tiere in Sachsen-Anhalt*. Rangsdorf: Natur und Text.
- TÄUSCHER L., 2016 — Algen (Cyanobacteria et Phycophyta). In: FRANK D. & SCHNITTER P. (Eds) *Pflanzen und Tiere in Sachsen-Anhalt Ein Kompendium der Biodiversität*. Rangsdorf: Westermann Druck Zwickau, pp. 63-112.
- TEMNISKOVA D., STOYNEVA M.P. & KIRJAKOV I.K., 2008 — Red list of the Bulgarian algae. I. Macroalgae. *Phytologia Balcanica* 14(2): 193-206.
- THIERS B., 2017 — Index Herbariorum: A global directory of public herbaria and associated staff. *New York Botanical Garden's Virtual Herbarium*. Retrieved December 19, 2017 from: <http://sweetgum.nybg.org/ih/>.
- VILIČIĆ D., 1980 — Contribution to the knowledge of flora and vegetation of algae in the river Kupa. *Biosistematika* 6: 1-14.
- WAERN M., 1938 — Om *Cladophora aegagropila*, *Nostoc pruniforme* och andra alger i Lilla Ullevifjorden, Mälaren. *Botanische noten* 1938: 129-142.
- WEHR J.D., 2003 — Brown algae. In: WEHR, J.D. & SHEATH R.G. (eds), *Freshwater Algae of North America. Ecology and Classification*. San Diego: Academic Press, Elsevier, pp. 757-773.
- WEHR J.D., 2015 — Brown Algae. In: WEHR J.D., SHEATH R.G. & KOCIOLEK J.P. (eds) *Freshwater algae of North America, ecology and classification*. 2nd edition. San Diego: Academic Press, Elsevier, pp. 851-871.
- WEHR J.D. & PERRONE A.A., 2003 — A new record of *Heribaudiella fluviatilis*, a freshwater brown alga (Phaeophyceae) from Oregon. *Western north American naturalist* 63: 517-523.

- WEHR J.D. & STEIN J.R., 1985 — Studies on the biogeography and ecology of the freshwater phaeophycean alga *Heribaudiella fluviatilis*. *Journal of phycology* 21: 81-93.
- WHITTON B.A., JOHN D.M., KELLY M.G. & HAWORTH E.Y., 2003 — *A coded list of freshwater algae of the British Isles. Second Edition*. World-wide Web electronic publication. URL: <http://www.nhm.ac.uk/our-science/data/uk-species/checklists/NHMSYS0000591449/index.html>
- YOSHIZAKI M., MIYAJI K. & KASAKI H., 1984 — A morphological study of *Heribaudiella fluviatilis* (Areschoug) Svedelius (Phaeophyceae) from central Japan. *Nankiseibutu Nanki biological society* 26: 19-23.
- ŽULJEVIĆ A., KALEB S., PEÑA V., DESPALATOVIĆ M., CVITKOVIĆ I., DE CLARCK O., LE GALL L., FALACE A., VITA F., BRAGA J.C. & ANTOLIĆ B., 2016 — First freshwater coralline alga and the role of local features in a major biome transition. *Scientific reports* 6:19642.