



# First record of *Paralemanea torulosa* (Roth) Sheath & A.R. Sherwood and new findings of *Lemanea fluviatilis* (Linnaeus) C. Ag. and *Hildenbrandia rivularis* (Liebmann) J. Agardh (Rhodophyta) in Serbia

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**ABSTRACT:** The paper presents the first record of the freshwater red alga *Paralemanea torulosa* and new findings of the species *Lemanea fluviatilis* and *Hildenbrandia rivularis* in Serbia. The existence of all three species was recorded in the upper reaches of clean fast-flowing rivers and brooks belonging to the basin of the Danube River. *Lemanea fluviatilis* was found in the Dojkinačka River in Eastern Serbia, while *Paralemanea torulosa* was recorded in the Drina River and *Hildenbrandia rivularis* in the Cvetića Brook and Bioštanska Banja Brook in Western Serbia. These reports are important for conservation of the biodiversity of Serbia, since it is well known that freshwater red algae are endangered and rare species (taxa) in many countries. In Serbia they are under strict protection of the law.

**KEYWORDS:** *Hildenbrandia*, *Lemanea*, *Paralemanea*, Rhodophyta, algae, Serbia

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## INTRODUCTION

Rhodophyta, or red algae, represent an interesting group of organisms, mostly inhabiting marine ecosystems. However, some of them can be found living in freshwater habitats (mostly in streams and rivers, as well as in lakes and ponds), and several representatives are adapted to live on wet ground (ELORANTA & KWADRANS 2004). The majority of freshwater taxa are benthic forms that live attached to various hard substrates. According to SHEATH & HAMBROOK (1988), red algae have ecological inclinations and biomechanical adaptations for life in

lotic environments. One third of the total number of the world's freshwater red algae (ca. 60 taxa) are recorded in Europe, and one third of these European taxa have been detected at a small number of locations or only at one location. The northern part of Europe, more precisely the Nordic countries of Sweden and Finland, have the highest diversity of freshwater red algae (27 and 28 taxa, respectively), while the number of taxa is significantly lower in the central and southern parts of Europe (ELORANTA & KWADRANS 2004; KWADRANS & ELORANTA 2010). As for Serbia, 15 species from seven genera have been recorded so far (CVIJAN *et al.* 2003;

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SIMIĆ *et al.* 2010; SIMIĆ & ĐORĐEVIĆ 2017). This group of algae is susceptible to frequent changes in taxonomy due to intensive research, mostly involving molecular analyses (KUČERA & MARVAN 2004).

Freshwater red algae are considered endangered species in many European countries, and in Serbia all of them are strictly protected (SGRS 5/2010, 47/2011). In general, this algal group can be found within a wide range of trophic states in aquatic habitats (SHEATH & HAMBROOK 1990), but most of them live attached to a clean substrate in oligotrophic waters. It is worth mentioning that red algae represent indicator species with different preferences for physical, chemical and biological variables (SIMIĆ 2002; ELORANTA & KWADRANS 2004).

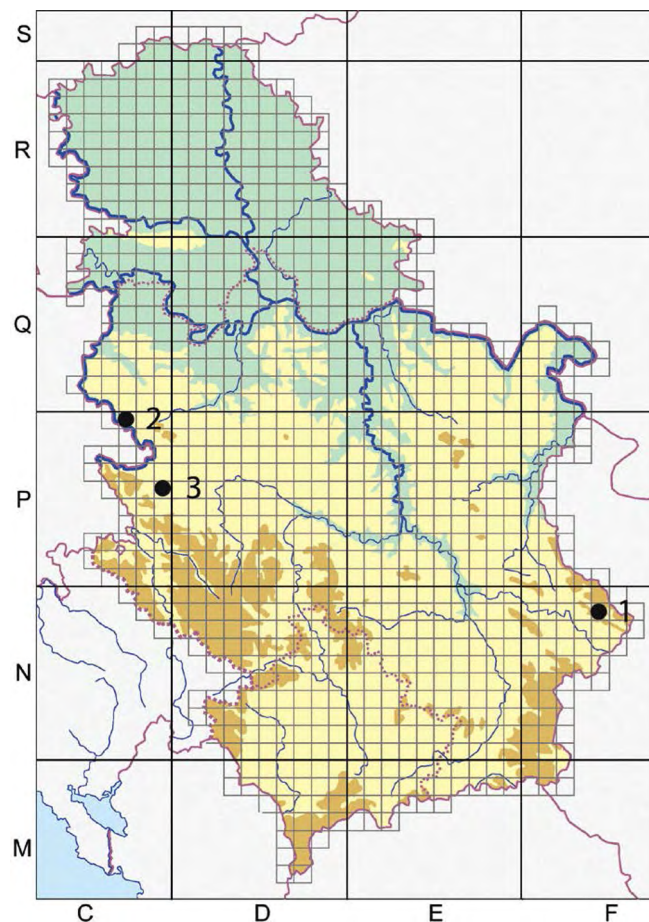
The IUCN Red List of Threatened Species (<http://www.iucnredlist.org/>) contains 58 species of marine red algae from the class Florideophyceae, but freshwater red algae have so far not been included in this list. In Europe freshwater red algae are found in regional red lists; for example *Lemanea fluviatilis* (Linnaeus) C. Ag. is labelled as vulnerable in Poland and Germany (SIEMIŃSKA 1992; LUDWIG *et al.* 1996) or as endangered in Slovakia (HINDAK 2001), while *Paralemanea torulosa* (Roth) Sheath & A.R. Sherwood is in a group of near threatened species in Bulgaria (TEMNISKOVA *et al.* 2008). *Hildenbrandia rivularis* (Liebmann) J. Agardh is included on the Red List of Threatened Algae in Slovakia as an endangered plant (MARHOLD & HINDAK 1998), while it is considered a vulnerable plant in Poland and Germany (SIEMIŃSKA 1992; LUDWIG *et al.* 1996) and a near threatened species in Finland and Bulgaria (RASSI *et al.* 2001).

Evaluation of phytobenthos is one of the components of ecological status assessment. As important representatives of phytobenthos, red algae should be involved in the assessment and monitoring of ecological status because although most of the WFD (Water Framework Directive) member states use diatoms for assessing phytobenthos, diatom analysis is costly and time-consuming. The potential of using macroalgae has therefore been recognised as a method complementary to current diatom and macrophyte techniques (KELLY *et al.* 2015). Freshwater red algae are rare in Central and Southern Europe because in densely populated countries many streams and rivers are eutrophicated and canalised with dams and river bars (KWADRANS & ELORANTA 2010).

The purpose of our study was to report new localities for two red algal species already recorded before, as well as to present one species documented for the first time on the territory of Serbia. The investigated species belong to two families: Lemnaceae and Hildenbrandiaceae.

## MATERIALS AND METHODS

**Plant material.** Eight samples of red algae were taken from three different aquatic areas on the territory of Eastern and Western Serbia during the period between



**Fig. 1.** Wider areas of localities from which red algae were collected from 2010 to 2014: 1) Dojkinačka River, 2) Drina River, 3) Cvetića Brook and Bioštanska Banja Brook.

2010 and 2014: 1. the Dojkinačka River in Eastern Serbia (locality a - Lilina česma, locality b - Čedina česma, locality c and locality d); 2. the Drina River in Western Serbia (Vrhpolje and Ljubovija); and 3. tributaries of the Vruci Reservoir in Western Serbia (the Cvetića Brook and the Bioštanska Banja Brook) (Fig. 1). The GPS coordinates were marked, and samples were deposited in the collection of the Institute of Botany and Botanical Garden "Jevremovac" of the Faculty of Biology under collection numbers 4313, 4314, 4316, 4319, 4907, 4908, 5143 and 5166, as shown in Table 1. Identification was performed using standard keys for identification of red algae according to ELORANTA *et al.* 2011.

Algological material was collected from the rocks on which it was found with a blade and a brush and then placed in plastic bottles and fixed in 4% formalin. Anatomical and morphological characteristics of red algae were observed in the laboratory under a stereo microscope (Zeiss Stemi DV4), while free hand cross-sections were observed under a light microscope with a digital camera (AxioCam MRC5 with AxioVision 4.8 software). Cross-sections were made with a razor blade

**Table 1.** Physical and chemical parameters measured at the study sites from July 2010 to October 2014 and the species that were found.

Aquatic area	Dojkinačka River			Drina River			Tributaries of Vrutci Reservoir		
Locality	Locality a (Lilina česma)	Locality b (Čedina česma)	Locality c	Locality d	Vrhpolje	Ljubovija	Cvetića Brook	Biošćanska Banja Brook	
Sample's coll. number	4313	4314	4316	4319	4907	4908	5143	5166	
Date	26 July 2010	25 July 2010	25 July 2010	25 July 2010	11 August 2013	11 August 2013	26 September 2014	21 October 2014	
GPS coordinate	N43°15'43.3" E22°46'23.2"	N43°15'24.5" E22°46'31.3"	N43°15'02.0" E22°46'32.6"	N43°15'32.0" E22°46'37.8"	N44°08'38.69" E19°27'23.99"	N44°10'51.96" E19°22'50.40"	N43°50'19" E19°42'43"	N43°50'43.98" E19°40'54.64"	
Altitude (m)	1015.5	1000.5	972	924	182	174	645	672	
Depth (m)	0.30	0.30	0.30	0.30	2.50	2.50	0.20	0.15	
Transparency	To the bottom	To the bottom	To the bottom	To the bottom	To the bottom	To the bottom	To the bottom	To the bottom	
Water temperature (°C)	12.5	15.0	16.0	17.0	15.0	15.0	13.20	12.10	
pH	7.0	7.50	8.0	7.0			8.47	8.23	
Dissolved oxygen (mg/L)							9.53	9.52	
O <sub>2</sub> (%)							97.70	95.60	
BOD <sub>5</sub> (mg/L)							2.74	0.63	
Phosphate (mg/L)							0.08	0.32	
Nitrate (mg/L)							0.21	1.83	
Arsenic (µg/L)							20.00	20.00	
Mercury (µg/L)							1.00	1.00	
Nickel (µg/L)							16.93	11.48	
Species		<i>Lemanea fluviatilis</i>			<i>Paralemanea torulosa</i>		<i>Hildenbrandia rivularis</i>		

and put into glycerine. We analysed the stalks and branches, plant length, spermatangial zone diameter, spermatangial papilla organisation and presence of cortical filaments.

**Study area.** The Dojkinačka River is a fast-running mountain river, a tributary of the Visočica River, which belongs to the basin of the Southern Morava River. The Dojkinačka River flows through canyons up to several tens of metres high, and through valleys. Gravel and stones cover the bottom of the Dojkinačka River. Around 20 larger and smaller streams flow into the Dojkinačka River (Ilić 2011). In July of 2010, four samples of red algae were collected from the rocky bed of the Dojkinačka River in the „Stara Planina“ Nature Park.

All of the Dojkinačka River localities were characterised by high water transparency. Locality a (Lilina česma) was overshadowed by beech (*Fagus sylvatica* L.) trees. There were mosses and ferns on the river bank. At locality b (Čedina česma), the river was predominantly surrounded by beech trees and herbaceous plants such as wild mint (*Mentha piperita* L.), angelica (*Angelica silvestris* L.) and a thick blanket of mosses. The locality of the third sample was exposed to the sun, and of higher plants we noticed white willow (*Salix alba* L.), elecampane (*Inula helenium* L.) and butterbur [*Petasites hybridus* (L.) G.Gaertn., B.Mey. & Scherb.]. The locality of the fourth sample was in the shade of beech trees.

Samples of red algae were collected from the rocky shores of the Drina River at two localities (Vrhpolje and Ljubovija).

Biofilm was collected from stones in the Cvetića Brook and the Bioštanska Banja Brook, tributaries of the Vruci Reservoir. Both brooks are located in the forest of a *Querceto-Fagetum* community.

**Physical and chemical analysis.** Transparency, water temperature and the pH level were estimated *in situ* with a Secchi disk, a thermometer and a PHTESTR 30 EUTECH pH meter, respectively. For water from the Dojkinačka River and Drina River, only temperature, transparency and pH data were taken. However, for water from the Bioštinska Banja Brook and Cvetića Brook, physical and chemical analyses of dissolved oxygen concentration and saturation, BOD<sub>5</sub> and concentrations of phosphate, nitrate, arsenic, mercury and nickel were carried out at the Institute for Development of Water Resources “Jaroslav Černi” according to standard methods (SMEWW 21<sup>st</sup>. 4500, SMEWW 21<sup>st</sup>. 5210, SRPS EN 12260: 2008, SMEWW 21<sup>st</sup>. 3120).

## RESULTS

The results of physical and chemical analyses of water from the sites where red algae were collected are given

in Table 1. The genera *Paralemanea* and *Lemanea* were distinguished on cross-sections using light-microscopy according to SHEATH (2003) and ELORANTA *et al.* (2011).

*Lemanea fluviatilis* (Linnaeus) C. Ag.

This species was found at four sites in the Dojkinačka River, at an altitude above 1000 m. Temperature of the water was between 12.5 and 18°C, and pH ranged from 7.0 to 8.0. *Lemanea fluviatilis* thalli were attached to stones.

Plants of *L. fluviatilis* were olive in colour; thalli were narrowed towards the base and transformed into a thin cylindrical stalk. The thalli were 8-13 cm long and nodes were fairly distinct (Fig. 2a). The spermatangial zone was substantially cylindrical with papillae measuring 500-600 µm in diameter. *Lemanea fluviatilis* had a uniaxial pseudoparenchymatous gametophyte thallus with internal carposporophytes. Carposporogonial branches developed all over the generative filaments. The carpospores were spherical, individual or arranged in rows (Fig. 2b).

*Paralemanea torulosa* (Roth) Sheath & A.R.Sherwood

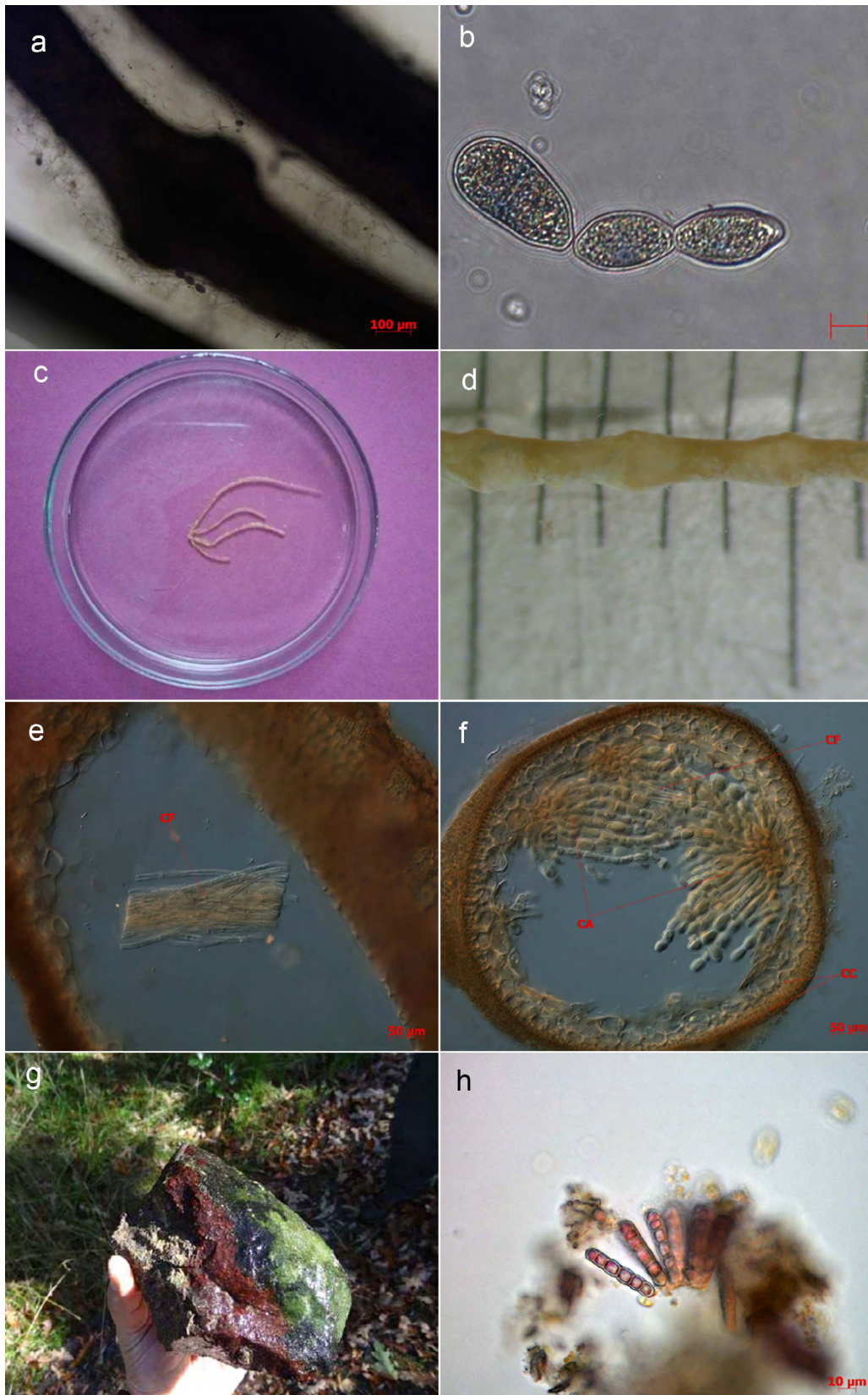
This species was found at two localities in the Drina River, at low altitudes (174 m and 182 m a.s.l.). The thallus was attached to the river bottom at a depth of 2.5 m, which may be associated with changes in the water-level because the Drina's flow is regulated by numerous dams. The bottom of the Drina River at the given localities was composed of small stones and gravel. Water temperature was 15°C. *Myriophyllum spicatum* L. and *Cladophora* Kütz. sp. were present along with *Paralemanea torulosa*.

Plants of *P. torulosa* were olive-green in colour, unstalked and unbranched, 5-8 cm long, 0.5-0.7 mm in diameter and with almost parallel internodes (Fig. 2c, d). Spermatangial zones formed broken rings. The thallus cross-section clearly showed the presence of cortical filaments (Fig. 2e). Axial filaments were surrounded by cortical filaments. Carposporophytes with carpospores are developed within the tubular thallus (Fig. 2f).

*Hildenbrandia rivularis* (Liebmann) J.Agardh

*Hildenbrandia rivularis* was recorded in shaded upland streams (645 m and 672 m a.s.l.). An intense red crust was noticed in places where the water overflows stones (Fig. 2g). The streams were cold (12.1 and 13.2°C) and oxygenated (9.53 and 9.52 mg/L). There was a relatively high nitrate concentration (1.83 mg/L) in the Bioštanska Banja Brook and a high content of heavy metals (arsenic and mercury) in the Cvetića Brook and Bioštanska Banja Brook.

The plants were bright crimson in colour, forming circular discs with a diameter of up to 2.5 cm. Neighbouring discs often fused into extensive crusts over the stones. The discs consisted of a basal layer composed of radiating rows and densely aggregated erect filaments (Fig. 2h). Erect filaments were simple or



**Fig. 2.** Species of red algae found from 2010 to 2014: a) *Lemanea fluviatilis*, node; b) *Lemanea fluviatilis*, carpospores in a row (scale bar 10  $\mu\text{m}$ ); c) thallus of *Paralemanea torulosa*; d) *Paralemanea torulosa*, nodes; e) *Paralemanea torulosa*, cortical filaments (CF); f) cross-section of *Paralemanea torulosa*, cortical cell layers (CC) and carposporophytes with carpospores (CA); g) *Hildenbrandia rivularis*, the red patches on stone; h) *Hildenbrandia rivularis*, side view of filaments (erect filaments).

rarely dichotomously branched and composed of almost isodiametric cells, 7–9 µm in diameter.

## DISCUSSION

According to SHEATH (2003), the most useful characteristics for identification of *Lemanea* and *Paralemanea* species are the organisation of nodal spermatangial sori and the presence or absence of cortical filaments and spermatangial rings. Readily discernible in Fig. 2e, the cortical filaments allowed us to distinguish clearly and separate the genus *Lemanea* from the genus *Paralemanea*. According to KUČERA & MARVAN (2004), *Lemanea* is characterised by the presence of a central axis with or without cortical filaments and by regular formation of spermatangia in rings that can be dependent on age. However, GANESAN *et al.* (2015) maintained that these attributes are not consistent. The generic status of *Paralemanea* as different from *Lemanea* has thus been under suspicion. Attempts by various authors to use some anatomical and morphological features to differentiate *Lemanea* and *Paralemanea* have not been definitive. Molecular analyses suggested that *Paralemanea* is a paraphyletic group. It is obvious that more molecular, morphological and ecological data are needed to make a clear distinction between the species of *Lemanea* and *Paralemanea* (GANESAN *et al.* 2015).

Species of the family Lemnaceae have been found to occur in clean streams, mainly in the Northern Hemisphere (VIS *et al.* 1992; ELORANTA *et al.* 2016), with a few records from the Southern Hemisphere (NECCHI & ZUCCHI 1995). The distribution of certain *Lemanea* species is insufficiently clear and precise, possibly as a consequence of a large number of synonyms mentioned in the literature (KUMANO 2002). According to GANESAN *et al.* (2015), certain authors incorrectly identified the species they found as *Lemanea fluviatilis*. In different countries of Southeast Europe, *L. fluviatilis* was the most often recorded species of freshwater red algae (SIMIĆ & RANKOVIĆ 1998; CARAUS 2002; SIMIĆ 2007).

Our study showed that the most favourable habitats for *L. fluviatilis* were in the cold, fast-running, neutral or slightly alkaline water of a mountain river (the Dojkinačka River) at an altitude of from 924 to 1015 m. However, this species has not only been found in mountain regions, but also at altitudes ranging from 305 to 888 m (KUČERA *et al.* 2008).

Previous investigations confirmed that *L. fluviatilis* requires a high concentration of dissolved oxygen (SHEATH 1984; SABATER *et al.* 1989; NECCHI *et al.* 1999). Many other authors have reported that this species prefers oligotrophic waters (ROTT *et al.* 1999; ELORANTA & KWADRANS 2007) and is sensitive to eutrophication (NEWMAN *et al.* 1997; AFNOR 2003; GUTOWSKI *et al.* 2004). However, there have been records of its presence in waters with moderate pollution (DELL'UOMO 1991).

Although nutrient data were not obtained in the present study, it can be stated that the Dojkinačka River is a clear river without anthropogenic or agricultural pressure. Results of some studies suggested that *L. fluviatilis* can tolerate a fairly wide range of temperature and oxygen values (ELORANTA & KWADRANS 2002; CESCHIN *et al.* 2013). As for electro-conductivity, some authors reported that this is a stenovalent species (VIS *et al.* 1992; SIMIĆ 2002), while others (ELORANTA & KWADRANS 1996) suggested that *L. fluviatilis* is very widely tolerant with respect to this physical parameter. Also, *L. fluviatilis* appeared to tolerate some heavy metal pollution (LIN & BLUM 1977). It is interesting that recent studies in Northern Europe (ELORANTA *et al.* 2016) showed that this species can be found across the whole of Sweden, in variable ecological conditions.

To date, *P. torulosa* has been recorded in Germany (LUDWIG & SCHNITTLER 1996), the Czech Republic (GARDAVSKÝ *et al.* 1995; KUČERA & MARVAN 2004), Romania (CARAUS 2002) and Turkey (AYSEL 2005) as *Lemanea torulosa*. In Belgium, the Czech Republic, France, Germany, Ireland and Switzerland (ELORANTA *et al.* 2011), as well as in Britain (WHITTON *et al.* 2003; ELORANTA *et al.* 2011; JOHN *et al.* 2011), Bulgaria (TEMNISKOVA *et al.* 2008) and Romania (CARAUS 2012), it was recorded as *Paralemanea torulosa*. As has already been mentioned, findings of this red alga in Serbia were documented in the Drina River, which is characterised by cold fast-flowing water with transparency equal to the water depth. Members of the genus *Lemanea* require a higher current velocity in comparison with those of the genus *Paralemanea* (MANNINO *et al.* 2003). Our data indicate that *P. torulosa* prefers fairly deep cold rivers with clean water.

*Hildenbrandia rivularis* is found worldwide, and its distribution has been confirmed by many authors (SIMIĆ 2008; CESCHIN *et al.* 2013). New findings of *H. rivularis* on the Balkan Peninsula have been reported from mountainous areas in Bosnia and Herzegovina, Macedonia and Bulgaria (SIMIĆ 2008); Slovenia (VRHOVŠEK *et al.* 2006); Romania (CARAUS 2012); and in the Dragovištica River near the town of Bosilegrad in Serbia (SIMIĆ 2008). There is evidence that *H. rivularis* persists over a wide range of nutrient concentrations in UK rivers (KELLY *et al.* 2015).

We found *H. rivularis* in streams with cold, highly oxygenated, slightly alkaline water with moderate velocity, where it was adherent to submerged stones in the shade. Its ecological preference for low light intensity has been confirmed (ELORANTA & KWADRANS 2004; CESCHIN *et al.* 2013). This species was found to prefer soft and alkaline water (SHEATH & SHERWOOD 2002; ELORANTA & KWADRANS 2004; ŽELAZNA-WIEZOREK & ZIUŁKIEWICZY 2008) with moderate conductivity (ROTT *et al.* 1999) where the variation of water temperature during the year was not higher than 10°C (STOYNEVA

*et al.* 2003). It has been recorded that the species can utilise carbon dioxide as a carbon source, but may also use bicarbonate (WEHR *et al.* 2015). According to our results, *H. rivularis* tolerates elevated heavy metal content in the water. The concentrations of these heavy metals in the streams where the species was found were far beyond the limits established for Class II surface waters (SGRS 50/2012). Due to recent rainfall in the Vruci Reservoir's catchment, there were slightly increased concentrations of phosphates and nitrates. Some authors considered this species to be a bioindicator of good water quality (DELL'UOMO 1991), in contrast to GUTOWSKI *et al.* (2004), who reported that it tolerates high levels of nitrates (NO<sub>3</sub>-N) (up to 12.5 mg/l), but is more sensitive to phosphate (P) enrichment [total phosphorus (TP) below 0.5 mg/l]. According to SCHMEDTJE *et al.* (1998), *H. rivularis* occurs in oligomesotrophic to eutrophic conditions, with the optimum in mesotrophic ones.

## CONCLUSION

This study gives a general overview of three representatives of the Rhodophyta group found in Serbia, viz., *Lemanea fluviatilis*, *Paralemanea torulosa* and *Hildenbrandia rivularis*. The species *P. torulosa* is here reported for the first time in Serbia. The habitats of species of the genus *Lemanea* and *Paralemanea* are mainly located in protected natural areas with limited human activities. In that sense, the presence and physiological condition of these organisms can serve as excellent indicators of a healthy environment, i.e., as potential bioindicators. The mechanisms of their adaptation in response to environmental impulses are poorly understood so far. Significant efforts in research on this group of algae are therefore needed, along with strict protection of their habitat.

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**Authors' contribution.** All authors contributed equally to the manuscript entitled "First record of *Paralemanea torulosa* (Roth) Sheath & A.R. Sherwood and new findings of *Lemanea fluviatilis* (Linnaeus) C. Ag. and *Hildenbrandia rivularis* (Liebmann) J. Agardh (Rhodophyta) in Serbia".

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## BOTANICA SERBICA



### REZIME

# Prvi nalaz *Paralemanea torulosa* (Roth) Sheath & A.R. Sherwood i novi nalazi *Lemanea fluviatilis* (Linnaeus) C. Ag. i *Hildenbrandia rivularis* (Liebmann) J. Agardh (Rhodophyta) u Srbiji

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Rad predstavlja prvi nalaz slatkovodne crvene alge *Paralemanea torulosa* i nove nalaze vrsta *Lemanea fluviatilis* i *Hildenbrandia rivularis* u Srbiji. Prisustvo sve tri vrste je bilo zabeleženo u gornjim delovima čistih, brzih reka i potoka, koji pripadaju dunavskom slivu. *Lemanea fluviatilis* je nađena u Dojkinačkoj reci (Istočna Srbija), *P. torulosa* je zabeležena u Drini, dok je *H. rivularis* nađena u Cvetića i Bioštanska Banja potoku (Zapadna Srbija). Ovi nalazi su značajni za zaštitu biodiverziteta Srbije, s obzirom da je dobro poznato da su mnoge vrste slatkovodnih crvenih algi ugrožene i retke vrste u više zemalja. U Srbiji su pod strogom zakonskom zaštitom.

**KLJUČNE REČI:** *Hildenbrandia*, *Lemanea*, *Paralemanea*, Rhodophyta, alge, Srbija

