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Morphology, distribution and ecology of the freshwater red algae *Paralemanea* (Batrachospermaceae, Batrachospermales, Rhodophyta) in Serbia

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Abstract: This paper describes the morphology, distribution and ecology of 15 populations of *Paralemanea* collected from 2004 to 2011 in 12 rivers in Serbia. On the basis of morphological and reproductive characteristics, two species were identified: *P. annulata* (12 populations) and *P. catenata* (3 populations). Morphological (presence of a stalk, thalli length, nodal diameter (ND), internodal diameter (ID), node and internode diameter ratio (ND:ID) and reproductive (arrangement of spermatangial sori, length and diameter of carpospores, presence of *Chantransia* stage) features described in the literature are generally confirmed in the populations from Serbia. True branching was observed in six populations of *P. annulata* in the gametophyte stage. False branching (whorled branching) occurred in five populations of both species observed. In the Pčinja (P2), Ibar (IB5) and Crnovrška rivers (CR10), the number of whorled branching was 6-11 (*P. annulata*). For *P. catenata* the number of such branching was 3-5 in the Nišava River (N8) and Sokobanjska Moravica River (SM12). False branching appears at damaged thalli, somewhat repairing it. Algae belonging to the *Paralemanea* genus were found at altitudes from 160 to 780 m (*P. annulata*), and from 240 to 400 m (*P. catenata*), at water temperatures ranging from 11.5 to 29°C (*P. annulata*) and from 12.6 to 17.4°C (*P. catenata*), in neutral and weakly alkaline waters, with a high level of oxygen concentration, with conductivity ranging from 70 to 433 μS/cm for *P. annulata*, and 260 to 440 μS /cm for *P. catenata*. It was also observed that *P. annulata* and *P. catenata* often grow in oligotrophic conditions and rarely in eutrophic conditions.

Key words: distribution; ecology; morphology; *Paralemanea*; Rhodophyta

INTRODUCTION

Algae belonging to the *Paralemanea* genus (Batrachospermaceae, Rhodophyta*)* are freshwater and macroscopic, thalli have a pseudoparenchymatous tube construction and interwoven medullary filaments are absent. The genus *Paralemanea* includes thalli that lack stalks [1]. It was placed in the family Lemaneaceae [2], but based on molecular data, Entwisle et al. [3] proposed a major revision of Batrachospermales and amended the circumscriptions of the family Batrachospermaceae to include Lemaneaceae and Psilosiphonaceae. The genus *Paralemanea* was retained pending further investigations, noting the possibility that *Paralemanea* is paraphyletic in relation to *Lemanea* [3-6]. According to Vis and Sheath [2] and Carmona and Necchi [7], in order to separate species within the genus *Paralemanea* the following morphological and diacritic characteristics were considered: length of thallus, width of node, width and shape of internode, branching, shape of spermatangial sori, position and the way of formation of carposporangia. Point of formation, height, length and width of *Chantransia* stage cells as well as the monospores formed in this stage are also considered [2,8-10]. Kučera and Marvan [9] described the diacritical features for distinguishing the different species within the genus *Lemanea*, and the positioning of the genus was done at the molecular level [3,11].

According to morphological and reproductive characteristics, two species of *Paralemanea* are actually recognized in Europe: *P. annulata* (Kützing) Vis et Sheath and *P. catenata* (Kützing) Vis et Sheath [10]. They were found in a small number of localities, mainly in central and southern Europe [12], Bulgaria [13,14], Italy [15], Czech Republic [9,11,16], Spain, the Iberian Peninsula [17] and Serbia [18,19]. The details of the findings in western Europe are limited. These species have been recently recorded in Belgium [20]. The presence of *P. annulata* and *P. catenata* in a few

localities with specific ecological conditions contributed to considering these algae as vulnerable (VU), and therefore including them in the Red List of algae in Bulgaria [14]. In Serbia, *P. annulata* is also marked as vulnerable (VU), and *P. catenata* as an endangered taxon (EN) (based on the data of its distribution, population densities and real and potential degree of habitat endangerment) [21].

In certain localities of Serbia, *Paralemanea* is reported only as a presence but without detailed data on its morphology and ecology [18,19]. The aim of this research was to contribute to the knowledge on the morphology, distribution and ecology of this red alga based on the analysis of 15 populations of *Paralemanea* found in rivers of Serbia.

MATERIALS AND METHODS

Collection of samples

Samples of *Paralemanea* were collected from 2004 to 2011 at 15 localities in 12 rivers of Serbia. Approximately 1/2 of the localities are in rivers under a certain degree of protection (Fig. 1, Table 1) [22]. The samples were collected from April to September (Table 1). Thalli of *Paralemanea* and other visible algal growths were collected from each locality. Each sampling locality consisted of a stream segment 10 m in length along the river bed. The segments were divided into five equal parts including the main stream microhabitats. At each locality, 25 mature thalli of *Paralemanea* and other microalgae were collected. All the collected *Paralemanea* thalli were measured. The percentage cover was evaluated according the methodology of Necchi and Moreira [23] and Ramírez-Rodríguez et al. [24] (Table 1). Samples of algae were instantly fixed in 4% formaldehyde. The preserved material was stored in the collection of the Department of Biology and Ecology in Kragujevac, Serbia.

Morphological characterization

In the laboratory, the following morphological characteristics were recorded: presence of a stalk, presence and incidence of branched thalli, thalli length, nodal diameter (ND), internodal diameter (ID), node and internode diameter ratio (ND:ID), presence of axial cortical filaments, arrangement of spermatangial sori,

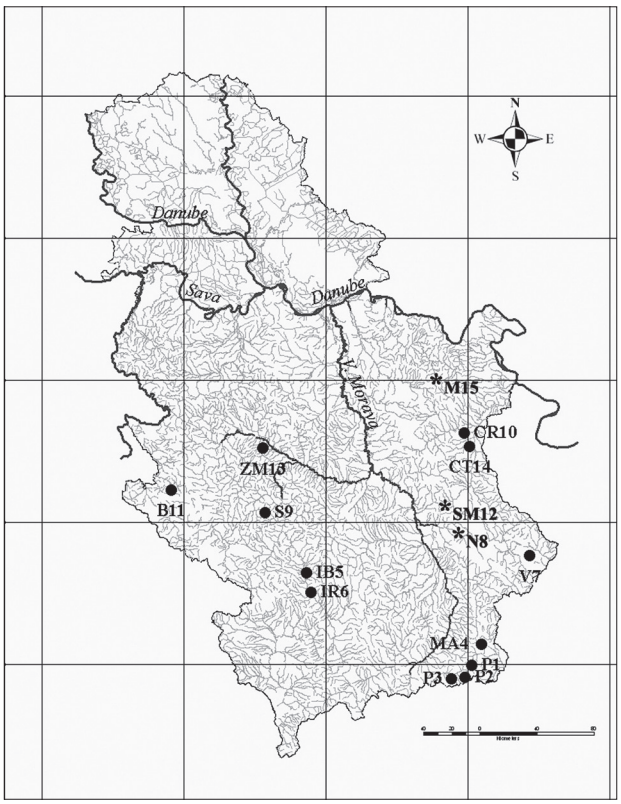

Fig. 1. Distribution of *Paralemanea annulata* and *P. catenata* in several rivers in Serbia. (•) *Paralemanea annulata*: River (population code): Pčinja (P1), Pčinja (P2), Pčinja (P3), Masurička River (MA4), Ibar (IB5), Ibar (IR6), Visočica (V7), Studenica (S9), Crnovrška River (CR10), Bučevka River (B11), Zapadna Morava (ZM13), Crni Timok (CT14). (*) *Paralemanea catenata*: River (population code): Nišava (N8), Sokobanjska Moravica (SM12), Mlava (M15).

length and diameter of carpospores, presence of *Chantransia* stage; branching of *Chantransia* (unilaterally or alternately) [2,8,10]. In addition, epiphytic and aquatic invertebrates were identified from each thallus in order for the species associations to be examined [25].

Environmental variables

The substratum type and water depth (cm) where thalli were present were determined at each location. The following environmental variables were measured for each site: temperature (°C), pH, oxygen concentration (mg/L), conductivity (μS/cm) and total hardness (dH) (measured with a digital conductometer of the HANA EP-3 type), ammonium ions, nitrates, phosphates (mg/L) [26].

Table 1. Geographical coordinates, physical and chemical characteristics, percentage cover of *Paralemanea annulata* and *P. catenata* (%) and associated species of macroalgae for each locality.

Species	River (population code)/Date	Locality	Protected areas*	Geographical coordinates	Altitude (m)	Temperature (°C)	pH	O_2 (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	Hardness (dH)	Cond.(µS/cm)	Percent coverage (%)	Associated macroalgae
Paralemanea annulata	Pčinja (P1) 13/07/2011	near the weekend place	LOQ	42° 20' 02" N 21° 54' 23" E	455	22.2	8.35	7.83	0.23	1.2	0.09	161	280	10	*Cladophora* sp. -
	Pčinja (P2) 12/07/2011	monastery	LOQ	42° 19' 95" N 21° 54' 04"E	447	23.0	8.76	9.10	/	/	/	100	260	50	*Cladophora* sp. -
	Pčinja (P3) 12/07/2011	border	LOQ	42° 18' 96" N 21° 53' 48"E	432	24.9	8.38	6.86	0.2	1.2	0.04	119	239	10	*Cladophora* sp. -
	Masurička River (MA4) 15/08/2004	Masurica	no	42° 38' 50" N 22 °10' 51"E	624	13.7	7.1	8.49	/	/	/	/	70	5	*Nostoc* sp. - *Lemanea* sp. -
	Ibar (IB5) 23/06/2011	Batrage	no	42°57' 85" N 20 °20' 88"E	780	19.3	9.0	10.94	0.35	1.8	1.17	158	317	10	*Vaucheria* sp.+
	Ibar (IR6) 9/08/2007	Ribariće	no	42°57' 67" N 20 °25' 98"E	699	17	8.9	/	/	/	/	162	331	20	*Cladophora* sp. + *Vaucheria* sp. +
	Visočica (V7) 12/08/2006	above accumulation Zavoj	NP	43°17' 59" N 22 °36' 41"E	612	15.2	8.56	13	/	/	/	/	98	80	*Cladophora* sp. +
	Studenica (S9) 09/2005	near monastery	BR	43° 29' 96" N 20° 32'12"E	490	11.5	7.46	/	/	/	/	/	150	10	*Cladophora* sp. + *Vaucheria* sp. -
	Crnovrška River (CR10) 08/2006	Crni vrh, waterfall	NP	43° 33' 21" N 22° 15' 26"E	700	12.0	7.7	/	/	/	/	/	100	5	*Nostoc* sp. -
	Bučevka River (B11) 04/2011	Goleša	no	43° 33' 29" N 19° 25' 76"E	422	11.7	8.16	9.58	0.12	0.6	0.15	136	274	1	-
	Zapadna Morava (ZM13) 7/07/2011	Talpe	no	43° 53' 54"N 20° 18' 41"E	259	22.6	9.23	8.13	/	/	/	161	318	1	-
	Crni Timok (CT14) 28/07/2004	below Gamzigradska Spa	no	43° 55' 05"N 22° 10' 10"E	160	29	7	8.98	/	/	/	/	433	20	*Vaucheria* sp. - *Cladophora* sp. -
Paralemanea catenata	Nišava (N8) 27/06/2005	Sićevo	NR	43°20' 06" N 22°05'19"E	240	17.4	8.05	10.4	0.31	15.1	5.5	213	400	20	*Cladophora* sp. +
	Sokobanjska oravica (SM12) 23/05/2004	Lepterija	NR	43° 39' 11"N 21° 52' 02"E	294	13.2	7.2	9.35	/	/	/	/	440	30	*Cladophora* sp. + *Nostoc* sp. -
	Mlava (M15) 8/06/2004	near the monestery Gornjak	no	44° 26' 58"N 21° 54' 30"E	400	12.6	7.6	9.04	/	/	/	/	260	70	*Cladophora* sp.+ *Vaucheria* sp. -

*Protected areas: LOQ-landscape of outstanding qualities; NP-nature parks, NR-nature reserve, BR-Biosphere reserve

+ Thalli of the macroalgae in the same sample with thalli *P. annulata* or *P. catenata*

- Thalli of the macroalgae not in the same sample with thalli *P. annulata* or *P. catenata*

RESULTS

Paralemanea annulata (Kützing) Vis et Sheath

Basionym: *Lemanea annulata* Kützing (M.D. Guiry in Guiry, M.D. & Guiry, G.M.)

Heterotypic synonyms: *Lemanea australis* Atkinson, *Lemanea grandis* Wolle (Carmona, Necchi)

Morphological analysis

Thalli in turfs (Figs. 2a-c and e; 4a). Uniaxial and pseudoparenchymatous gametophyte thallus, unstalked. The length of thallus was from 1.3 to 12 cm. Unbranching (Table 2, Fig. 2a) and true branching (first, second and third order of branching) were present (Table 2, Fig. 2c and d). In the populations recorded at the localities P2, IB5, CR10 and in the regenerated parts of the thallus at the point where the thallus was torn, a false branching (whorled branching) was observed. The number of such "branches" in the whorl was from 6 to 11 (Fig. 2e and f).

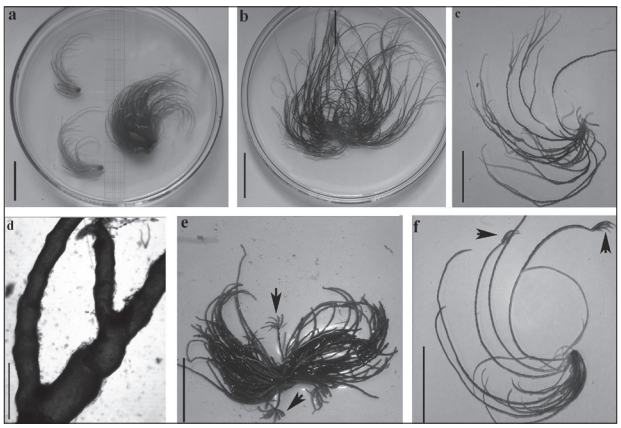

Fig. 2. Morphological features of *Paralemanea annulata*: General view (**a** – P1; **b** – IR6); True branching (**c** – IB5; **d** – IB5); whorled branching – arrows (**e** – CR10; **f** – P2); scale bars: 2 cm for plates a-c, e and f; 100 μm for plate d.

The nodal diameter (ND) was from 130 to 1200 μm, and the internodal diameter (ID) from 100 to 860 μm. Internodes rarely cylindrical, were mostly concave. Nodal/internodal diameter ratio (ND/ID) was from 1.1 to 1.6 (Table 2). In the middle of the thallus,

Table 2. Characteristics of populations of *Paralemanea annulata* and *P. catenata* from Serbia.

Species	River (population code)	Number of true branches / number of branches which start from the whorl*	Plant length (cm)		Nodal diameter (μm)		Internodal diameter (μm)		ND:ID	Spermatangial sori arrangement R-regular I-irregular		Carpospores (μm)				*Chantransia* a-alternately u-unilaterally	Hypertrophy on the cortex of thallus /Thalus covered limestone coatings
												Length		Diameter			
			min.	max.	min.	max.	min.	max.		R	I	min.	max.	min.	max.		
Paralemanea annulata	Pčinja (P1)	0/0*	2.2	5.4	250	600	200	400	1.3	+	+	25.0	41.0	13.0	22.0	-	no/no
	Pcinja (P2)	0-2/6*	4.4	8.9	130	980	100	690	1.2	+	+	29.0	47.0	13.0	26.0	-	no/no
	Pčinja (P3)	0/0*	1.4	6.0	320	620	280	480	1.2	+	+	29.7	39.6	16.5	26.4	-	yes/no
	Masurička River(MA4)	0/0*	1.4	9.0	500	740	400	640	1.1	+	-	26.4	33.3	16.5	26.4	+/a	yes/no
	Ibar (IB5)	0- 1-3/11*	3.7	8.1	400	700	270	400	1.5	-	+	-	-	-	-	+/a	no/no
	Ibar (IR6)	0-1-3/0*	5.7	12	410	500	250	315	1.6	-	+	22.0	30.0	12.0	21.0	+/a	no/yes
	Visočica (V7)	0-1/0*	2.0	8.0	580	790	250	600	1.6	+	+	-	-	-	-	+/a	yes/yes
	Studenica (S9)	0-1-3/0*	1.5	5.0	430	700	300	640	1.2	+	+	26.4	33.3	16.5	19.8	+/a	yes/no
	Crnovrška River (CR 10)	0/8-11*	1.8	8.0	600	1200	480	860	1.5	+	+	26.3	33.3	16.5	26.4	-	yes/no
	Bučevska River (B11)	0-1-4/0*	2.5	4.5	450	700	300	500	1.4	-	+	21.0	40.0	13.0	26.0	+/ u	no/no
	Zapadna Morava (ZM13)	0/0*	1.3	5.6	580	720	460	560	1.3	+	+	28.0	38.0	15.0	25.0	+/u	no/no
	Crni Timok (CT14)	0/0*	1.3	5.2	450	800	310	510	1.4	+	+	15.0	35.0	15.0	25.0	+/u	no/no
Paralemanea catenata	Nišava (N8)	0/3*	3.0	10	600	1000	400	800	1.5	-	+	29.7	49.5	13.2	26.4	-	no/yes
	Sokobanjska Moravica (SM12)	0/5*	6.5	21.0	740	1150	500	800	1.4	-	+	23.1	33.3	13.8	26.4	+/ a/u	no/yes
	Mlava (M15)	0/0*	3.5	17	520	1200	440	700	1.5	-	+	23.0	33.3	13.8	26.4	+/a/u	no/no

+ Present; - Not present

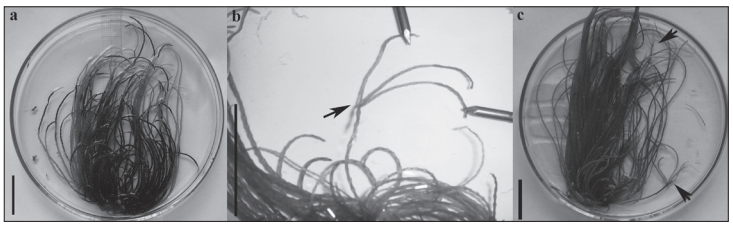

Fig. 3. Morphological features of *Paralemanea catenata*: General view (**a** – N8; **c** – SM12); whorled branching – arrows (**b** – N8; **c** – SM12); scale bars 2 cm.

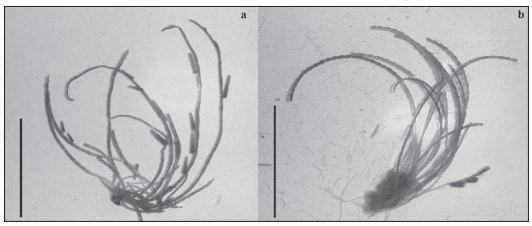

Fig. 4. Thallus of *Paralemanea* as microhabitat. **a** – Trichoptera (*Oligoplectrum maculatum*) on the thallus of *P. annulata* (P3). **b** – Diptera (*Simulium* sp.) on the thallus of *P. catenata* (M15); scale bar 2 cm.

the central axis (axial filament) was wrapped in cortical filaments. From them, radial cells start, consisting of two layers – the proximal does not touch the outer cortex, while the distal is generally Y-branched and attached to the cortex. The nodes have spermatangia, in spermatangial sori. These sori are regular or rarely irregular, and sometimes both regular and irregular sori are present in the same population (Table 2).

The carposporophyte zone was in the center of internode. Carposporangia were in chains (up to 13), oval or cylindrical, (length 15-47 μm, width 12-26.4 μm) (Table 2). Carposporangia were in chains, either branched or unbranched.

The *Chantransia* stage was present in the base. The *Chantransia* branched alternately or unilaterally. Monospores were observed (only in IR6 populations) in August (Table 2).

On the thalli collected in the localities P3, MA4, V7, S9 and CR10, hypertrophies of the cortex were present along the whole thallus in the form of papules with regular or irregular edges (Table 2).

Distribution and ecology

Thalli of *P. annulata* were collected from 12 localities in 9 rivers of central, western and eastern Serbia (Fig. 1, Table 1). In the Crnovrška River (CR10), the thalli were beneath a waterfall. They were found at altitudes from 160 to 780 m, in rivers whose width varied to over 20 m. The populations were found in fully sunlit localities, rarely partially shaded, always surrounded by deciduous vegetation. They were always found on rocky substrate (mostly bedrock), in places where the depth was max. 50 cm. They were found in waters with temperatures ranging from 11.5 to 29°C, pH from 7 to 9.23, well aerated (oxygen concentration from 6.86 to 13 mg/L), with concentration of nitrates from 0.12 to 0.35 mg/L. Phosphates were from 0.04 to 1.17 mg/L. Water hardness was from 119 to 162 dH, and conductivity was from 70 to 433 μS/cm) (Table 1).

The thalli coverage varied from 5 to 80% (Table 1). *Cladophora* sp., *Vaucheria* sp., *Nostoc* sp. and *Lemanea* sp. were often found in the localities along with the thalli of *P. annulata* (Table 1). Sometimes other macroalgae were found in the same samples with *P. annulata* (intertwined) but not always. Thalli collected on rocks in the water-air zone in localities IR6 and V7 were covered by grey-green coatings, 0.5 to 3-4 mm thick (Table 1). Coatings, consisting of lime clusters together with silicate shells of Bacillariophyceae and threads of Cyanobacteria, were observed. In the locality P3, some juvenile forms of *Oligoplectrum maculatum* (Trichoptera) were also found on the thalli (Fig. 4a).

Paralemanea catenata (Kützing) Vis et Sheath

Basionym: *Lemanea catenata* Kützing

Synonyms: *Lemanea catenata* Kützing; *Lemanea pleocarpa* Atkinson; *Lemanea nodosa* Kützing; *Paralemanea pleocarpa* (Atkinson) Vis & Sheath.

Morphological analysis

Thalli in turfs, rarely individually (Figs. 3a and c; 4b). Uniaxial and pseudoparenchymatous gametophyte thallus, unstalked. The length of the thallus was 3-21 cm. True branching was not present. Whorled branching in populations N8 and SM12 was observed (Fig. 3b, c). New "branches" in the whorl start from where

the thallus was torn. The number of such "branches" in the whorl was from 3 to 5 (Table 2, Fig. 3b, c).

The ND was from 520 to 1200 μm, and the ID from 400 to 800 μm. Internodes were mostly concave. The ND/ID ratio was from 1.4 to 1.5 (Table 2). In the middle of the thallus, the central axis (axial filament) was wrapped in cortical filaments. From them, radial cells start, consisting of two layers – the proximal does not touch the outer cortex, while the distal is generally Y-branched and attached to the cortex. The nodes have spermatangia, in spermatangial sori. These sori are irregular or rarely regular, and sometimes both regular and irregular sori are present in the same population.

The carposporophyte zone was in the center of internode. Carposporangia were in chains (up to 13), oval or cylindrical, (length 23-49.5 μm, width 13.2-26.4 μm) (Table 2). Carposporangia were in chains, either branched or unbranched.

The *Chantransia* stage was present in the base (SM12 and M15 populations), and occasionally along the thallus (SM12 population). The *Chantransia* branched alternately and/or unilaterally. Monospores were observed only in the SM12 populations in May (Table 2).

Distribution and ecology

Thalli of *P. catenata* were collected from 3 localities in 3 rivers of eastern Serbia (Fig. 1, Table 1). They were found at altitudes from 240 to 400 m, in rivers whose width varied over 20 m. They were always found on stable bases, large stones or rocks, in places where the depth was max. 50 cm. The populations were found in fully sunlit localities, rarely partially shaded, always surrounded by deciduous vegetation. They were found in waters with temperatures ranging from 12.6 to 17.4 °C, pH 7.2-8.05, well aerated (oxygen concentration from 9.04 to 10.4 mg/L), with a high concentration of biogenic elements in locality N8 (nitrates 15.1 mg/L and phosphate 5.5 mg/L). Water hardness was 213 dH, and conductivity was from 260 to 440 μS /cm) (Table 1).

The thalli coverage varied from 20 to 70% (Table 1). *Cladophora* sp., *Vaucheria* sp. *Nostoc* sp. were often found in the localities along with the thalli of *P. catenata* (Table 1). Some samples of *P. catenata* were intertwined with other macroalgae in the same sample. Thalli collected on rocks in the water-air zone in localities N8 and SM12 were covered in grey-green coatings, 0.5 to 1-2 mm thick (Table 1). Coatings, consisting of lime clusters together with silicate shells of Bacillariophyceae and Cyanobacteria, were observed. In the locality M15, some juvenile forms of *Simulium* sp. (Diptera) were also found on the thalli (Fig. 4b).

DISCUSSION

In the current literature, differences between the species *P. annulata* and *P. catenata* are based on diacritic characteristics, mainly the length and width of the thallus, branching, node and internode diameter ratio, the shape of spermatangial sori, the presence of *Chantransia* stage as well as the branching pattern [2,8,10,12. Based on morphological and molecular data, Kučera and Marvan [9] suggested the inclusion of *P. annulata* in *P. catenata*. The two basic differences given in the keys (length of thallus and shape of spermatangial sori), are considered to be unreliable. Kučera and Marvan [9] concluded that the height of the plant obviously depends on the size of the sample, season and environmental conditions, especially flow rate and illumination.

Our research confirms the difficulties in distinguishing *P. annulata* from *P. catenata* by using the descriptions available in the literature and the abovementioned characteristics. Indeed, only small differences in length, nodal diameter, internodal diameter, nodal/internodal diameter ratio, as well as in the shape of the node were observed. Kučera and Marvan [9] noticed a similar situation and explained it as being a result of the variation of the thallus age and the reproductive maturity.

Previous studies have suggested that the branching of thalli is an important characteristic in distinguishing *P. annulata* from *P. catenata* [2,7,9]. Vis and Sheath [2] consider both species unbranched. According to Carmona and Necchi [7], branching is not present or rarely present in *P. annulata*, while *P. catenata* is considered unbranched by Kučera and Marvan [9]. The examination of our material has shown that true branching was present only in the populations of *P. annulata*. An interesting phenomenon is the false

branching we found in both populations in places where the thallus was torn. A similar phenomenon has been described in *P. mexicana* as whorled branching and given as a characteristic, on the basis of which this species was differentiated as a separate species within the genus *Paralemanea* [2,7,8,27]. Carmona et al. [27] suggest that the frequently whorled branches are the results of a false branched pattern and are apparently exclusive to *P. mexicana.* The number and branching pattern are variable and correlated with environmental changes, especially with changes in current velocity.

Carmona et al. [27,28] suggested that the morphological differences are the result of adaptation to environmental conditions, especially to the rate of water. Previous studies conducted in Europe indicate that this alga was found in mountain rivers at altitudes of 1400 m in Italy [15], 700 m in Spain 17, as well as lowland-highland rivers of Czech Republic at altitudes from 214 to 497 m [9]. Based on analysis of the described environmental conditions, it is concluded that the prerequisites for the development of these algae are sunlit or partially shaded localities, a stable base, fast or moderately fast waters (higher than 35 cm/s), relatively small depth (1-60 cm), neutral or alkaline waters (pH 6.3-8.1), temperatures ranging from 5 to 24°C, with a high oxygen concentration and oxygen saturation (around 100% and more), low conductivity (under 300 μS/cm) [9,7,17]. The conditions suitable for the development of these algae in Serbia were found in both mountain/highland rivers and in physicochemical conditions similar to those described in the abovementioned studies. The only exception, up to a point, were the environmental conditions recorded in locality CT14, in the part of the Crni Timok River floating under a thermo-mineral spa (Gamzigradska Spa), with several springs of water whose temperature ranged from 38 to 43°C. At the altitude of 160 m (being the lowest altitude *P. annulata* has been recorded so far) the highest water temperature was measured (29°C), and the highest conductivity (over 400 μS/cm) was recorded. The populations of *P. catenata* in locality N8 and *P. annulata* in IB5 were found in eutrophic waters with high levels of biogenic elements. Recent data indicate that *P. catenata* appears with a high percent coverage and well-developed thalli in waters enriched by nutrients, namely mesotrophic and eutrophic waters [17].

The finding of slight morphological differences between *P. annulata* and *P. catenata*, the occurrence of branching and especially the phenomenon of false branching, indicate that morphological and reproductive characteristics are insufficient to distinguish between the two species. Therefore, the results of this study require further confirmation by molecular analyses.

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