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New data on the morphology, reproduction
and distribution of a freshwater brown alga
Porterinema fluviatile (Porter) Waern
(Phaeophyceae)

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New data on the morphology, reproduction and distribution of a freshwater brown alga *Porterinema fluviatile* (Porter) Waern (Phaeophyceae)

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ABSTRACT

This paper provides information on the morphology, reproduction and distribution of a freshwater brown alga, *Porterinema fluviatile* (Porter) Waern (Phaeophyceae). The alga was recorded in oligotrophic water from Mlava Spring, a karst limnocrone in eastern Serbia (44°11'298"N, 21°47'021"E). This is the first record of freshwater brown algae in general in Serbia, and the first record of *P. fluviatile* in southeastern Europe. The thallus of *P. fluviatile* formed small, dark-brown tufts, with multicellular, uniseriate filaments that were irregularly branched. An anastomosis was noticeable between some filaments. The vegetative cells of the young filaments were cylindrical (17-38 µm long × 2.5-4.0 µm wide). The cells had 1 or 2, rarely 3, plate-like parietal plastids. The reproductive organs of *P. fluviatile* (asexual reproduction) originate from the transformation of vegetative cells. Elongated vegetative cells develop inflated vegetative cells (5-10 µm in diameter) with lipid droplets. Further transformation of these cells leads to the formation of intercalary plurilocular sporangia with 4 to 8 (rarely 16) cells. Nonmotile, round aplanospores, which develop by protoplast division in elongated vegetative cells, are described for the first time in this species.

KEY WORDS

Freshwater,
Phaeophyceae,
lipid droplets,
plurilocular sporangia,
aplanospores.

RÉSUMÉ

Nouvelles données sur la morphologie, la reproduction et la distribution d'une algue brune d'eau douce Porterinema fluviatile (Porter) Waern (Phaeophyceae).

Cet article fournit des informations sur la morphologie, la reproduction et la distribution d'une algue brune d'eau douce, *Porterinema fluviatile* (Porter) Waern (Phaeophyceae). L'algue a été observée dans les eaux oligotrophes de la source Mlava, une source limnocène karstique de Serbie orientale (44°11'298"N, 21°47'021"E). Il s'agit du premier signalement d'algues brunes d'eau douce en général en Serbie et du premier signalement de *P. fluviatile* en Europe du Sud-Est. *Porterinema fluviatile* forme de petites touffes brun foncé, avec des filaments multicellulaires, unisériés, à ramifications irrégulières. Une anastomose est perceptible entre certains filaments. Les cellules végétatives des jeunes filaments sont cylindriques (17-38 µm de long × 2,5-4,0 µm de large). Les cellules présentent 1 ou 2, rarement 3, plastes pariétaux en forme de plaquettes. Les organes reproducteurs de *P. fluviatile* (reproduction asexuée) proviennent de la transformation de cellules végétatives. Les cellules végétatives allongées développent des cellules végétatives gonflées (5-10 µm de diamètre) avec des gouttelettes lipidiques. La transformation ultérieure de ces cellules entraîne la formation de sporanges pluriloculaires intercalaires de 4 à 8 (rarement 16) cellules. Des aplanospores rondes non mobiles, qui se développent par division protoplastique dans des cellules végétatives allongées, sont décrites pour la première fois chez cette espèce.

MOTS CLÉS

Eaux douces,
dulcaquicole,
Phéophycées,
gouttelettes lipidiques,
sporangies pluriloculaires,
aplanospores.

INTRODUCTION

Brown algae (Phaeophyceae) are characterized by a large diversity of their species, which are almost exclusively marine organisms. Only seven species, currently classified in six genera, occur in freshwater habitats: *Bodanella lauterborni* W.M. Zimmermann, *Ectocarpus siliculosus* (Dillwyn) Lyngbye, *Heribaudiella fluviatilis* (Areschoug) Svedelius, *Pleurocladia lacustris* A. Braun, *Porterinema fluviatile* (Porter) Waern, *Sphacelaria fluviatilis* C.-C. Jao and *S. lacustris* Schloesser & Blum (van den Hoek *et al.* 1995; Eloranta *et al.* 2011; Wehr 2016). Other than in freshwaters, *E. siliculosus*, *P. lacustris* and *P. fluviatile* may colonize brackish and marine habitats (Waern 1952; Jaasund 1965; Wehr 2015).

According to the available literature, *P. fluviatile* has been recorded in marine waters, estuaries, and several putative freshwater habitats (Wehr 2015). The species is distributed across a small number of locations in Europe (Austria: Geitler 1970; Kusel-Fetzmann 2014; England: Belcher 1959; Germany: Porter 1894; McCauley & Wehr 2007; Sweden: Waern 1952; Norway: Jaasund 1965; Netherlands: Dop & Vroman 1976; Dop 1979) and North America (Wilce *et al.* 1970; Rhodes 1972). The majority of these records are from brackish-water habitats (Wilce *et al.* 1970; Wehr 2016).

Porterinema fluviatile is the only member of the monotypic genus *Porterinema* (Eloranta *et al.* 2011), which is taxonomically enigmatic, as it has been insufficiently explored and has several synonymies, making this a problematic genus (Wehr 2015). The species was described originally as *Streblonema fluviatile* (Porter 1894). In 1946 Waern collected a very similar alga from coastal waters in the Öregrund archipelago and identified it as *Porterinema fluviatile* (Waern 1952). Waern compared his finding with Porter's description and concluded that these two specimens were the same species (Waern 1952). Belcher (1959) also collected a similar alga, identified as

Apistonema pyrenigerum, which Dop (1979) regarded to be a synonym for *P. fluviatile*. Jaasund (1965) collected a marine specimen of *Porterinema* from the upper sublittoral of Lakesfjord in northern Norway, naming it a new species, *P. marina*, separated from *P. fluviatile* on the basis of its morphological characteristics and marine habitat. Jaasund (1965) established *P. marina* on the strength of the filaments which were more uniform and did not exhibit variation in size, similar to those of *P. fluviatile*. As the species was found inside of marine algae *Punctaria plantaginea* (Roth) Greville and *Dictyosiphon chordaria* Areschoug, Jaasund (1965) considered the species to be different from species already identified. Wilce *et al.* (1970) and Rhodes (1972) regard these species as conspecifics, with *P. marina* as a synonym of *P. fluviatile*. Report of the new genus *Pseudobodanella* Gerloff in Europe (Gerloff 1967), which was done by the holotype species *Pseudobodanella peterfi* Gerloff, was also regarded as identical to *P. fluviatile* (Wehr 2015). Even after Waern (1952) had unified several of these taxa, Geitler (1970) collected the alga from Neusiedler Sea and identified it as *Apistonema expansum*. Subsequently, Dop (1979) and Bourrelly (1981) both suggested the earlier findings of *A. pyrenigerum* (Belcher 1959), *A. expansum* (Geitler 1970) and *P. marina* (Jaasund 1965) to be synonyms of *P. fluviatile*.

The classification of *P. fluviatile* is also problematic. When first described, it was classified in the order Ralfsiales (Porter 1894). According to the terminal unilocular sporangia resembling those of *Lithoderma* and *Sorapion*, Waern (1952) classified it in the family Lithodermataceae. Tanaka & Chihara (1981) suggested that *P. fluviatile* does not belong to Lithodermataceae or Ralfsiales on the basis of its unique plurilocular sporangia, thalli composed only from creeping filaments attached to the substratum, and the lack of clearly erect filaments. According to a recent molecular analysis (Kusel-Fetzmann & Schagerl 1992; McCauley & Wehr 2007) *P. fluviatile* is considered to be “*insertae sedis*” in the class Phaeophyceae.

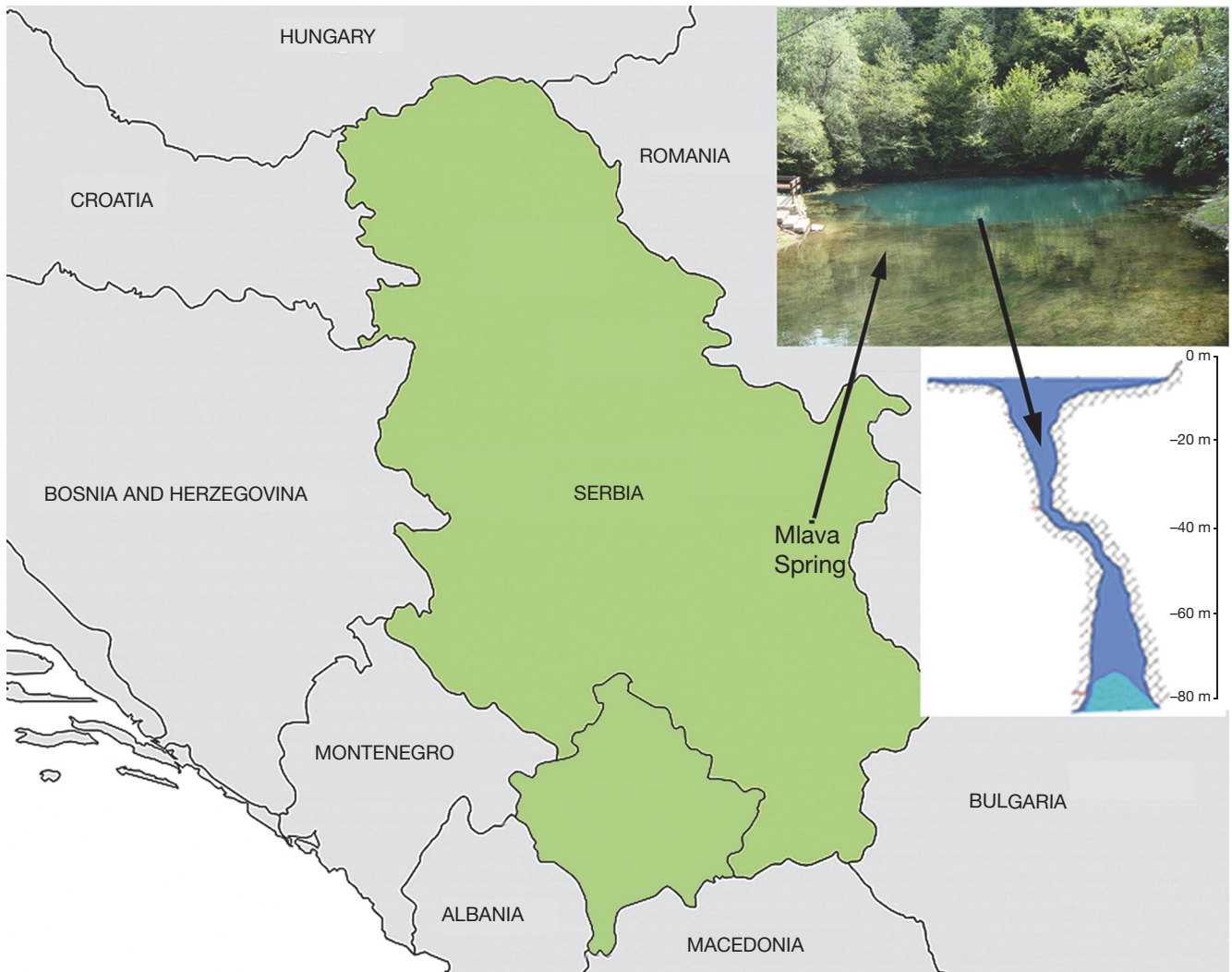


FIG. 1. — Study area map. Location of the karst limnocrene Mlava Spring and cross section through the siphonal channel.

Because of the limited and occasionally contradictory information on this species, the goal of this study was to provide new information on the morphology, reproduction and distribution of *P. fluviatile*, based on a newly-discovered population from a freshwater karst spring in eastern Serbia.

MATERIAL AND METHODS

Algal material was collected in karst limnocrene Mlava Spring, located in eastern Serbia (44°11'298"N, 21°47'021"E), southeastern Europe (Fig. 1), at an altitude of 314 m. This spring receives water from the karst aquifer of the calcareous massif of the Beljanica Mountains, with a diluted system of underground canals. Water reaches the surface through an inlet siphonal channel from a depth of more than 80 m (Kojadinović 2013).

This research was carried out in October 2014, August and October 2015 and in October 2016, 2017 and 2018. The samples of water were collected from a siphonal channel at

a depth of 5, 10 and 20 m (Ruttner bottle, 2 L), a spring lake (plankton net, macrophytes) and part of the brook near the spring (scraping from rocks, pipette along the sand surface, tear off the thalli from the substrate with tweezers, macrophytes). The samples were fixed with 4% formaldehyde solution and they are stored at the Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia.

The morphological characteristics of the algal material were analyzed under a Motic BA310 microscope with a magnification of up to 800-1000× and photographed with a Bresser (9MP) and MicroCamLab. The macroalgal tufts were identified according to Eloranta *et al.* (2011) and Wehr (2015) while the diatom and cyanobacterial samples were identified according to Krammer & Lange-Bertalot (1991a, b) and Komárek & Anagnostidis (2005), respectively.

The physical and chemical parameters of the water were measured in the field according to the American Public Health Association (1995), while the bicarbonate and carbonate concentrations (mg L⁻¹) were measured at the Institute of Public Health in Kragujevac.

TABLE 1. — Comparative review of the morphological and reproductive features of *Porterinema fluviatile* (Porter) Waern.

Characteristics of species	Sample from Mlava Spring (Serbia)	Eloranta <i>et al.</i> 2011	Wehr 2015
Thalli	Brown and dark in colour; loosely arranged, irregularly branched filaments; anastomoses are noticeable; axial cells of filaments are elongated, gradually narrowing to the top	Creeping thalli form loose discs of radiating branched filaments; short erect filaments	Thalli are monostromatic, brown disc-shaped plates or loosely arranged, spreading filaments; creeping, irregularly branched uniseriate filaments, with short erect filaments, produced infrequently
Cellular hairs	not present	Multicellular, with basal growth; 3–8 µm wide, up to 200 µm long and sheathed at the base	Multicellular; 3–8 µm wide, 100–200 µm long; may be sheathed at their base
Cylindrical vegetative cells	2.5–4.0 µm width	no data	no data
Inflated vegetative cells	17–38 µm length		
Plastids	5–10 µm in diameter	6–8 µm in diameter	6–12 µm in diameter
Pyrenoids	1–2 (rarely 3) plate-like, parietal	2 (occasionally 3) plate-like	1 to 3 lobed, golden-brown, parietal chloroplasts
Sporangia	not observed	only visible using a transmission electron microscope	pyrenoids obscure; visible via electron microscopy
Zoospores	Intercalary plurilocular sporangia; 4–8 (rarely 16) cells per sporangia; sessile or on unicellular pedicels; unilocular sporangia were not observed	Pear-shaped unilocular sporangia; plurilocular sporangia intercalary, sessile, on one or two pedicels, or terminal	Plurilocular sporangia, intercalary (occasionally terminal); occasionally in four-celled clusters or “crowns” on pedicels or sessile; sometimes produced in clusters of up to 32 sporangia; unilocular sporangia were rare
Aplanospores	not observed	present	present
	Develops by protoplast division of elongated vegetative cells; 2–7 round aplanospores per cell; mature aplanospores 5.0–6.5 µm in diameter	not observed	not observed

RESULTS

MORPHOLOGY AND REPRODUCTION OF SPECIMENS

The macroscopic thalli of *P. fluviatile* were dark-brown, up to 3 mm in size, composed of loosely arranged, irregularly branched filaments (Table 1). The filaments were composed of elongate vegetative cells (17–38 µm long × 2.5–4.0 µm wide) (Fig. 2A; Table 1). An anastomosis was noticeable between some filaments (Fig. 2A; Table 1). The vegetative cells had 1–2 (rarely 3) plate-like parietal chromatophores (Fig. 2B; Table 1).

The vegetative cells transform into reproductive organs by shortening and spreading the elongated vegetative cells (Fig. 2C, D). The cell wall later thickens, while within the cells there is an accumulation of cellular content and gradual formation of a transverse partition (12–25 µm long × 4.0–7.5 µm wide; 2.5–4.5 µm wide in narrow sections) (Fig. 2D). From this, two new inflated (oval-shaped) vegetative cells (5–10 µm in diameter) with a large number of lipid droplets (Fig. 3A, B; Table 1) develop from the original elongated vegetative cell. Further transformation of these newly formed, inflated vegetative cells leads to the formation of dark, roughly oval cells with thick walls (5.5–8.0 µm long × 5.0–7.5 µm wide) (Fig. 3A, B). The intercalary plurilocular sporangia (12.5–39.0 µm long × 10–17 µm wide) (Fig. 3C, D) develop by means of the division of these cells. The plurilocular sporangia may be constructed from

4–8 (rarely 16) cells (Table 1). Cells inside the plurilocular sporangia are usually 5–10 µm in diameter (Fig. 3E). The plurilocular sporangia may be sessile (Table 1) or on unicellular pedicels (Fig. 3E; Table 1).

The axial cells of the filaments are elongated, gradually narrowing to the top (Fig. 3A, B; Table 1). In most cells comprising the apical filaments, the protoplast is divided into two to seven sections (Table 1). On each of these sections, a cell wall is formed while they are developing in a vegetative cell (Fig. 4A, B). This is how nonmotile aplanospores (Fig. 4C) are formed. Aplanospores are round, 2–3 µm in diameter while they are contained within a vegetative cell; they are released by rupturing of the cell wall. The diameter of the released aplanospores is 2.5–3.5 µm (Fig. 4C). Mature aplanospores, from which the new thallus develops, are 5.0–6.5 µm in diameter (Fig. 4D; Table 1).

DISTRIBUTION AND ECOLOGY

Porterinema fluviatile was found in a submerged siphonal channel of the freshwater Mlava Spring at a depth of 20 m in October 2014. However, during a research conducted in August and October 2015 and October 2016, 2017 and 2018 thalli of *P. fluviatile* was not found.

The physical and chemical parameters of water are presented in Table 2. In the sample of collected water, diatom species *Cyclotella ocellata* Pantocsek, *Gomphonema* sp., *Melosira varians* C. Agardh, and *Nitzschia dissipata* (Kützinger) Raben-

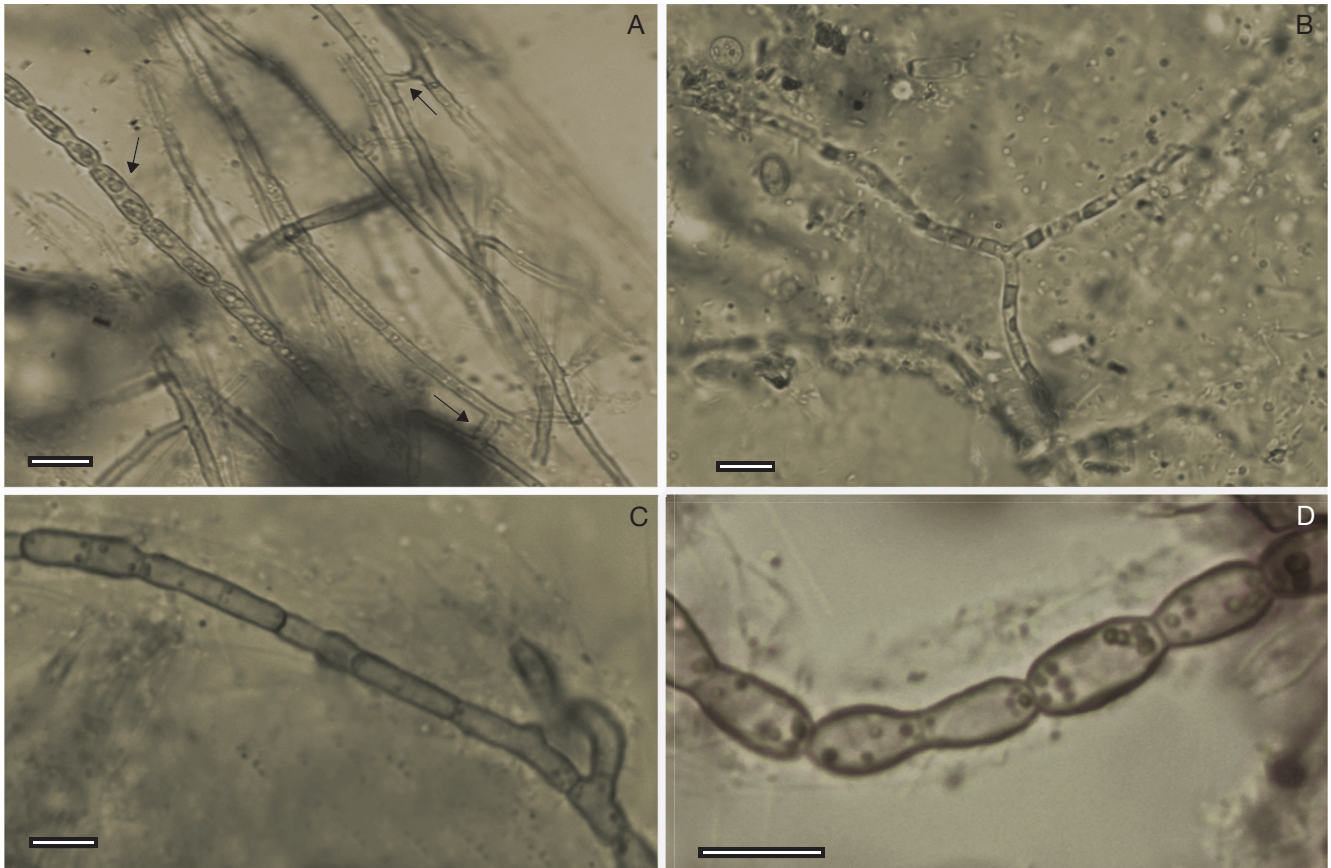


FIG. 2. — **A**, Young filaments composed from elongated vegetative cells with an anastomosis (→) between their filaments. Filament with elongated vegetative cells in the transformation process (→); **B**, young filaments composed from elongated vegetative cells with plastids; **C**, **D**, first phase of the transformation of elongated vegetative cells to inflated vegetative cells. Scale bars: 10 μm.

horst were also recorded. The cyanobacterial species *Romeria leopoliensis* (Raciborski) Koczwara (Fig. 5) develops as an epiphyte on some parts of the *P. fluviatile* thalli.

DISCUSSION

Data on the morphology, reproduction, ecology and distribution of *P. fluviatile* are very scarce and incomplete because there have only been a small number of collections (Wehr 2016).

The thalli of *P. fluviatile* collected from the Mlava Spring were composed of loosely arranged, irregularly branched filaments. An anastomosis was noticeable between some of the filaments (Table 1). Cellular hairs described by Eloranta *et al.* (2011) and Wehr (2015) (Table 1) were not recorded in the present material, but the axial cells of the filaments were elongated, gradually narrowing to the top (Fig. 3A, B; Table 1). Previous studies did not record the shape or dimensions of the young vegetative cells, but only the larger, inflated vegetative cells (Porter 1894; Waern 1952; Rautenberg 1960; Jaasund 1965; Reith 1969; Geitler 1970; Wilce *et al.* 1970; Dop 1979, Eloranta *et al.* 2011; Wehr 2015). Previous studies describe the number of plastids as

1 to 2 per cell (rarely 3), which are plate-like or lobed, also described by (Eloranta *et al.* 2011; Wehr 2015) (Table 1). No pyrenoids were noticed in our material. According to Eloranta *et al.* (2011) and Wehr (2015), pyrenoids are visible only via an electronic microscope (Table 1). The same authors described inflated vegetative cells 6–8 μm or 6–12 μm in diameter. In our material, the inflated cells were more or less oval, but similar in size, 5–12 μm in diameter (Table 1).

According to available data, the reproduction of *P. fluviatile* is asexual, with biflagellate zoospores produced in plurilocular, rarely unilocular, sporangia. It is suggested that critical identification of *P. fluviatile* is based on its unique four-celled (crown-shaped) sporangia (Porter 1894; Waern 1952; Rautenberg 1960; Jaasund 1965; Reith 1969; Geitler 1970; Wilce *et al.* 1970; Dop 1979). In material from Mlava Spring, only intercalary plurilocular sporangia were observed, which were composed of 4–8 (rarely 16) cells. More than four cells in the plurilocular sporangia were also observed by Dop (1979) and Kusel-Fetzmann (2014). Dop (1979) suggested that there are two types of quite different plurilocular sporangia: the intercalary type, which develops by the partition of cells whose cell wall eventually surrounds the whole sporangia, and the crown-shaped terminal type that occurs as a result of cleavage of the cells, whereby four

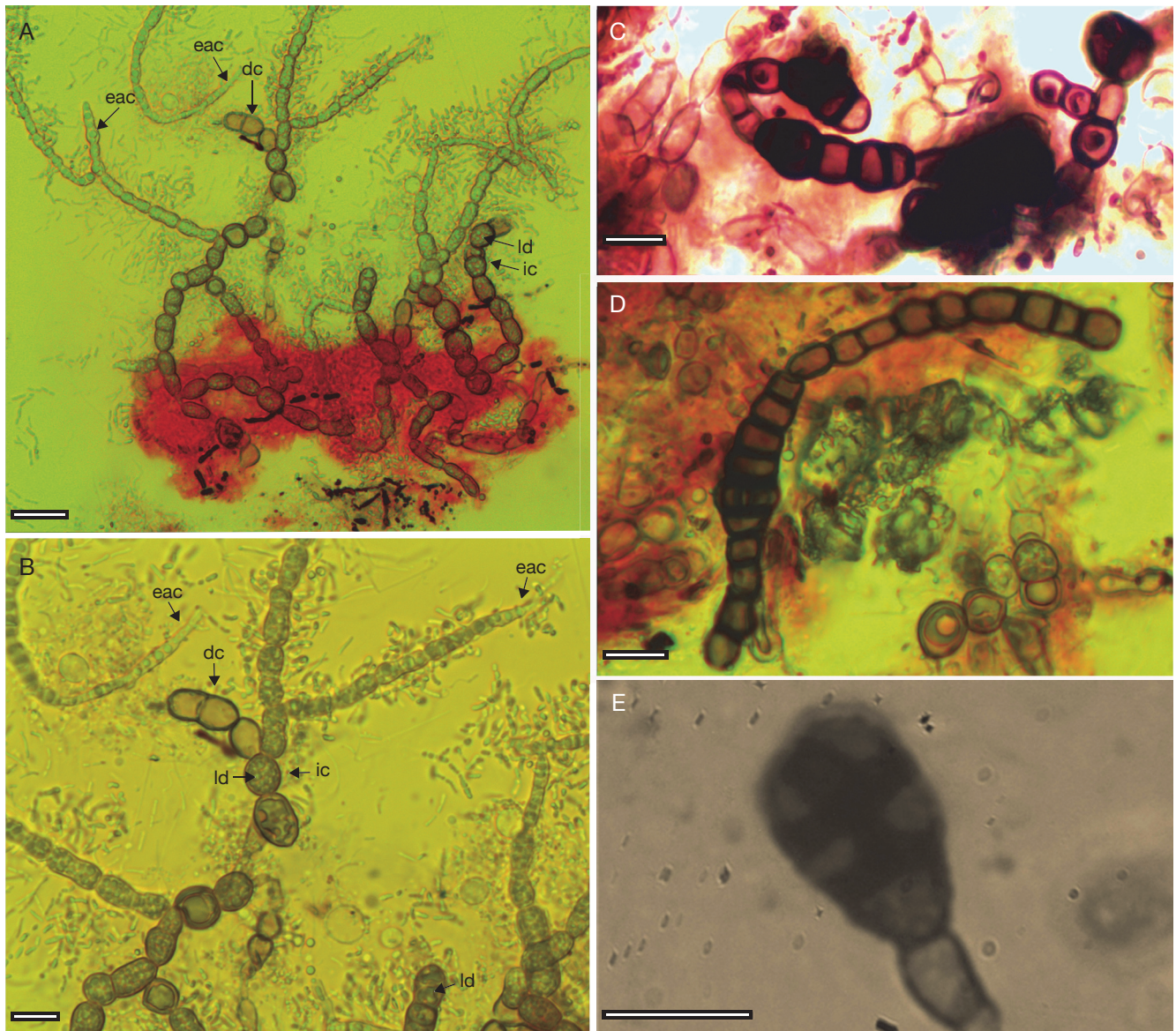


FIG. 3. — **A, B**, Newly formed inflated vegetative cells (**ic**) with thick walls and a large number of lipid droplets (**ld**), formation of the approximately oval dark cells (**dc**) with thick walls, elongated axial cells (**eac**) of filaments, gradually narrowing to the top; **C, D**, developing intercalary plurilocular sporangia; **E**, intercalary plurilocular sporangia on the pedicels. Scale bars: 10 µm.

loosely connected spores occur on a common pedicel. In the material from the Mlava Spring, no crown-shaped terminal plurilocular sporangia were observed, while intercalary sporangia were abundant (Table 1). Dop (1979) suggested that elevated chloride concentration affects the growth, thalli structure and production of sporangia, but not their formation. According to Wehr (2016) plurilocular sporangia develop in a large number while unilocular sporangia are rare. In our material, unilocular sporangia, as well as zoospores, were not observed (Table 1).

In the material from the Mlava Spring, we recorded the division of the protoplast into several parts and the forming of immobile unicellular aplanospores in the axial cells of the filaments. The number of aplanospores is two to seven per cell (Table 1). The reproduction of *P. fluviatile* with nonmotile

aplanospores has not yet been described. In the filamentous brown alga *Sphacelaria*, asexual propagation without flagellated spores may occur, and a new thallus can develop from multicellular triradiate propagules after they are released from the thallus (Eloranta *et al.* 2011).

Data on the geographical distribution and ecology of *P. fluviatile* are very scarce. This species has been collected from approximately 14 localities in Europe and North America, usually in brackish waters, and rarely from marine or freshwater habitats (Wehr 2015). Our collection of *P. fluviatile* represents the first occurrence of this species in Serbia, as well as the first record in southeastern Europe. It has been observed that *P. fluviatile* occurs in channels (Rhodes 1972), streams (Gerloff 1967), ditches (Dop & Vroman 1976; McCauley & Wehr 2007), ponds (Dop & Vroman 1976),

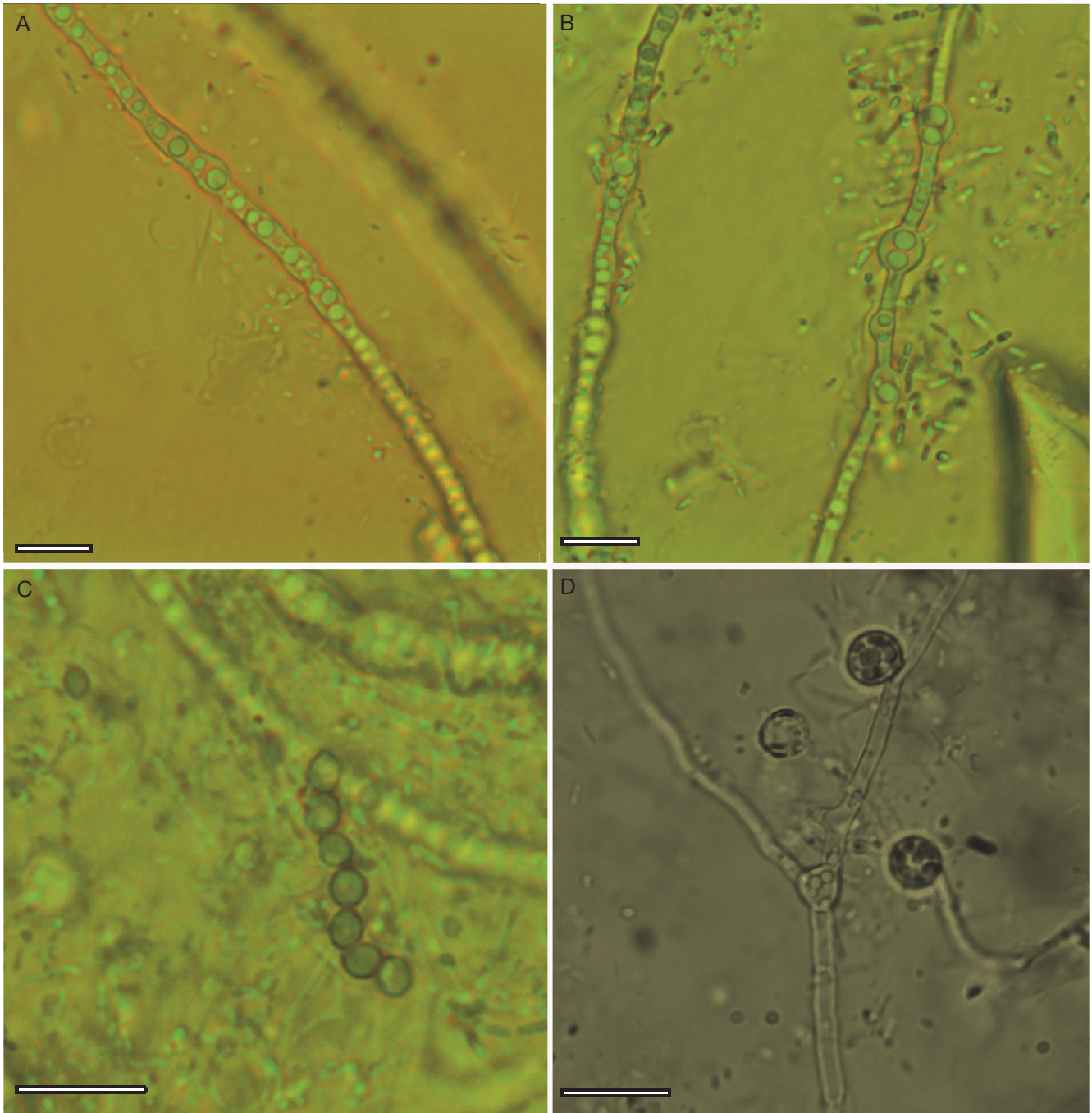


FIG. 4. — **A, B**, Protoplast division into several parts in apical filament cells resulting in the formation of aplanospores; **C**, nonmotile aplanospores; **D**, new thalli developing from aplanospores. Scale bars: 10 µm.

gravel pits (Belcher 1959), marshes (Wilce *et al.* 1970; Rhodes 1972), lakes (Gerloff 1967; Geitler 1970; Kusel-Fetzmann 2014), fjords (Jaasund 1965), estuaries (Porter 1894; Wilce *et al.* 1970) and marine habitats (Waern 1952). According to Porter (1894), Waern (1952), Belcher (1959), Jaasund (1965), Gerloff (1967), Geitler (1970), Wilce *et al.* (1970), Rhodes (1972), Dop & Vroman (1976), Dop (1979) and Kusel-Fetzmann (2014) *P. fluviatile* is a benthic alga which develops as epiphytic, endophytic, epilithic or epizoic. Finding

the *P. fluviatile* in a plankton sample is unusual. As the species is considered to be a benthic alga, we suppose that it was cut off from the rocky substrate of the siphonous channel walls. Another theory is that it was brought by ground water from another habitat, which presumably ties in with the fact that we did not find it in our later research and that we found a very small amount of the material.

Based on the first finding of *P. fluviatile* by Waern in 1952, the species was considered an exclusively brackish

TABLE 2. — The physical and chemical parameters of water from the Mlava Spring in October 2014 at a depth of 20 m.

Parameter	Mlava Spring (20 m depth)
Temperature (°C)	11.30
Conductivity ($\mu\text{S cm}^{-1}$)	400
pH	8.39
Dissolved oxygen (mg L^{-1})	9.64
Oxygen saturation (%)	91.7
Nitrate (mg N L^{-1})	< 0.02
Nitrite (mg N L^{-1})	< 0.01
Ammonium (mg N L^{-1})	< 0.02
Phosphate (mg P L^{-1})	0.19
Secchi depth (transparency, m)	3
Bicarbonate (HCO_3) (mg L^{-1})	234.8
Carbonate (CO_3) (mg L^{-1})	0

species, which was a logical assumption at that time. Subsequent findings in freshwater and marine habitats indicate that *P. fluviatile* is extremely tolerant to salinity, since it was found in different salinity conditions (0 to 35.5 ‰ in natural habitats) (Eloranta *et al.* 2011; Wehr 2016). The karst limnocrone Mlava Spring is clearly non-saline, which compared to previous data supports the fact that *P. fluviatile* has a wide ecological valence in regard to salinity (Dop 1979). Freshwater members of the Phaeophyceae class tend to occur in neutral to slightly alkaline systems and span oligotrophic to eutrophic waters, but they are not tolerant to organic pollution (Eloranta *et al.* 2011). In our investigation, the species was found in oligotrophic, slightly alkaline and well aerated water, rich with soluble ionizing substances (Table 2). According to Kusel-Fetzmann (2014) *P. fluviatile* inhabits eutrophic water.

CONCLUSION

This study represents a significant contribution to the knowledge of the morphology and especially the reproduction of *P. fluviatile*. The asexual reproduction and transformation of vegetative cells into plurilocular sporangia is described in detail. Also, asexual reproduction with nonmotile aplanospores is described for the first time for this species. Subsequent molecular analysis of the material could contribute to the clearer phylogenetic position of this species. Our record is the first record of freshwater brown algae in general in Serbia, and accordingly the first record of *P. fluviatile* in southeastern Europe.

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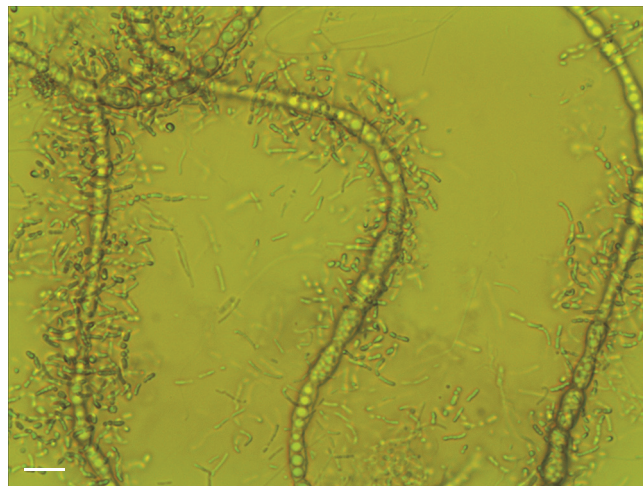


FIG. 5. — *Romeria leopoliensis* (Raciborski) Koczwara (Cyanobacteria), an epiphyte on *Porterinema fluviatile* (Porter) Waern. Scale bar: 10 μm .

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