Botanica SERBICA 43 (1): (2019) 97-102
*Original Scientific Report*

# Rediscovery of *Chara canescens* Loiseleur in Serbia

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**ABSTRACT**: *Chara canescens* is the only charophyte performing parthenogenetic reproduction. Although most recently found populations consist solely of parthenogenetically reproducing females, bisexual populations exist as well, making the species a unique example of the sympatric occurrence of both reproductive modes. In Serbia, *C. canescens* was found only once for certain, near Prokuplje in 2005. The purpose of the present study is to report a reliable new finding of *C. canescens* in Serbia, in a stable parthenogenetic female population. Sampling was conducted on 7 July 2018 from the Plava Banja pond near the city of Kikinda. Results of water analyses revealed high concentrations of almost all measured parameters, but especially high levels of salinity and alkalinity markers. Very high concentrations of sulphates and chlorides in the Plava Banja pond pointed to extreme ion anomalies. On the basis of the concentration of nutrients in it, the Plava Banja pond can be characterised as a eutrophic water body. *Chara* specimens collected from this pond were identified as *C. canescens*, and only females in the reproductive phase were detected. The environmental features of this habitat are typical in relation to preferences of the given species for light and salinity (ion concentration). This record is of great importance because *C. canescens* has been declared to be probably *extinct in the wild* in Serbia.

**Keywords**: charophyte, parthenogenesis, Kikinda, Plava Banja pond

Received: 05 February 2019 Revision accepted: 08 March 2019

UDC: 582.263.3:581.163: 581.95 (497.11 Kikinda)
DOI: https://doi.org/10.2298/BOTSERB1901097T

*Chara canescens* Loiseleur (Charales, Charophyceae) is a small to medium-sized „brush-like" charophyte. Being the only European species of the genus with a haplostichous cortex, it is easily recognisable (Krause 1997; Mouronval *et al.* 2015; Urbaniak & Gąbka 2015; Calero & Rodrigo 2018). A very unique peculiarity of *C. canescens* within the genus *Chara* is the occurrence of parthenogenesis, which, moreover, is just an option to sexual reproduction (Krause 1997). However, bisexual populations are rare and most of the populations known in Europe consist of parthenogetic female individuals exclusively (Küster *et al.* 2004; Schaible *et al.* 2011). In a parthenogenetic population of *C. canescens*, asexual reproduction is obligatory and irreversible; specimens cannot reproduce vegetatively, but only by oospore germination. Being an annual species, growth is fast and reproduction relies on semelparity for sexual as well as parthenogenetic individuals (Blindow & Schubert 2004; Schaible *et al.* 2012). Extant bisexual populations are extremely rare and limited to a few localities in Europe – Austria (the border area between Austria and Hungary) and Spain (Cirujano *et al.* 2008; Schaible & Schubert 2008; Schaible *et al.* 2011). An extinct bisexual population is also well documented by herbarium material collected close to Montpellier in France (Corillion 1957, 1975).

With respect to its ecology, the species *C. canescens* can be described as being "heliophilic", typical of shallow (< 1 m) brackish water habitats (with salinity in the range of from 1.5 to 21 g/L), indifferent toward nutrient content and able to tolerate and resist extreme ion anomalies (Blindow & Schubert 2004; Küster *et al.* 2004; Schubert *et al.* 2017). *Chara canescens* is typically distributed throughout the Northern Hemisphere, but it

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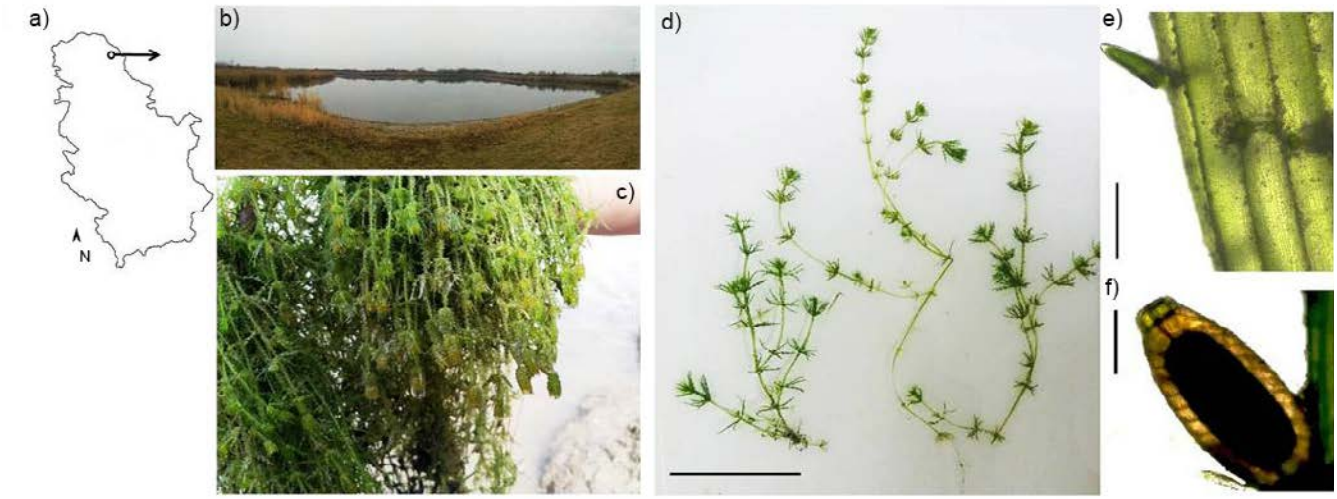


**Fig. 1.** a) Location of the study area in Serbia, b) the Plava Banja pond, c) *Chara canescens* on a rake, d) habitus of *C. canescens* (scale bar 5 cm), e) haplostichous cortex of *C. canescens* with solitary and twinned spines (scale bar 200 μm), f) oogonia (scale bar 200 μm). Photographs by D. Predojević.

has also been recorded in Australia (Casanova & Nikol 2009). Despite the relatively wide range of the species, populations of *C. canescens* are isolated, and it is considered to be threatened/endangered and rare (Casanova & Nikol 2009; Korsch *et al.* 2013).

The only record of *C. canescens* in Serbia documented by herbarium material is from a pond near Prokuplje in 2005; subsequently, Blaženčić (2014) considered this species to be probably *extinct in the wild* [EW(?)] according to the IUCN criteria because afterwards it could not be found in Prokuplje or anywhere else in Serbia. Published records of *C. canescens* exist for the Vrbas-Bezdan Canal (near Mali Stapar) in the Vojvodina province (Stojanović *et al.* 1994). However, because of the lack of herbarium material available for revision and habitat conditions different from the niche requirements reported so far for the species, Vesić *et al.* (2016) expressed doubts about this. After Stojanović *et al.* (1994), *C. canescens* has never again been found at the mentioned locality or elsewhere in the Vojvodina province (Vesić *et al.* 2016).

The purpose of the present study is to report a reliable finding of *C. canescens* in an abundant and healthy parthenogenetic female population near the city of Kikinda in the Vojvodina province, which significantly contributes to knowledge about the distribution of this species in Serbia and Europe, and also enables us to update its threat status in Serbia.

Near the city of Kikinda (Vojvodina province, Serbia) (Fig. 1a), there are several excavated ponds that were made in the process of clay digging and subsequently abandoned by a local company (Toza Marković, Kikinda). One of them is the popular city recreation area and swimming place called the Plava Banja pond (Fig. 1b). After the process of exploitation was completed, part of the shore was covered with gravel to make a beach, while more than half of the shoreline remained untouched and gradually became overgrown with reed. Thus, only a small part of the bottom of this aquatic ecosystem is pebbled, while most of the bottom is clay. For the last 10 years, the Plava Banja pond has not been used for swimming, primarily due to the high concentration of sulphates and chlorides. The main use of this ecosystem today is as a source of drinking water for cattle, whose presence unquestionably represents an additional source of nutrients for this shallow water ecosystem.

The Plava Banja pond was visited on 7 July 2018. Macrophytes were sampled from the region of the shoreline (up to a depth of 1 m) using a rake and grapnels. Except for a fragmented population of *Phragmites australis* (Cav.) Trin. ex Steud. along the shore, no other emergent vegetation was detected. *Zannichellia palustris* L. (Cook *et al.* 1974) was noticed growing together with charophytes. Charophyte samples were placed in plastic bags, sealed and transported to the laboratory of the Department of Algology, Mycology and Lichenology of the Institute of Botany and Botanical Garden "Jevremovac" (Faculty of Biology, University of Belgrade), where material was identified according to the standard literature (Wood & Imahori 1965; Gollerbah & Krasavina 1983; Krause 1997; Mouronval *et al.* 2015; Urbaniak & Gabka 2015). Following its identification, the material was deposited in the collection of Charophyta at the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade (BEOU).

Results of water chemistry analyses were kindly provided by the Institute of Public Health in the city of Kikinda. Water samples were taken on 11 July 2018. Water quality parameters were compared to the limit-

**Table 1.** Environmental parameters measured in the Plava Banja pond in July of 2018. **Bold** - measured values which exceed the limit of class V water quality for surface waters (Službeni glasnik RS, 50/2012).

| Parameter | Unit | Measured value | Limit of class V water quality for surface waters |
|---|---|---|---|
| Ph | | **9.4** | >8.5 |
| Total hardness | ° dH | 72.3 | |
| Conductivity | μS/cm | **8060** | >3000 |
| Total dissolved solids | mg/l | **6132** | >1500 |
| Bicarbonates $HCO_3^-$ | mg/l | 747.9 | |
| Carbonates $CO_3^{2-}$ | mg/l | 397.2 | |
| Sulphates $SO_4^{2-}$ | mg/l | **3590** | >300 |
| Chlorides $Cl^-$ | mg/l | **445.4** | >250 |
| TN | mg/l | 0.9 | >15 |
| TP | mg/l | 0.03 | >1 |

ing values for pollutants in surface water according to Serbian legislation (Službeni glasnik RS 50/2012). The values of Carlson's trophic indices based on total phosphorus [TSI (TP)] and total nitrogen [TSI (TN)] were calculated and used to characterise the trophic status of the Plava Banja pond (Carlson & Simpson 1996).

The quality of water in the Plava Banja pond was declared to be unsuitable for swimming by the Institute of Public Health in the city of Kikinda in June of 2018 (http://www.zavodki.org.rs). Our results confirmed that in July of 2018, the value of pH, concentrations of chloride and sulphate ions, total content of dissolved solids and conductivity still exceeded the limits for class V water quality in the Plava Banja pond, which seems to be the permanent state of conditions in it (Table 1). The measured parameters indicate a bad ecological potential of the water, which means that it cannot be recommended for any use (Službeni glasnik RS 50/2012). According to the values of nutrient content measured in our study (Table 1), this pond can be classified as a eutrophic water body [Carlson's trophic indices were: TSI (TP) = 53 and TSI (TN) = 53] (Carlson & Simpson 1996).

After careful examination, *Chara* specimens from the Plava Banja pond were identified as *C. canescens* (Fig. 1c). Specimens were small to medium-sized, about 10-15 cm in height (Fig. 1d). In the whole sample (around 50 specimens collected), no male plants were detected, so the rediscovered population can be characterised as a parthenogenetic female population. Plants were bright-green and not incrusted (Fig. 1d). Typical morphological characteristics of *C. canescens* were documented (Fig. 1e, f). Axis diameter was 0.5 – 1 mm, and 6-9 branchlets could be observed in a whorl. A haplostich cortex was clearly distinguishable (Fig. 1e), with numerous spine cells, solitary or twinned, acute and variable in length (Fig. 1e). Stipulodes were well developed, arranged in two rows, acute. Since only female specimens were detected, only well developed oogonia were documented (Fig. 1f). Gametangia were solitary, had the form of a long ellipse (650-700 μm long, 330-390 wide) and showed 11-14 convolutions, with the short coronula about 70 μm long (Fig. 1f). Oospores were dark-brown to black. Morphological characteristics of the *C. canescens* specimens found in the Plava Banja pond are in agreement with published data (Wood & Imahori 1965; Gollerbah & Krasavina 1983; Krause 1997; Mouronval *et al.* 2015; Urbaniak & Gabka 2015; Schubert *et al.* 2016), confirming uniformity of morphological traits in populations of this species in Europe. According to Schubert *et al.* (2016), plants first appear in early spring (March-April), while oospore maturation takes place in the period of July-August, after which the plants die. The period when we found *C. canescens* perfectly fits into this template. Still, the ecology of this species is extremely interesting and should be further investigated, since its phenology differs depending on the seasonal environment (Calero & Rodrigo 2018). The opportunistic nature of the species (its status as an annual) is challenged by Calero & Rodrigo (2018), as well as by Blindow & Schubert (2004) and Mouronval *et al.* (2015), who confirmed the existence of overwintering individuals. Mild temperatures during winter and autumn favour overwintering phenomena and more than one reproduction cycle in *C. canescens* (Calero & Rodrigo 2018). This is an interesting issue that needs to be explored in the Plava Banja

pond in the future, considering evident recent changes in the typical continental climate of Serbia (Vuković *et al.* 2018).

As suggested by Schubert *et al.* (2017), our results in general confirm that *C. canescens* indeed resists extreme ion anomalies, specifically toward very high sulphate and chloride ion concentrations such as were detected in the Plava Banja pond (Table 1), in contrast to other submerged macrophyte species (only *Z. palustris* was discovered growing together with *C. canescens* in this pond). Indifference of *C. canescens* toward nutrient content (Blindow & Schubert 2004) is also confirmed by our results, which indicated that the Plava Banja pond is a eutrophic water body. The stated tolerance of *C. canescens* toward the ecosystem´s trophic status (Blindow & Schubert 2004) can be discussed in terms of inability of the vascular flora and/or periphyton and phytoplankton to develop abundantly under conditions of high ion concentrations. In our study, no diverse or abundant emergent or submergent flora was detected, *C. canescens* plants were not overgrown by periphyton, and preliminary analyses pointed to low biomass of phytoplankton in the Plava Banja pond (Predojević, personal communication). In the same context, the "heliophilic" behaviour of *C. canescens* (Blindow & Schubert 2004), can be explained as its adaptation to high irradiance due to restriction to sites lacking competitors for light. Considering the presence of several more waterbodies of the same origin as the Plava Banja pond near the city of Kikinda and many more in the wider area of the Vojvodina province, we emphasise that the distribution of *C. canescens* should be carefully investigated. Such mapping and monitoring should be conducted especially in order to check for the existence of sexually reproducing populations such as have been observed at sites with a comparable climatic background (Corillion 1957, 1975; Cirujano *et al.* 2008; Schaible *et al.* 2011). Possible confirmation of male specimens would make such a population one of very few in Europe (Schaible *et al.* 2011).

This record is of great importance since the species in question is considered to be almost *extinct in the wild* in Serbia. Further investigations on the distribution of *C. canescens* could also be of significance for designing potential (and necessary) plans for the conservation of charophytes in Serbia.

**Acknowledgements** – This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. OI 176020) and by the Rufford Foundation (funds were made available through a Rufford small grant for realisation of the project „Guardians of the Fragile Equilibrium in Shallow Ecosystems of Ramsar Sites in Serbia: Stonewort Diversity and Distribution"). The authors give special thanks to the administration of the city of Kikinda; to the Institute of Public Health in the city of Kikinda; and especially to Dr. Sanja Brusin Beloš for providing data of chemical analyses of water in the Plava Banja pond. Many thanks to Dr. Jasmina Šinžar Sekulić for help in the process of identification of vascular macrophytes. The authors would also like to thank Mr. Nebojša Krištof for technical and field support. In addition, they greatly appreciate the patience, support and expertise of Milica Petrović Đurić in the task of charophyte identification. Finally, we acknowledge the helpful comments on the manuscript offered by two anonymous reviewers.

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Botanica SERBICA

**REZIME**

# Ponovno otkriće vrste *Chara canescens* Loiseleur u Srbiji

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*Chara canescens* je jedina vrsta pršljenčica kod koje je poznata partenogenetska reprodukcija. Iako je većina populacija ove vrste sačinjena samo od partenogenetskih ženki, postoje i dvopolne populacije, te se kod ove vrste beleži i jedinstven primer simpatričkog pojavljivanja oba reproduktivna oblika. U Srbiji je do sada zabeležen samo jedan pouzdan nalaz vrste *C. canescens* i to u blizini Prokuplja 2005. godine. Cilj ove studije je da se opiše novi nalaz stabilne, ženske, partenogenetske populacije *C. canescens* u Srbiji. Uzorkovanje je izvršeno 7. jula 2018. godine na jezeru Plava Banja (napušteni kop) u blizini Kikinde. Rezultati hemijskih analiza ukazali su na visoke vrednosti gotovo svih izmerenih parametara, pri čemu se izdvajaju indikatori saliniteta i alkaliteta. Izuzetno visoke koncentracije sulfata i hlorida ukazale su na ozbiljne jonske anomalije u ovom vodnom telu. Na osnovu koncentracije nutrijenata, jezero Plava Banja je okarakterisano kao eutrofno vodno telo. Pršljenčice sakupljene iz ovog napuštenog kopa identifikovane su kao *C. canescens* i pri tom su zabeležene samo ženske jedinke u reproduktivnoj fazi. Zabeležene karakteristike staništa u potpunosti odgovaraju ekološkim preferencama ove vrste, u pogledu svetlosti i saliniteta (koncentracije jona). Ovaj nalaz je od velike važnosti imajući u vidu da je *C. canescens* opisana kao *izumrla u divljini* u Srbiji.

**Ključne reči**: harofita, partenogeneza, Kikinda, Plava Banja