



Rediscovery of the endemic species *Chara rohlenae* Vilh. 1912 (Characeae) - believed extinct - on the Balkan Peninsula

Jelena BLAŽENČIĆ* and Branka STEVANOVIĆ

University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden „Jevremovac“, Takovska 43, 11000 Belgrade, Serbia

ABSTRACT: The species *Chara rohlenae* was described more than a hundred years ago (in 1912) as a species new to science on the basis of herbarium specimens collected from the Mratinje locality in Montenegro. In addition, there were some other herbarium specimens of this charophyte originating from Greece (collected in 1885) and also ones from Bosnia and Herzegovina (collected in 1925), which, however, were taxonomically determined in different ways and not clearly identified as belonging to the species *C. rohlenae*. For such a long period of time thereafter, no new data on the presence of the given species in the Balkans were recorded, and for this reason the species was considered to be extinct (EX glob ?) in accordance with IUCN criteria. However, during botanical surveys conducted in 2010 and 2012, *C. rohlenae* was rediscovered on the Balkan Peninsula, in the Mokra Gora Mountain (a spur of the Prokletije massif) in Serbia. This finding confirms existence of the species in the wild. Morphological characteristics of the newly found specimens of *C. rohlenae* from Serbia are investigated in the present study.

KEYWORDS: Charophyta, new records, endemic species, *Chara rohlenae*

Received: 6 April 2017

Revision accepted: 16 August 2017

UDC: 497:582.2.271
DOI: 10.5281/zenodo.1173564

INTRODUCTION

The species *Chara rohlenae* was first described by J. VILHELM in 1912 based on his analysis of specimens collected by J. Rohlena at the beginning of 20th century in Montenegro. He named this new plant species after its collector. The specimens were gathered in the Mratinje stream at the foot of Mt. Maglič. This *locus classicus* in Montenegro is presently flooded by a reservoir servicing the Mratinje hydroelectric power plant.

The taxonomic status of this charophyte should be re-examined and revised if necessary. Until recently, relevant morphological characters have been considered sufficient for the recognition and identification of charophyte species. If this assumption is accepted, *C. rohlenae* should be regarded as a good species, judging from its detailed description by Vilhelm in 1912, which was based

on the plant material collected in Montenegro. However, a review of the subsequently published relevant charological literature clearly indicates that the taxonomic status of *C. rohlenae* has been differently interpreted and understood by various researchers in the past and still is. Thus, twenty years after Vilhelm's identification of this new species of charophyte, FILARSZKY (1931) relegated the species *C. rohlenae* to the rank of a form as *C. gymnophylla* f. *rohlenae* (Vilh.) N. Filarszky on the basis of herbarium material collected by T. Heldreich in Greece in 1885 and by K. Maly in Bosnia and Herzegovina in 1925. This taxonomic status was also accepted by TORTIĆ NJEGOVAN (1956). Subsequently, WOOD (1962) changed the name and rank of this taxon from *C. gymnophylla* f. *rohlenae* (J. Vilhelm.) N. Filarszky to *Chara vulgaris* var. *gymnophylla* f. *rohlenae* (J. Vilhelm) R.D.Wood. Recently, LANGANGEN (2005-2006) and BLAŽENČIĆ *et*

*correspondence: jelenablazencic@gmail.com

al. (2006a, b) restored the original rank of this arguable taxon as determined by Vilhelm by recognising the species name *C. rohlenae* Vilhelm. In view of such different opinions, it is clear that only an integrated study taking into account morphological variability, phenological changes, and molecular findings will help us to arrive at a precise determination of the taxonomic status of this charophyte. In any event, investigations carried out so far have always been performed on herbarium specimens of plants, whether collected in Montenegro, Greece, or Bosnia and Herzegovina. At present, it appears reasonable enough to consider this charophyte as being endemic to the Balkans, since its presence at localities other than ones on the Balkan Peninsula has not been reported as yet.

Meanwhile, the species was declared and classified as extinct (EX glob ?) in accordance with IUCN criteria, since the relevant data are rather old, and new confirmed findings have been lacking for more than 80 years (BLAŽENČIĆ *et al.* 2006a). However, during field surveys in the summers of 2010 and 2012, the species was rediscovered on the Balkan Peninsula and found for the first time in Serbia, where it was collected by the young Serbian botanist P. Lazarević in a puddle in the Mokra Gora Mountain at an altitude of 1600 m a.s.l. Detailed analyses of morphological features and ecological requirements of the discovered specimens support the opinion that this taxon is still present in the wild and indicate that a new estimation of its threatened status is needed.

In the present study, morphological features of *C. rohlenae* from Serbia are analysed for the first time using fresh specimens. The aim of this study was to obtain relevant results on fresh plant material and compare them with earlier herbarium data. This might confirm the permanent presence of the given charophyte on the Balkan Peninsula and contribute to elucidation of its taxonomic status as a good species.

MATERIAL AND METHODS

Specimens of *C. rohlenae* were collected in a small mountain puddle at the Ponor locality in the Mokra Gora Mountain, the northern spur of the Prokletije massif in Serbia, at an altitude of 1600 m during the summers of 2010 and 2012. The shallow puddle, on a limestone substratum, was formed by water from a nearby spring situated in a forest clearing (a former forest community of *Picea abies* and *Pinus peuce*) (Fig. 1). The plants of *C. rohlenae* grew in water 20 to 30 cm deep, forming a dense carpet-like cover together with the species *C. contraria* var. *nitelloides* A. Braun, which was dominant in this algal stand. The largest part of the charophyte's thallus was in the water, whereas the thallus tips were above the water.

Our analyses of morphological characteristics of *C. rohlenae* pertained to plant size, cortex structure, ar-

rangement of stipulodes, number and characteristics of branchlets, and form and size of oogonia, oospores, and antheridia. Measurements of morphological parameters were carried out using our own fresh plant material. Seventeen specimens were included in the analyses of gametangia, from which about 35-40 oospores and 10-15 antheridia were measured.

After measurements, the specimens were preserved in 4% formalin solution and deposited in the charological collection (col. No 2312 from 2010 and col. No. 2319 from 2012) of the herbarium of the Institute of Botany and Botanical Garden "Jevremovac" (BEOU), Faculty of Biology, University of Belgrade. In our experience, plant material in formaldehyde retains its elasticity and natural colours for a longer period of time, which makes it more suitable for further morphological and anatomical analyses. We thoroughly washed the material in running water before performing lab analyses.

RESULTS AND DISCUSSION

The species *C. rohlenae* is considered to be a Balkan endemic charophyte based on the fact that the oldest, earlier, and most recent specimens were all collected only in some part of the Balkan Peninsula. The localities where the plant has been recorded in Montenegro, Bosnia and Herzegovina, Greece, and Serbia are presented on the map of its distribution shown in Fig. 2. At all these sites, specimens of *C. rohlenae* were found in shallow waters of mountain springs, puddles, or ponds.

Morphological analyses carried out on fresh specimens of *C. rohlenae* collected in Serbia (in the Mokra Gora Mountain) showed that the plant is monoecious and olive-green in colour, small, up to 4 cm high, heavily encrusted, forming a compact and richly branched shrub-like habit (Fig. 3). The axes are slender, 500-550 μm in diameter with the internodes 0.6-0.8 cm long; their length shortens progressively toward the top. They are about as long as branchlets or shorter than them. The axial cortex is irregular, tylacanthous to isostichous, rarely aulacanthous. Spine cells are solitary, small and papilliform, 55-60 μm long and 50 μm wide. Stipulodes are short and arranged in two rows; cells in the upper row are larger (up to 110 x 70 μm) than those in the lower one (up to 60 x 50 μm). The branchlets are completely ecorticated, being 8-9 in the node, up to 1.0 cm long with 2-3 segments. The branchlets in the lower nodes are simple, reduced to a single segment. The terminal segment of branchlets consists of mucronate end cells and inflated allantoid cells below them (Fig. 4). The mucronate end cells are very short, curved, and claw-shaped (Fig. 4). The two bract-cells and two bracteoles are as long as the oogonium or a little longer. Gametangia are conjoined at the lowest 1-2 branchlet nodes. On branchlet nodes there are 1-2 oogonia, 650-700 μm long and 380-400 μm wide, with 12-13 convolutions. The convoluting cells of



Fig. 1. View of a forest clearing in the Mokra Gora Mountain with a puddle inhabited by *C. rohlenae* and *C. contraria* var. *nitteloides* (photo by Predrag Lazarević).

the oogonium envelope below the coronula are bubble-shaped. The coronula is 80-95 μm long and 170-175 μm wide. Oospores are golden-brown, 450-480 μm tall and 270-290 μm wide, with 11 ridges. The oospore membrane is light-brown, diffusely spotted (Fig. 5). The sparse antheridia are 270-280 μm in diameter.

Almost identical findings concerning the general appearance of a low, bushy, highly encrusted thallus, as well as similar measurements of morphological structures, were reported for *C. rohlenae* in the publications of authors (VILHELM 1912; FILARSZKY 1931; TORTIĆ- NJEGOVAN 1956; WOOD & IMAHORI 1964, 1965; LANGANGEN 2005-2006) who studied herbarium specimens of this charophyte collected at different localities on the Balkan Peninsula (Montenegro, Greece, and Bosnia and Herzegovina). We also had the opportunity to analyse and measure herbarium specimens from Greece collected as far back as the end of the 19th century, which were sent to us by A. Langangen with the request to give our opinion as to the taxonomic status of the species *C. rohlenae*.

From the description of the locality in Greece that was provided with the specimens collected, it can be concluded that this habitat was similar to the habitat where specimens of the given charophyte were discovered in Serbia. To be specific, *C. rohlenae* in Greece was found in spring water or puddles formed around mountain springs, mostly at altitudes above 1500 m. Sometimes this species was also found at altitudes of less than 1000 m, as at the localities in Montenegro (at the foot of Mt. Maglič), as well as those in Bosnia and Herzegovina (in Koševo near Sarajevo). Some of those previously studied localities no longer exist in their original form, being dried up (Koševo near Sarajevo) or flooded (the Mratinje hydroelectric power plant in Montenegro), whereas the locality in Greece where the species was found in 1885 has never been verified again. However, given that the species was up to now found in small water basins in difficultly accessible high mountain regions, it seems possible that new localities of this charophyte will be discovered on the Balkan Peninsula in the future.

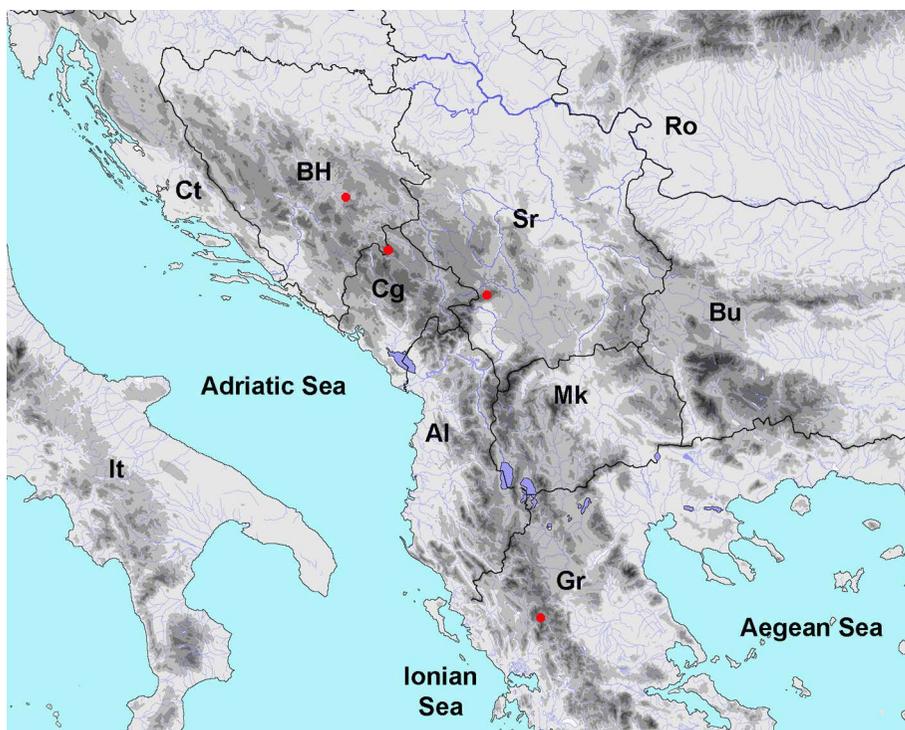


Fig. 2. Map of the distribution of *C. rohlenae*.



Fig. 3. General aspect of *C. rohlenae* specimens.



Fig. 4. Apical segment of *C. rohlenae* branchlet – inflated allantoic cells (a) and small mucronate end cells (m) that are curved and claw-shaped.

Taxonomic inconsistencies of the species *C. rohlenae* stem from its specific similarity with *C. gymnophylla* A. Braun [= *C. vulgaris* var. *gymnophylla* (A. Braun) Nyman], which was observed by Vilhelm as long ago as in 1912 (VILHELM 1912), when he performed a comparative morphological analysis of specimens of these two taxa. Based on the established morphometric differences, he described and named *C. rohlenae* as a species new to science. The main differences between these two species are as follows: (a) the axis cortex of *C. gymnophylla* (*C. vulgaris* var. *gymnophylla*) is regularly diplostichous or aulacanthous, while the axis cortex of *C. rohlenae* is irregular, tylacanthous to isostichous, rarely aulacanthous; (b) the branchlets of *C. rohlenae* are always completely ecorticated, unlike those of *C. gymnophylla* (*C. vulgaris* var. *gymnophylla*), in which the lower one or two segments can be corticated; and (c) the terminal segments are allantoic and mucronate in *C. rohlenae*, while in *C. gymnophylla* (*C. vulgaris* var. *gymnophylla*) they are longish, slender, and blunt. The distribution of these two species is also different. The species *C. gymnophylla* (*C. vulgaris* var. *gymnophylla*) is widely distributed, particularly in the Mediterra-

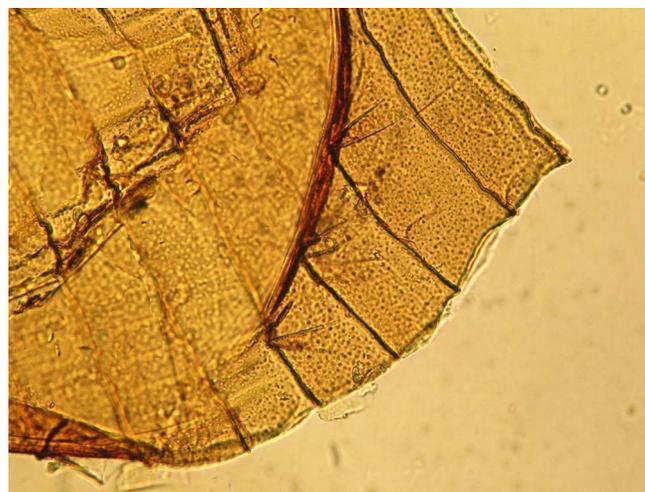


Fig. 5. The oospore membrane is diffusely spotted.

nean region of Europe and Africa, as well as in Asia, inhabiting slowly running water of streams and small rivers. On the other hand, *C. rohlenae* has until now been recorded in shallow puddles around montane and mountain springs, but only on the Balkan Peninsula. However, AHMADI *et al.* (2012), in exploring charophytes from central and western Iran, reported that they found a species which they named *C. gymnophylla* var. *rohlenae* (Vilhelm) Ahmadi stat. nov. In view of this finding, it might be considered that the status of the species *C. rohlenae* has been changed once again. But in our opinion, this is not only disputable, but also unacceptable, inasmuch as the authors only briefly mentioned the species found without giving a detailed description of it, and even wrote as follows: "... rarely fertile branchlets with 1 corticated segment" (AHMADI *et al.* 2012), which is a characteristic of the species *C. gymnophylla* A. Braun [= *Ch. vulgaris* var. *gymnophylla* (A. Braun) Nyman] and not of the species *C. rohlenae*.

CONCLUSION

The re-discovered specimens of *C. rohlenae* on the Balkan Peninsula were found for the first time in Serbia. At this new locality in Serbia (in the Mokra Gora Mountain), the species inhabited a shallow puddle around a mountain spring at 1600 m a.s.l. Our analyses showed that this charophyte is distinguished by a number of specific morphological features, such as: an irregular axial cortex with tylacanthous to isostichous (rarely aulacanthous) primary rows, completely ecorticated branchlets, "inflated" cells on the apical segments of branchlets, and claw-shaped mucronate end cells at the top of the branchlets. These structural characteristics support the opinion that *C. rohlenae* should be regarded as a good species. Still it is clear that further

detailed field investigations, experimental studies on the phenology and morphological variabilities of this species, and molecular analyses are all needed in order to resolve the conflicting opinions as to the taxonomic status of *C. rohlenae*.

The specimens of this charophyte newly (re)found in the high mountain region of Serbia confirm that *C. rohlenae* is not globally extinct. The re-discovery of this species contributes to the indisputable floristic richness of the Balkan stoneworts. In addition to this, the newly found locality of *C. rohlenae* in Serbia points to its broader distribution in the Balkans. Because *C. rohlenae* is an extremely rare species, one which, moreover, is found only in small populations in fairly marginal habitats, efficient protection measures are urgently required.

Acknowledgements — The paper is dedicated to Professor Vladimir Stevanović in recognition of his important contributions to the chorology and conservation of charophytes on the Balkan Peninsula. We express our gratitude to him for constructing the distribution map and for valuable advice and successful collaboration over many years. We would also like to thank Dr. Predrag Lazarević for supplying us with fresh charophyte specimens and photos of their habitat. The authors greatly appreciate the expert technical assistance of Milica Petrović Đurić. Finally, we acknowledge the helpful comments on the manuscript offered by two anonymous reviewers.

REFERENCES

- AHMADI A, RIAHI H, SHEIDAI M & VAN RAAM J. 2012. Some Charophytes (Characeae, Charophyta) from central and western of Iran including *Chara kohrangiana* *Species nova*. *Cryptogamie, Algologie* **33**(4): 359-390.
- BLAŽENČIĆ J, STEVANOVIĆ B, BLAŽENČIĆ Ž & STEVANOVIĆ V. 2006a. Distribution and ecology of charophytes recorded in Western and Central Balkan. *Cryptogamie, Algologie* **27**(4): 311-322.
- BLAŽENČIĆ J, STEVANOVIĆ B, BLAŽENČIĆ Ž & STEVANOVIĆ V. 2006b. Red date list of charophytes in the Balkans. *Biodiversity and Conservation* **15**: 3445-3457.
- FILARSZKY N. 1931. Adatok Horvát-Szlavonország és a Balkán néhány más orszádanak Chara- vegetációjához. *Magyar Botanikai Lapok* **30**(1-12): 81-99.
- LANGANGEN A. 2005-2006. *Chara rohlenae* Vilhelm (Charales) found in Greece. *Allionia* **40**: 31-33.
- TORTIĆ- NJEGOVAN M. 1956. *Chara gymnophylla* i neke druge haraceje Jugoslavije. *Acta Botanica* **14-15**: 145-162.
- VILHELM J. 1912. Zweiter Beitrag zur Kenntnis der Charophyten flora von Montenegro und Bulgarien. *Hedwigia* **53**: 23-35.
- WOOD RD. 1962. New combination and taxa in the revision of Characeae. *Taxon* **11**(1): 7-25.
- WOOD RD & IMAHORI K. 1964. *A revision of the Characeae. II. Iconography of the Characeae*. Verlag J. Cramer, Weinheim.
- WOOD RD & IMAHORI K. 1965. *A revision of the Characeae. I. Monograph of the Characeae*. Verlag J. Cramer, Weinheim.

Botanica SERBICA



REZIME

Ponovno otkriće endemične vrste *Chara rohlenae* Vilh. 1912 (Characeae) – smatrane iščezlom - na Balkanskom poluostrvu

Jelena BLAŽENČIĆ i Branka STEVANOVIĆ

Vrsta *Chara rohlenae* je opisana pre više od sto godina, tačnije 1912. godine, kao nova vrsta za nauku na osnovu herbarskih primeraka sakupljenih na lokalitetu Mratinje u Crnoj Gori početkom 20. veka. Pored toga, postoje herbarski primerci harofite koji potiču iz Grčke (sakupljeni 1885. godine), kao i oni iz Bosne i Hercegovine (sakupljeni 1925. godine) koji su taksonomski različito određivani, odnosno nisu jasno identifikovani da pripadaju vrsti *Chara rohlenae*. Od poslednjeg nalaza prošao je čitav niz godina bez novih podataka o prisustvu ove vrste na Balkanskom poluostrvu, zbog čega je, u skladu sa IUCN kriterijumima, ova harofita smatrana iščezlom vrstom (EX glob ?).

Medjutim, tokom botaničkih istraživanja 2010. i 2012. godine, *C. rohlenae* je ponovo otkrivena na Balkanskom poluostrvu, na planini Mokra Gora (ogranak masiva Prokletija) u Srbiji. Ovaj nalaz potvrđuje činjenicu da vrsta i dalje postoji u prirodi. U radu su analizirane morfološke odlike novonadjanih primeraka vrste *C. rohlenae* iz Srbije.

KLJUČNE REČI: Charophyta, novi nalaz, endemična vrsta, *Chara rohlenae*

