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ABSTRACT
A new site for stonewort *Chara kokeilii* A.Braun was recorded in a Mediterranean shallow lake Prološko Blato Retention, Croatia, SE Europe. The species was associated with the vegetation alliance *Potamogetonion graminei* Westhoff & Den Held. This record has been compared with four other sites (Albania, Bulgaria, Greece and Montenegro) from which the species was recorded in SE Europe, regarding the species morphological traits, environmental parameters and vegetation types. It is possible that the main distribution area of this species is in Southeast Europe, a statement that is supported by previous micropaleontological research. This research provides new data on distribution and ecological affiliation of the critically endangered *C. kokeilii* (IUCN Red List Categories and Criteria).

KEY WORDS
Characeae,
macroalgae,
macrophytes,
Prološko Blato Retention,
Croatia.

RÉSUMÉ

Distribution actualisée de la rare Characeae Chara kokeilii *A.Braun (Charophyta) en Europe du Sud-Est, avec des notes sur son environnement.*

Une nouvelle localité pour la characeae *Chara kokeilii* A.Braun a été enregistrée dans un lac méditerranéen peu profond, Prološko Blato Retention, Croatie, Europe du Sud-Est. L'espèce était associée à l'alliance végétale *Potamogetonion graminei* Westhoff & Den Held. Cet enregistrement a été comparé à quatre autres sites (Albanie, Bulgarie, Grèce et Monténégro) où l'espèce a été recensée en Europe du Sud-Est, en ce qui concerne les traits morphologiques de l'espèce, les paramètres environnementaux et les types de végétation. Il est possible que la principale zone de distribution de cette espèce se situe en Europe du Sud-Est, une affirmation qui est soutenue par des recherches micropaléontologiques antérieures. Cette recherche fournit de nouvelles données sur la distribution et l'affiliation écologique du *C. kokeilii*, espèce en danger critique d'extinction (catégories et critères de la Liste rouge de l'UICN).

MOTS CLÉS
Characeae,
macroalgae,
macrophytes,
Prološko Blato Retention,
Croatie.

INTRODUCTION

The herbarium of the State Museum of Carinthia houses a stonewort that Friedrich Kokeil discovered in Gräben am Wörthersee near Klagenfurth (Austria) in 1846. In his honour, Alexander Braun named the stonewort *Chara kokeilii* A.Braun (1847), while Ganterer (1847) provided the first detailed illustrations of the species, which he described as "very peculiar". Migula (1897) have assessed *C. kokeilii* as an uncertain morphotype, rather than a species. Further records of the species, almost nine decades after its discovery, originate from Szeged in Hungary (Filarszky 1934) and from Lake Ohrid in North Macedonia (Kostić 1936), but both authors gave only brief description of the species. In their "Monograph of the Characeae", Wood and Imahori list the species for Germany and Denmark (Wood & Imahori 1965); however, in 1999 the species was assessed as extinct in Germany (Weyer & Raabe 1999). Blaženčić & Blaženčić (1983), provided a new locality for *C. kokeilii* in Lake Skadar, along with a detailed description of the lake vegetation and its environmental characteristics, which prompted additional visits the locality and a monograph on *C. kokeilii* from Lake Skadar in Montenegro (Krause 1990). The lake is located in the border area between Montenegro and Albania, and until 2013, *C. kokeilii* was recorded only in Plavnica, a locality in Montenegro. Later on, the species was recorded also in the Albanian part of the lake in Jubice (Blaženčić *et al.* 2006b).

With the gradual extension of desultory and limited historical data and the accumulation of large amount of literature on European stoneworts, e.g., the publication of the monograph "Charales (Charophyta)" (Krause 2008) as part of a "Süßwasserflora von Mitteleuropa", the species was recorded, in additional localities namely in Southeast Europe. In 2009, on the Greek island of Evia, the species has been recorded in two mining lakes, located close to each other, north of town of Prokopi (Langangen 2010). The species is also listed for Bulgaria in "Red list of the Bulgarian algae" (Temniskova *et al.* 2008). It was recorded in fresh stagnant water in the Primorsko Region at the Black Sea Coast, without a more precise locality. General studies of the stoneworts in SE Europe can be described as detailed, selective and intensive, especially during the period from the 1980s until the first decade of the 21st century, but the extent of the research varies from country to country (Blaženčić & Blaženčić 1988, 2002, 2003; Blaženčić *et al.* 1995, 1998; Bruinsma *et al.* 2000; Urbanc-Berčić 2003; Langangen 2010). Culmination of the research is summarised in Red Data List of Charophytes in the Balkans (Blaženčić *et al.* 2006b). In Croatia, the studies on stoneworts were done mostly in the last three decades (Blaženčić & Ranđelović 1994; Blaženčić & Blaženčić 2002; Blaženčić *et al.* 2006). The group was also studied within the biomonitoring (Alegro *et al.* 2016a, b), conducted according to the Water Framework Directive (European Community 2000).

Since the branches of *C. kokeilii* appear smooth, translucent like glass and fresh green, the term "nitellized Chara" is often used when describing its morphology. However, having pointed, monopodial branchlets up to 7 mm long, which protrude from the branch on all sides, it can be easily distinguished from any *Nitella* C.Agardh, 1824 species. When removed from the water, the alga keeps its shape despite its delicate, fine-branched nature, usually branching out several times from the bottom. *Chara kokeilii* forms an inconspicuous translucent cortex on shoots, covered with short spines. It is triplostichous, meaning that the number of cortex cell rows is three times that of the branches forming the whorl. The spiked stipulodes at the base of the branches are inconspicuous. *Chara kokeilii* is a monoecious species with gametangia sited the branchlets' nodes. When ripened, the oospores retain their brown colour, in contrast with black oospores of most other species. Mature oospores are surrounded by an easily breakable gyrogonite (Krause 1990).

The aim of this study is to update the current distribution of *C. kokeilii* in Southeast Europe with a new finding from Croatia, examine morphological features of Croatian population, identify its habitat and ecology and compare Croatian findings with other populations from SE Europe.

MATERIAL AND METHODS

Fieldwork was performed in July 2016 and 2019 at Prološko Blato Retention dam (Dalmatia, Croatia, SE Europe); 43°28'29.0"N, 17°07'15.2"E; 268 m a.s.l. (Fig. 1). The dam covers an area of 2.11 square kilometres and is approximately 4.5 meters deep. The water body is a part of a larger wetland area situated in the western part of the karst field, Imotski Field, southwest of the town of Imotski. The whole wetland area covers 10.24 square kilometres, with only a part being flooded during the whole year (several lakes including Prološko Blato Retention). The entire area is flooded only during high waters, mostly during winter, when water depth reaches a maximum of 6 meters in some parts of the area. This is a typical periodically flooded karst field in Dalmatia, some of which are today destroyed by draining in order to expand agricultural land. The area is important in both aesthetic and ecological terms as waterfowl habitat, mainly during the winter and overflow. The area is geomorphologically interesting with amphitheater-looking hills around the fields where downy oak trees and scrub are growing. Prološko Blato belongs to sub-Mediterranean vegetation belt. The same area was protected in 1971 as a significant landscape and is a part of the NATURA 2000 network (National Gazette 124/13; 105/15).

The water body was surveyed for macrophytes along multiple 6 × 100 m transects. In less-accessible areas of the water body, the bottom was raked to reach the macrophytes, using either a long pole or a rope with a rake. Cover and abundance of all recorded plant species were estimated using the expanded nine-degree Braun-Blanquet scale: r = one individual; + = up to 5 individuals; 1 = up to 50 individuals; 2m = more than 50 individuals, coverage < 5%; 2a = coverage between 5 and 15%; 2b = coverage between 15 and 25%; 3 = coverage between 25 and 50%; 4 = coverage between 50 and 75%; 5 = coverage over 75% (Barkman *et al.* 1964, Braun-Blanquet 1964, Dierschke 1994). Finding sites were geocoded using an e*T*rex 30x GPS device and a distribution map was created using ArcGIS 10.5 software.

The stoneworts were examined under 10-400× magnification, photographed and identified with the use of Krause (2008) and Urbaniak & Gąbka (2014). Collected specimens of the stoneworts were conserved in 50% ethanol with 30% of glycerol added and deposited in herbarium ZA (Thiers 2020). Nomenclature follows EURO+MED (2020) for vascular plants and AlgaeBase (Guiry & Guiry 2020) for Charophytes.

The water was sampled for basic physicochemical parameters and nutrients. Conductivity, salinity and pH were measured *in situ* with SevenMulti Modular Meter System (Mettler Toledo) under standard conditions. Dissolved oxygen was measured *in situ* with Hach HQ40D Portable Multi Meter using an optical sensor. Water samples for alkalinity measurement were stored at 4-5°C and alkalinity was determined by titration within 24 hours from sampling. Water samples for total phosphorus analysis were preserved with sulphuric acid and analysed with a UV-VIS spectrometer (Perkin Elmer Lambda 25). Water samples for total nitrogen were stored at 4-8°C in dark bottles filled to the top, and analysed within 24 hours using a Shimadzu TOC-VCPH equipped with an analyser for total nitrogen. Water samples for ammonium, nitrate and orthophosphate analysis were immediately filtered on glass filters with 0.45 µm pores, stored at 4-8°C and analysed within 24 hours on ionic chromatographer (Dionex 3000).

Additionally, all available literature was summarized and a detailed overview of the distribution and ecological preferences of *C. kokeilli* in SE Europe is provided.

RESULTS

Altogether 43 lakes were investigated in the period from 2009 to 2020 within the project of Water Frame Directive biomonitoring, out of which 36 artificial lakes and reservoires and 7 natural lakes. Out of all investigated lakes in Croatia, new site of stonewort *Chara kokeilii* was recorded only in Prološko Blato Retention, namely near its southeast margin. During the field work it was obvious that the water regime of the dam is variable (Fig. 1). The water from the water body is used for irrigation of the surrounding agricultural land and it is occasionally used for fishing. The riparian vegetation surrounding the lake is mostly composed of *Scirpus lacustris* (L.) Palla and less abundant *Bolboschoenus maritimus* (L.) Palla, with smaller species, such as *Eleocharis palustris* (L.) Roem. & Schult. and *E. acicularis* (L.) Roem. & Schult. alternating in shallow water of the same vegetation belt. In the shallow part of the retention, zonation of helophytic vegetation (*Gratiola officinalis* L., *Polygonum amphibium* (L.) Gray, *Agrostis stolonifera* L., *Alisma plantago-aquatica* L., *Alisma lanceolatum* With., *Mentha aquatica* L. *and Lythrum salicara* L.) and stoneworts (*Chara contraria* A.Braun ex Kützing, *C. kokeilii*, *C. vulgaris* L. and *Nitella hyalina* (DC.) C.Agardh) were observed. In the deeper part of the lake, dense meadows of aquatic vegetation with predominant pondweed vegetation (*Potamogeton gramineus* L., *P. pectinatus* L. and *P. crispus* L.) and water-nymphs (*Najas minor* All.) were recorded (Fig. 1). Full vegetation surveys of the SE part of the Prološko Blato are given in the Table 1.

The water in Prološko Blato is slightly basic and alkaline, with conductivity not exceeding 431 µS cm^{-1}. Measured concentration of reactive phosphorous was low, with annual average concentration ranging from 0.0015 to 0.009 mg P l^{-1}, indicating mesotrophic status of the retention. Measured concentration of ammonium was low, while concentration of nitrates was moderately raised, but still indicating good status regarding the eutrophication. According to the measured chlorophyll *a* concentration, the retention is oligotrophic to slightly mesotrophic. Water in the retention is mostly warm during the year, except during summer months when maximum temperature reaches 28°C classifying the lake as very warm. Measured concentration of dissolved calcium carbonates indicates that the water is hard through the year. Full physicochemical and chemical parameters of water in Prološko Blato Retention based on average annual measurements are given in the Table 2.

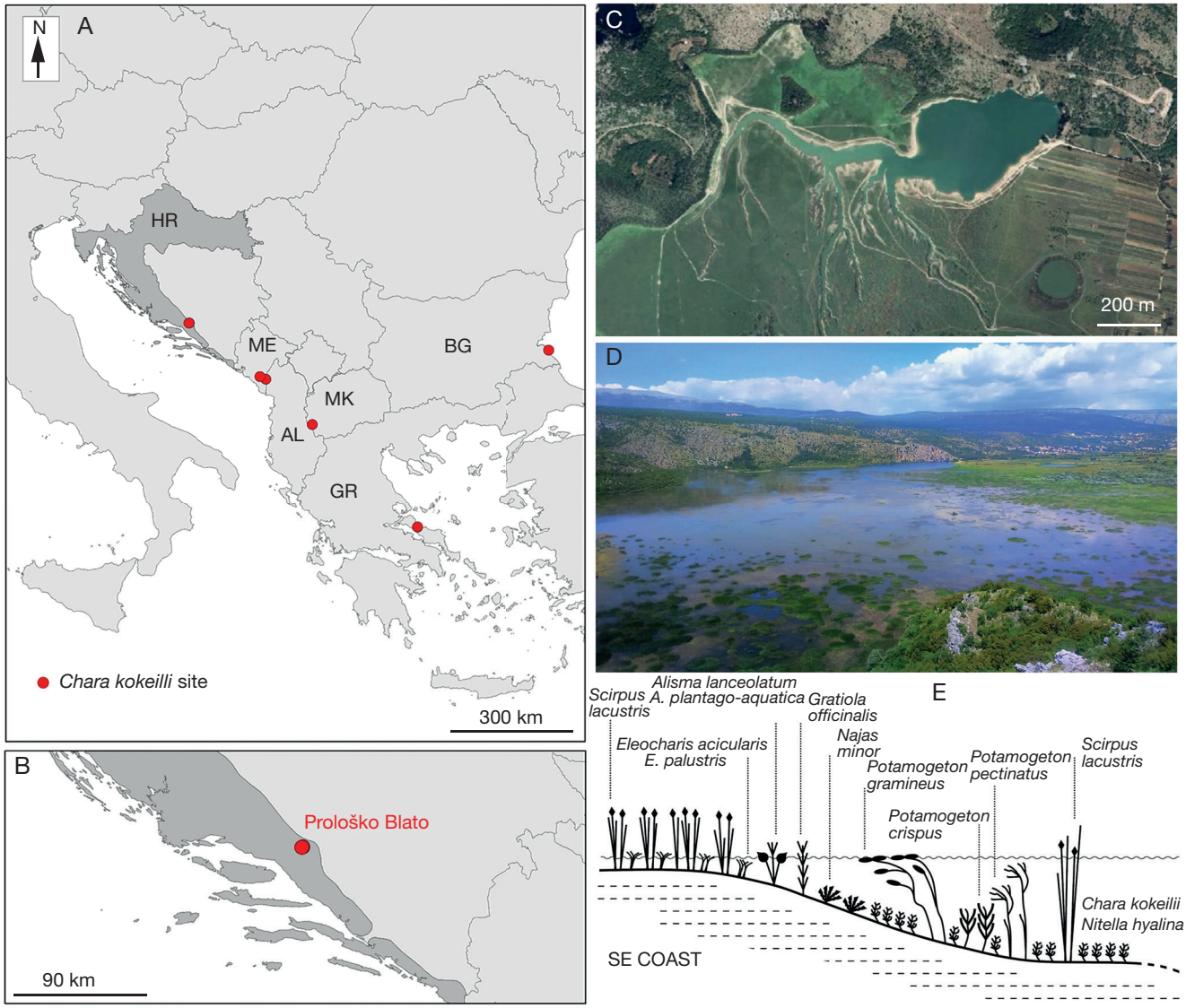

FIG. 1. — Distribution map of *Chara kokeilii* A.Braun in Southeast Europe with detailed location of Prološko Blato Retention in Croatia (**A**, **B**) (Abbreviations: **AL**, Albania; **BG**, Bulgaria; **GR**, Greece; **HR**, Croatia; **ME**, Montenegro; **MK**, North Macedonia). Satellite image of Prološko Blato Retention (**C**) with the picture of flooded Prološko Blato wetland (**D**) and sketched vegetation profile of the *C. kokeilii* finding site at the SE coast of the Retention (**E**).

In Prološko Blato Retention, population of *C. kokeilii* was recorded in July 2016 and in July 2019, rooting in the muddy substrate in 0.5-4 m deep, stagnant water near the SE margin of the retention. The species was associated with the vegetation of the alliance *Potamogetonion graminei* Westhoff & Den Held, 1969, dominated by *Potamogeton gramineus* and *P. pectinatus* in 2016 and *P. gramineus* and *Polygonum amphibium* in 2019. Furthermore, several other stonewort species were accompanying *C. kokeilii*, but with lower abundance: *Nitella hyalina* in 2016 and *Chara contraria* and *C. vulgaris* in 2019. *Chara kokeilii* grew within submerged meadows of vascular plants, intermixed with *Agrostis stolonifera* and *P. gramineus*.

The height of *Chara kokeilii* plants ranged from 5 to 20 cm, with the axes diameter from 490 to 580 µm with root-bulbils on the rhizoids. Internodes were from 1.7 to 2.4 cm long, slightly encrusted. All collected samples were triplostichous, isostichous, with 7 to 9 branchlets in a whorl, each with 3 to 4 segments, about 15 mm long. Terminal/distal segments were composed of 3 to 5 cells. The cortification was absent from lowest internodes and all branchlets, which was the main character for the species identification. Spine-cells were solitary, acute and short (0.3-0.5 × stem diameter), evenly distributed on the internodia. Stipulodes were divided in two rows, and were small and difficult to observe as they were only partly developed. The samples had developed antheridia and oogonia on the first three nodes of the branchlets. The oospores were approximately 450 µm long and 300 µm wide, while the antheridia were approximately 250 µm wide at their widest point. Anterior bract-cells were longer than the oogonium while the pos-

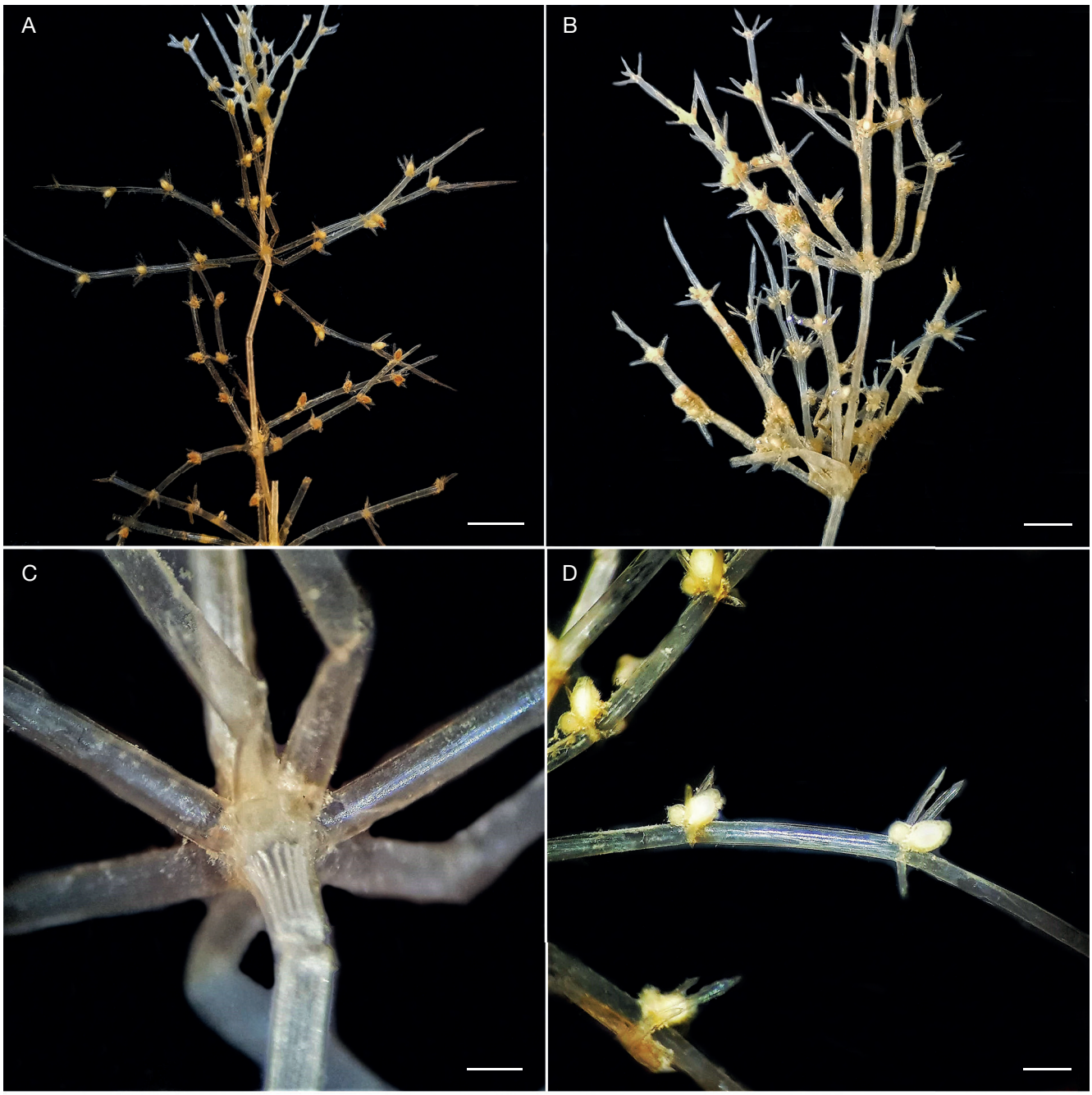

FIG. 2. — **A**, **B**, *Chara kokeilii* A.Braun habitus; **C**, detail of node with stipulodes, cortified main axis and branchlets in a whorl without cortex cells; **D**, detail of the branchlet with nucule, globule and bracteoles. Scale bars: A, 1 cm; B, 0.5 cm; C, 5 mm; D, 1 mm.

terior were short to papillose (Fig. 2). All collected samples were fertile and without visible incrustations.

To date, seven localities of this species have been confirmed for Southeast Europe; a single locality in Albania (Blaženčić *et al.* 2006b), Bulgaria (Temniskova *et al.* 2008), Montenegro (Blaženčić & Blaženčić 1983; Krause 1990) and North Macedonia (Kostić 1936), two close localities in Greece (Langangen 2010) and our most recently recorded locality from Croatia. Most of the findings were well documented, with detailed specimens, population and habitat descriptions. Map of *C. kokeilii* records in SE Europe is shown in Figure 1 and the characteristics of recorded populations, specimens and habitats are shown in Table 3.

DISCUSSION

Chara kokeilii is generally a rare stonewort with a small number of confirmed sites. According to all available data, the species is distributed in Europe (Albania, Austria, Bulgaria, Croatia, Greece, Hungary, Montenegro and North Macedonia) while according to Algaebase (Guiry & Guiry 2020) it has also

Table 1. — List of species at finding site of *Chara kokeilii* A.Braun at Prološko Blato Retention in Croatia, with the estimated cover and abundance according to expanded Braun-Blanquet scale.

	Prološko Blato	
	July 2016	July 2019
Agrostis stolonifera L.	2m	4
Alisma lanceolatum With.	2m	
Alisma plantago-aquatica L.	2m	
Bolboschoenus maritimus (L.) Palla	1	
Chara contraria A.Braun ex Kützing		1
Chara kokeilii A.Braun	2m	2a
Chara vulgaris L.		1
Drepanocladus aduncus (Hedw.) Warnst.		1
Eleocharis acicularis (L.) Roem. & Schult.	2a	
Eleocharis palustris (L.) Roem. & Schult.	2a	2m
Gratiola officinalis L.	2a	
Lythrum salicaria L.		2m
Mentha aquatica L.		2m
Najas minor All.	2a	
Nitella hyalina (DC.) C.Agardh	2m	1
Polygonum amphibium (L.) Gray	1	4
Potamogeton crispus L.	2m	
Potamogeton gramineus L.	2b	4
Potamogeton pectinatus L.	2b	
Ranunculus circinatus Sibth.		+
Scirpus lacustris (L.) Palla	3	2b

Table 2. — Basic physicochemical and chemical parameters of water in Prološko Blato Retention based on monthly measurements in 2017.

	average	range
water temperature (°C)	18.75	10.3-28.5
pH	7.75	7.61-8.21
conductivity (µS cm-1)	347.33	276-431
total suspended solids (mg L-1)	5.93	<0.53-17.6
total water hardness (mg$CaCO_3$ L-1)	185.93	145.7-225.1
dissolved oxygen (mgO_2 L-1)	9.14	4.6-11.4
oxygen saturation (%)	97.96	49.09-120.1
ammonium (mgN L-1)	0.12	<0.003-0.533
nitrites (mgN L-1)	0.0054	<0.001-0.011
nitrates (mgN L-1)	0.2122	<0.04-0.477
total nitrogen (mgN L-1)	0.6121	0.285-1.2
orthophosphates (mgP L-1)	0.0024	<0.001-0.005
total phosphorus (mgP L-1)	0.0037	0.0015-0.009
dissolved silicates (mgSiO_2 L-1)	0.0022	0.0015-0.005
chlorophyll *a* (µg L-1)	2.607	0.51-9.1

been recorded in Argentina (South America) (Tell 1985). In accordance with all available literature, it is possible that the main distribution area of this species is in Southeast Europe. This statement can also be supported with micropaleontological research, which revealed the presence of *C. kokeilii* gyrogonites (calcified oospores) in sediment cores from the Lake Skadar area (Albania/Montenegro) dating to 4500 BC. The gyrogonites were compared with SEM illustrations of rare and endangered Balkan Charophytes (Urbaniak & Blaženčić 2012), including *C. kokeilii*.

As it is a very rare species, little is known about its phytocoenological preferences. A more detailed description of the aquatic vegetation is available for Lake Skadar, where *C. kokeillii* was found within species-rich community *Potametum perfoliati* Miljan, 1933 of alliance *Potamogetonion* Libbert, 1931, a vegetation of rooted and floating macrophytes of freshwater bodies at low and mid-altitudes of temperate Eurasia (Mucina *et al.* 2016). Here, it was accompanied with dominant *Potamogeton perfoliatus* L., several other *Potamogeton* species (*P. lucens* L., *P. pectinatus*, P. *acutifolius* Link, *P. pusillus* L., *P. crispus*), *Myriophyllum spicatum* L., *Najas marina* L., *N. minor* and charophytes, such as *Chara globularis* Thuiller, *C. vulgaris*. *C. tenuispina* A.Braun, *Nitella syncarpa* (Thuillier) Chevallier, *N. gracilis* (J.E.Smith) C.Agardh , *N. capillaris* (Krocker) J.Groves & Bullock-Webster, *N. confervacea* (Brébisson) A.Braun ex Leonhardi, *Tolypella prolifera* (Ziz ex A.Braun) Leonhardi and *T. glomerata* (Desvaux) Leonhardi. On the other hand, the vegetation in Croatian locality is under great influence of water level fluctuations. The whole retention is rather shallow; therefore, a significant part dries out when the water level decreases. This favours the development of amphibious vegetation on the margins of the retention, which can withstand such conditions. Here, *C. kokelli* was found within the vegetation dominated by *Potamogeton gramineus* and *Polygonum amphibium*, species inhabiting fluctuating shallow water, developing both aquatic and terrestric forms. Both species are known to occur within the alliance *Littorellion uniflorae* Koch ex Klika, 1935 in a more mesotrophic conditions, along with other pondweed (*Potamogeton* spp.) and stonewort (*Chara* spp.) species. This amphibious vegetation is indeed known to develop in fluctuating shallow oligo-mesotrophic waters of temperate and boreal Europe, which according to some authors should include the alliance *Potamogetonion graminei* Westhoff & Den Held ,1969, i.e., vegetation of rooted macrophytes of nutrient-poor shallow freshwaters at mid-altitudes of Europe, dominated by *P. gramineus*. Furthermore, in mesotrophic conditions of Prološko Blato Retention, *C. kokeilii* was accompanied with relatively frequent and abundant *P. pectinatus*, a good indicator of greater nutrient load.

Information on affiliation and preference of *C. kokeilii* regarding the particular vegetation is rather scarce and incomplete since the species was so far recorded on only few occasions. However, it is quite evident from available data that the species actually uses the aquatic and coastal vegetation for protection from extreme environmental conditions, such as the impact of waves, water levels fluctuations, wind, landslides etc., as it has been recorded mainly at small depths, often within reeds or dense communities of submerged plants. For example, on island of Evia (Greece), *C. kokeilii* was the only species recorded in two lakes, former mining excavations, where aquatic and wetland vegetation was otherwise completely absent. Here, it occupied steep shores overgrown with shrubs, which were often partially submerged providing the shelter for *C. kokeilii*. Similarly, in Lake Ohrid, *C. kokeilii* was limited only to shallow and sheltered parts (Kostić 1936) of the lake.

As previously mentioned, the species seemingly survives only in SE Europe, as it has gone extinct in Central Europe where it was originally described (Weyer & Raabe 1999). One of the concerns regarding its survival in remote lakes is the rise of anthropogenic pressure, such as extensive tourism and eutrophication. One of the most disturbing events with the potential of wiping out entire populations is backfilling

TABLE 3. — *Chara kokeilii* A.Braun population, specimen and habitat characteristics from reported sites in SE Europe.

	Prološko Blato Retetion (Croatia)	Lake Skadar (Montenegro, Albania)	Lake Ohrid (North Macedonia)	mining lakes (Island of Evia, Greece)	Black Sea Coast (Bulgaria)
population size	from more than 50 individuals to covering up to 15% of research area	–	small, few specimens	small, few specimens	–
habitat description	gentle slope of the submerged coastal part of the retention, in riparian and between aquatic vegetation	coastal parts of the lake, protected against wind and water undulations by aquatic and helophytic vegetation	sheltered places along the edge of the lake, often in the reeds	step shores of former mining site, now lake with no other aquatic vegetation	stagnant water
substrate	mud	sand and mud	mud	lime rich sediment	–
water depth	0.5-4 m	1-2 m	0.8-1 m	–	–
plant height	5-20 cm	10-30 cm	6-8 cm in nature; 25 cm in culture	up to 12 cm	up to 30 cm
axis diameter	490-580 µm	500-800 µm	420-588 µm	500 µm	800 µm
internodia length	1.7-2.4 cm	–	0.5-6 cm	up to 2 cm	up to 4 cm
number of branchlets per whorl	7-9	–	9-10	10	9-11
number of branchlet segments	3-4	3-5	(1) 3-5 (8)	3-4	–
spine-cells length	0.3-0.5 × stem diameter	–	56-154 µm	0.3-0.5 × stem diameter	–
gametangia position	branchlet nodia 1, 2 and 3	branchlet nodia 2 and 3	–	branchlet nodia 2 and 3	–
oospores dimensions	450-300 µm	441-556 × 304-451 µm	700-743 × 434-448 µm	–	–
antheridia diameter	250 µm	250 µm	280-343 µm	–	–

and concreting the lakeshores, and mowing of coastal and aquatic vegetation. Site locations of *C. kokeilii* mentioned in this study are, most likely, the only ones remaining as safe zones for this species to occur. According to the IUCN Red List Categories and Criteria, *C. kokeilii* is classified as critically endangered (CR) with high risk of global extinction.

CONCLUSION

New site location of rare stoneworth *Chara kokeilii* has been documented in Croatia, SE Europe. Species was recorded in shallow Mediterranean retention, Prološko Blato. This record has been compared with all four other sites (Albania, Bulgaria, Greece and Montenegro) from SE Europe, regarding the species morphological traits, environmental parameters and vegetation types. The species was associated with the vegetation of the alliance *Potamogetonion graminei*. New data on the ecology and distribution of the species contribute to the aim of greater protection of this rare and endangered species.

REFERENCES

ALEGRO A., STANKOVIĆ I., ŠEGOTA V., VAN DE WEYER K. & BLAŽENIČ J. 2016a. — Macrophytic vegetation in the oligotrophic Mediterranean Lake Vrana (Island of Cres, Northern Adriatic) – New insight after 50 years. *Botanica Serbica* 40: 21-28.

ALEGRO A., ŠEGOTA V., RIMAC A., HRŠAK V., VUKOVIĆ N., KOLETIĆ N. & STANKOVIĆ I. 2016b. — Macrophyte vegetation of rivers in Croatia – diversity and ecological indication potential, *in* REŠETNIK I. & LJUBEŠIĆ Z. (eds), *Book of abstracts of the 5th Croatian Botanical Symposium with International Participation*. Croatian botanical society, Zagreb, 6 p.

BARKMAN J. J., DOING H. & SEGAL S. 1964. — Kritische Bemerkungen und Vorschläge zur quantitativen Vegetationsanalyse. *Acta Botanica Neerlandica* 13: 394-419. https://doi.org/10.1111/j.1438-8677.1964.tb00164.x

BLAŽENČIĆ J. & BLAŽENČIĆ Ž. 2003. — An overview of the existing data on living charophytes (Charales) of the Balkan Peninsula. *Acta Micropalaeontologica Sinica* 20: 103-110.

BLAŽENČIĆ J. & BLAŽENČIĆ Ž. 1983. — Fitocenološka studija zajednica *Charetum fragilis Corillion 1957* i *Chareto-Nitellopsidetum obtusae* J. Blaž. ass. nova kod Plavnice, na Skadarskom jezeru. *Glasnik republičkog Zavoda za Zaštitu Prirode - Prirodoslovni Muzej, Titograd* 16: 7-13. (in Serbian).

BLAŽENČIĆ J. & BLAŽENČIĆ Ž. 1988. — Floristic and chronological analysis of algae belonging to the division Charophyta in Bosnia and Herzegovina. *Zbornik referata naučnog skupa "Minerali, stijene, izumrli i* živi *svijet B&H", Sarajevo*: 283-293.

BLAŽENČIĆ J. & BLAŽENČIĆ Ž. 2002. — Rare and threatened species of charophytes (Charophyta) in Southeast Europe. *Phytologia Balcanica* 8: 315-326.

BLAŽENČIĆ J. & RANĐELOVIĆ V. 1994. — *Chara visianii* J. Blaženčić et V. Randelović sp. nov. (Characeae) from the river Krka (Croatia). *Cryptogamie, Algologie* 15: 73-79.

BLAŽENČIĆ J., BLAŽENČIĆ Ž. & LJALJEVIĆ M. 1995. — The contribution to the knowledge of the distribution and ecology of the Charophyta in Vojvodina (Serbia, Yugoslavia). *Ekologija* 30: 1-18.

BLAŽENČIĆ J., BLAŽENČIĆ Ž., LAKUŠIĆ D. & RANĐELOVIĆ V. 1998. — Phytogeographical and ecological characteristics of the charophytes of the East Adriatic coast. *Archives of Biological Sciences Belgrade* 50: 119-128.

BLAŽENČIĆ J., STEVANOVIĆ B., BLAŽENČIĆ Ž. & STEVANOVIĆ V. 2006a. — Distribution and ecology of charophytes recorded in the West and Central Balkans. *Cryptogamie, Algologie* 27 (4): 311-322.

BLAŽENČIĆ J., STEVANOVIĆ B., BLAŽENČIĆ Ž. & STEVANOVIĆ V. 2006b. — Red Data List of Charophytes in the Balkans. *Biodiversity and Conservation* 15: 3445-3457.

BRAUN A. 1847. — *Chara kokeilii*, eine neue deutsche Art. *Flora* 2: 17-27.

BRAUN-BLANQUET J. 1964. — *Pflanzensoziologie, Grundzüge der*

Vegetationskunde, ed. 3. Springer-Verlag, Berlin, 866 p.
Bruinsma J., Hospers A. & Hospers M. 2000. — Some Charophytes Records in Snežnik Regional Park. *Acta Biologica Slovenica* 44: 71-77.
Dierschke H. 1994. — *Pflanzensoziologie. Grundlagen und Methoden*. Eugen Ulmer Verlag, Stuttgart , 683 p.
Euro+Med 2020. — Euro+Med PlantBase. The information resource for Euro-Mediterranean plant diversity, Botanic Garden and Botanical Museum Berlin-Dahlem. Available from: http://ww2.bgbm.org/EuroPlusMed/ (accessed 3 June 2020).
European Community 2000. — Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Available from: eur-lex.europa.eu (accessed 3 June 2020).
Filarszky N. 1934. — Enumeratio specierum et formarum Characearum in regionibus oppidi Szeged hucusque congnitarum. *Folia Cryptogamica* 1 (10): 1307-1316.
Ganterer U. 1847. — *Die bisher bekannten österreichischen Charen*. Inaugural-AbHandlung, Wien, 36 p.
Guiry M. D. & Guiry G. M. 2020. — AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. – Available from: http://algaebase.org. (accessed 3 June 2020).
Kostić L. 1936. — Prinos poznavanju haraceja Ohridskog jezera i okoline (Ein Beitrag zur Kenntnis der Characeen des Orchridsees. *Acta Botanica Croatica* 11: 64-68.
Krause W. 1990. — Zum Neufund der Chara kokeilli A. Br. im Skautarisee. *Carinthia II* 180 (100): 705-713.
Krause W. 2008. — Charales (Charophyta), *in* Ettl H., Gärtner G., Heynig H. & Mollenhauer D. (eds), *Süßwasserflora von Mitteleuropa*, vol. 18. Spektrum Akademischer Verlag Heidelberg. Gustav Fischer, Jena, Stuttgart, 202 p.
Langangen A. 2010. — Some charophytes (Charales) collected on the island of Evia, Greese in 2009. *Flora Mediterranea* 20:149-157.
Migula W. 1897. — *Die Characeen*. Leipzig, 765 p.
Mucina L., Bültmann H., Dierssen K., Theurillat J.-P., Raus T., Čarni A., Šumberová K., Willner W., Dengler J., García R. G., Chytrý M., Hájek M., Di Pietro R., Iakushenko D., Pallas J., Daniëls F. J. A., Bergmeier E., Santos Guerra A., Ermakov N., Valachovič M., Schaminée J. H. J., Lysenko T., Didukh Y. P., Pignatti S., Rodwell J. S., Capelo J., Weber H. E., Solomeshch A., Dimopoulos P., Aguiar C., Hennekens S. M. & Tichý L. J. 2016. — Vegetation of Europe: Hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. *Applied Vegetation Science* 19 (1): 3-264.
National Gazette 124/2013, 105/2015. — Uredba o ekološkoj mreži (Regulation on the ecological network).
Tell G. 1985. — Catálogo de las algas de agua dulce de la República Argentina. Bibliotheca *Phycologica* 70: 1-283.
Temniskova D. T., Stoyneva M. P. & Kirjakov I. K. 2008. — Red List of the Bulgarian algae. I. Macroalgae. *Phytologia Balcanica* 14 (2): 193-206.
Thiers B. 2020. — Index Herbariorum. A global directory of public herbaria and associated staff, New York Botanical Garden's Virtual Herbarium. Available from: http://sweetgum.nybg.org/ih/ (accessed 3 June 2020).
Urbanc-Berčić O. 2003. — Charophytes of Slovies, their ecological characteristics and importance in aquatic ecosystems. *Hladnika* 15-16: 17-22.
Urbaniak J. & Blaženčić J. 2012. — SEM study of oospore characteristics in endemic and endangered Balkan Charophytes. *Cryptogamie, Algologie* 33 (3): 277-288.
Urbaniak J. & Gąbka M. 2014. — *Polish Charophytes. An Illustrated Guide to identification*. Uniwersytet Przyrodniczy we Wrocław, 120 p.
Weyer Van De K. & Raabe U. 1999. — Rote Liste der gefährdeten Armleuchteralgen-Gewächse (Charales) in Nordrhein-Westfalen. 1. Fassung, *in* Wolff-straub R. & Wasner U. (eds), *Rote Liste der gefährdeten Pflanzen end Tiere in Nordhein-Westfalen*. LÖBF-Schr.R. 17: 295-305.
Wood R. D. & Imahori K. 1965. — *A Revision of Characeae. I. Monograph of the Characeae*. J. Cramer, Weinheim, 904 p.

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