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Distribution and ecology of charophytes recorded in the West and Central Balkans

Jelena BLAŽENČIĆ [a], Branka STEVANOVIĆ [a], Živojin BLAŽENČIĆ [b] & Vladimir STEVANOVIĆ [a]*

[a] *Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Serbia*

[b] *Faculty of Veterinary Medicine, University of Belgrade, Serbia*

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Abstract — Long-term investigation of charophytes in the West and Central Balkans has confirmed an exceptional level of diversity of these macroalgae in the region. Charophytes have been observed in the fresh, brackish, salt and mineral waters of different aquatic environments from the Adriatic coast up to alpine zones. Forty two species of all extant genera of charophytes were identified, including 25 species the genus *Chara*, 10 species of *Nitella* and 4 of *Tolypella*. Only one species of the genera *Lychnothamnus, Lamprothamnium* and *Nitellopsis* has been found. Aquatic environments in the coastal Adriatic zone, such as the lakes of the Krka River, the estuary of the Neretva River in Croatia and Lake Skadar in Montenegro, are notable for the great abundance of various charophytes. This is no less true for the glacial lakes on Mts Zelengora and Durmitor in Montenegro and the deep and ancient lake of Ohrid in FYR Macedonia. All these habitats are centers of charophyte diversity and, as such, deserve particular attention and protection.

Charophytes / habitat conditions / ecological adaptability / floristic diversity

Résumé — Distribution et écologie des charophytes récoltés dans l'ouest et le centre des Balkans. Les longues recherches sur les charophytes du Balkan occidental et central ont confirmé un niveau exceptionnel de la diversité de ces macroalgues dans la région. On a observé des Charophytes dans les eaux douces, saumâtres, salines et les eaux minérales de différents environnements aquatiques de la côte adriatique jusqu'aux zones alpestres. Quarante deux espèces de tous les genres existants de charophytes ont été identifiées, dont 25 espèces du genre *Chara*, 10 espèces de *Nitella* et 4 espèces de *Tolypella*. Par contres les genres *Lychnothamnus, Lamprothamnium* et *Nitellopsis* ne sont représentés que par une seule espèce chacun. Les environnements aquatiques dans la zone adriatique côtière, tels que les lacs du fleuve de Krka, de l'estuaire du fleuve de Neretva en Croatie et du lac Skadar dans le Montenegro, sont remarquables par la grande abondance de divers charophytes. C'est aussi le cas pour les lacs glaciaires des Mts Durmitor et Zelengora du Montenegro et le lac profond et ancien d'Ohrid dans la FYR Macédoine. Tous ces habitats sont des centres de diversité de charophyte et, en tant que tels, méritent une attention particulière et une protection.

Adaptation écologique / Charophytes / conditions d'habitat / diversité floristique

* Correspondence and reprints: jblaz@eunet.yu

INTRODUCTION

The Balkan Peninsula (SE Europe) is well known as the most diverse region of the western Palaearctic regarding its flora and vegetation in general. The richness of charophytes, in particular, may be attributed to its abundant and varying aquatic habitats (Stevanović *et al.*, 1999). The evolutionary pressures and variations in habitat that have acted upon this part of Europe, from the Tertiary through the Ice Age up to the present day, have resulted in an exceptional diversity of stoneworts, including a large number of endemic species (Blaženčić & Blaženčić, 2003). The West and Central Balkans is an area rich in various types of lake, river, canal, pond, pool, spring, peat-bog, fishpond, mineral spring and thermal spring, reservoir and water meadow. These habitats are located both in lowland and highland regions, as well as along the Adriatic Seaboard. Charophytes thrive in these fresh to salty waters, with several species occurring in the many mineral and hot water springs, abundant throughout the area. The geomorphological, pedological and hydrological features of these aquatic habitats, as well as seasonal variations in water movement, temperature and transparency, have resulted in a diverse charophyte vegetation, i.e. in the formation of different algal canopy and/or multi- monospecific charophyte mats at various water depths.

It is also important to mention that the Balkan Peninsula is characterized by numerous "hot spots", or centers of diversity, and by the Important Plant Area (IPA), where stoneworts or charophytes are particularly important coenobionts. The aim of this study is to describe the presence and distribution patterns of charophytes in these regions according to their ecological demands.

MATERIAL AND METHODS

Studies were carried out in summer at diverse localities throughout the West and Central Balkans. During the collection of plant material, air and water temperatures were measured with mercury thermometers with a sensitivity of 0.2°C. The clarity of the water was established with a Secchi disk, while its acidity was determined with a paper indicator (Merck 6.4-8.0) or pH-meter. The physical features of the aquatic soil substrate and characteristics of bottom hydromorphological relief were recorded along transverse profiles.

In order to achieve a reliable insight into both floristic diversity and vegetation distribution, investigations were carried out by the method of transects in all directions. Samples were taken at a number of transect points, dependent on the ecosystem size and complexity. The richness and diversity centers of charophyte distribution in the region were mapped on a 50×50 km UTM square, as is the practice in the *Atlas Florae Europaeae* project carried out in Helsinki. The main data for the mapping were our own field results and the numerous literature and herbaria data on charophytes recorded throughout the former Yugoslavia.

Specimens were fixed at the collection site with 4% formaldehyde. Laboratory processing of samples was performed at the Institute of Botany of the Faculty of Biology, University of Belgrade, where the plant material is stored (BEOU).

Charophytes were identified using the keys developed by Corillion (1957, 1975), Wood & Imahori (1964, 1965), Gollerbah & Krasavina (1983), Krause (1997) and Schubert & Blindow (2003).

RESULTS AND DISCUSSION

Forty two species were recorded in the region surveyed (the former Yugoslavia), which confirms the floristic richness of stoneworts in the area studied, compared to other regions in Europe (Blaženčić *et al.*, 1990; Krause, 1997; Schubert & Blindow, 2003). Species of all the extant charophyte genera are present, viz. *Chara* – 25 species, *Nitella* – 10 species, *Tolypella* – 4 species, *Lamprothamnium* – 1 species, *Lychnothamnus* – 1 species and *Nitellopsis* – 1 species (Blaženčić & Blaženčić 2003).

The prevalence of certain charophyte species in some lakes is conspicuous to the extent that they are floristically classified, e.g. as a *Chara* type lake, such as Lake Ohrid in the FYR Macedonia, or a *Nitella* type lake like Lake Skadar in Montenegro (Stankovic, 1960; Krause, 1990). However, charophytes are particularly abundant in the oligotrophic mountain glacial lakes in Mts Durmitor, Prokletije, Zelengora (Montenegro) and in Mt. Triglav (Slovenia), as well as in the karst lakes of Plitvice and the Baćinska (Croatia). In these lakes they usually form dense underwater meadows at different depths.

The first data indicating the presence of charophytes in the West and Central Balkans were recorded more than 150 years ago (Visiani, 1842). Having taken into account all the established charophyte sites during the investigation process, we are in a position to show not only the general pattern of their distribution in the territory of the former Yugoslavia, but also the regions distinguished by a floristic richness of these plants (Fig. 1).

The majority of charophytes grow on silty or sand-silty substrate, like *Chara hispida* L., *C. virgata* Kütz., *C. tenuispina* A. Br., *Nitella tenuissima* (Desv.) Kütz. and others in different aquatic environments in the area studied. Smaller numbers of species inhabit detritus and rock crevices or grow between stones in the littoral zone. Excellent examples are *Chara strigosa* A. Br., which sprouts from crevices in submerged rocks in Lake Zminje (Mt Durmitor), or the species *C. aspera* Deth. ex Willd., *C. vulgaris* L. and *Nitella syncarpa* (Thuill.) Chevall, which develop on the shelves and crevices of the rocky littoral zone in the reservoir Slano near the city of Nikšić in Montenegro (Blaženčić & Blaženčić, 1997).

It is interesting to mention the species *Chara contraria* var. *nitelloides* A. Br. that was recorded on arsenic-rich substrate in ponds beside the river Bistrica in the vicinity of the mine (Alshar or Majdan) on Mt Kožuf in Macedonia. In sections of the river, where the current is slow, small "ponds" with silty bottoms are formed, and on their surface large masses of charophytes occur. As the whole region is rich in arsenic, the appearance of one charophyte in this habitat is particularly intriguing.

Thirty nine of the 42 species studied inhabit shallow water, at a depth of 1 m or less. The species number decreases gradually and is depth dependent (Fig. 2A). The species *C. braunii* C.C. Gmel., *C. conimbrigensis* Hy, *C. connivens-fragilis* G. da C., *C. muscosa* J. Gr. *et* B.-W., *C. strigosa*, *C. tenuispina*, *Lamprothamnium papulosum* (Wallr.) J. Gr., *Nitella capillaris* (Krock) J. Gr. *et*

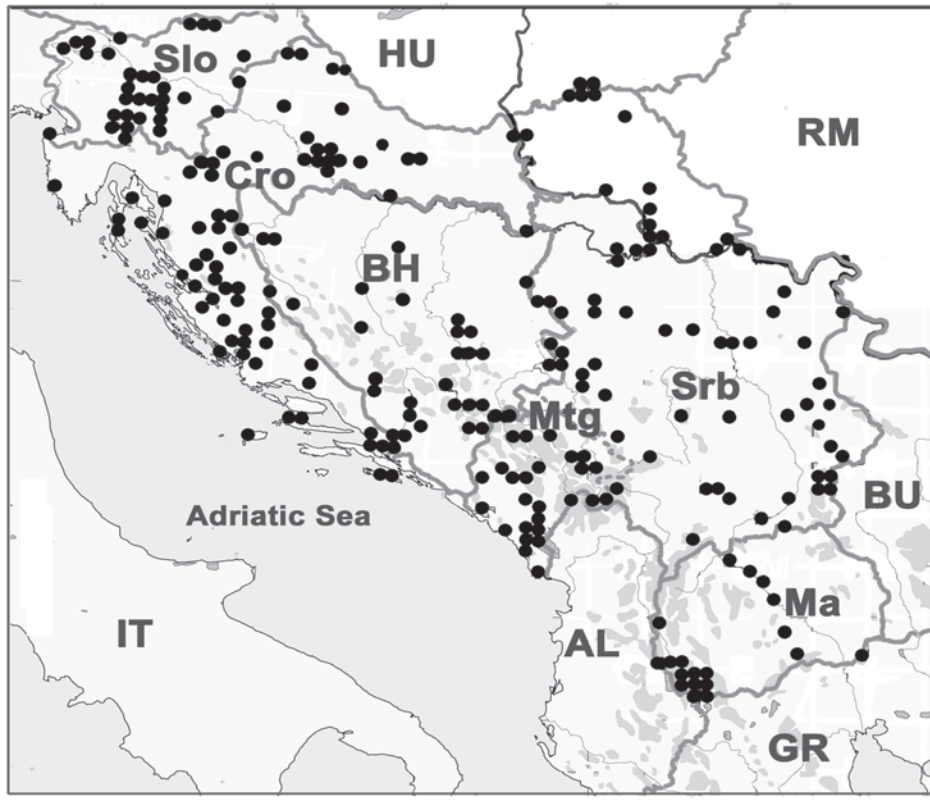

Fig. 1. Distribution of charophytes in the West and Central Balkans.

B.-W., *N. tenuissima*, *Tolypella nidifica* (Müller) A. Br., *T. prolifera* (Müller) A. Br. are present more or less only in shallow waters. In contrast, only 4 species were found at depths of about 20-40 m. They are *C. globularis* Thuill., collected at a maximum depth of 19 m in lake Kozjak in the National park Plitvička jezera as well as in Lake Ohrid, *C. ohridana* (Kostić) Krause at 20 m in Lake Ohrid, whereas in lake Vrana on the island of Cres in Croatia *C. polyacantha* A. Br. was recorded at 25 m and *Nitella opaca* Ag. at 38-40 m (Kostić, 1936; Golubić, 1960; Urbac-Berčič, 2003).

The species found in different environments near the Adriatic coast, such as *C. intermedia* A. Br. in A. Br., *C. corfuensis* J. Gr. ex Fil., *C. canescens* Desv. *et* Loisel. in Loisel., *Lamprothamnium papulosum* and *Tolypella nidifica*, are adapted to water temperatures of 25-30°C during the summer, which do not drop below 10°C in winter. Bearing in mind the wide distribution of these species throughout the world temperate zone (with the exception of *C. corfuensis*), it seems that they are adapted to very large temperature variations (Corillion, 1957; Wood & Imahori, 1965; Schubert & Blindow, 2003).

We established that the species *C. aspera, C. vulgaris, C. contraria* A. Br. ex Kütz. and few others, inhabiting low coastal to mountain regions (glacial lakes), endure greater extremes of temperature (Fig. 2B). In contrast, some species which also occur over a wide altitudinal range, i.e. *Nitella opaca* and *C. globularis*,

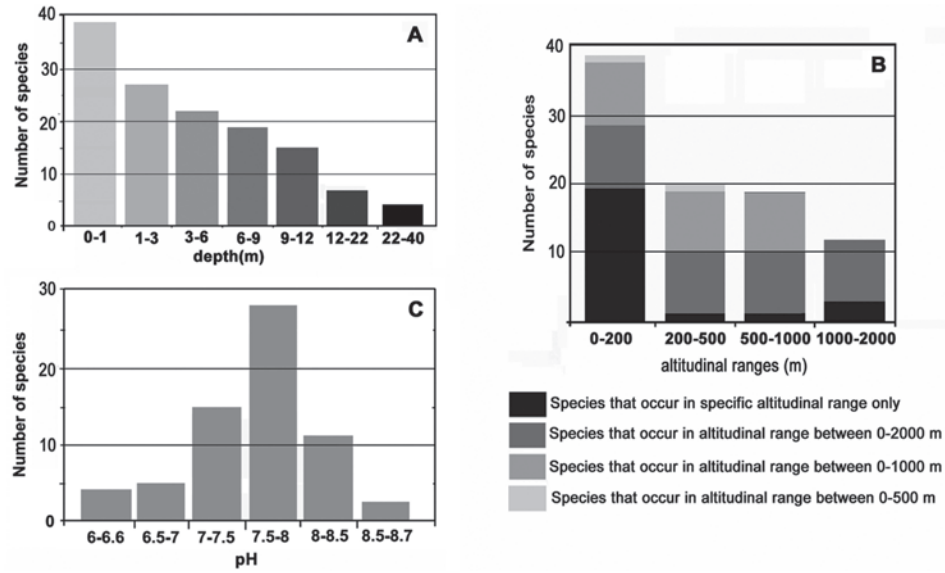

Fig. 2. Abundance of charophytes in relation to habitat conditions: **A.** at different water depth; **B.** along the altitudinal gradient; **C.** at various water pH.

mainly inhabit cold waters where underwater currents frequently prevail. Thus, in these types of lakes they are present at lower altitude (500-650 m), but also in the high mountain lake of Bijelo on Mt Zelengora at 1416 m above sea level. *C. muscosa* and *C. strigosa*, on the other hand, only grow in the glacial lakes of Mts Zelengora and Durmitor (Montenegro), and can be considered as markedly mountain cryophilic species, contrasting with *C. braunii, C. globularis, C. vulgaris, C. contraria, Nitella gracilis* (Js. Sm.) C. Ag., which grow in the hot mineral waters, occurring at many spas at temperatures between 28 and 32°C.

The charophytes in the region were found mainly in neutral to slightly alkaline waters, the pH of which varied between 7.5 and 8.0 (Fig. 2C). Hence, the largest number of recorded species exhibit a strong preference for calcareous waters, meaning they can be classified as calcicole. This refers in particular to the species of the genus *Chara* (*C. rudis* A. Br. in Leonh., *C. contraria, C. globularis*), which are the most reliable indicators of hard, calcium rich inland waters (Plitvice, Baćinska, Triglav and some other lakes). In the former Yugoslavia only the species *C. braunii* and *Nitella opaca* have been recorded in acidic, soft water (pH of 6.0-6.6) in one locality only, which is the peat-bog lake formed by the Vlasina reservoir (Blaženčić & Blaženčić, 1991). There, *C. braunii* inhabits in shallow water with higher concentration of chloride, sodium and potassium ions while in the deeper water grows *N. opaca.* It is well known that these two species tolerate wider variations in pH.

The charophytes prevalently inhabit freshwater habitats, and commonly in localities with neutral to mildly alkaline waters. Thus 32 out of 42 species in all were found in inland lakes, reservoirs, marshes, etc., four in brackish (*C. intermedia, C. canescens, C. baltica* Bruz. and *Tolypella nidifica*) and only one in salt water (*Lamprothamnium papulosum*), whereas five thrive in both fresh and brackish waters (*C. aspera, C. contraria, C. virgata, C. polyacantha* and *Nitella*

tenuissima) (Zavodnik, 1967; Firbas & Al-Sabti, 1995; Blaženčić & Blaženčić, 1997; Blaženčić *et al.*, 1998).

The species *Lamprothamnium papulosum* survives in extremely salty water although it is not a fully marine species. It was recorded in the shallow (up to 1 m deep) salt water of the pool-like marsh, Malo Blato near Šibenik in Croatia. It is situated in the immediate vicinity of the sea, being connected to it by a narrow channel. The salinity of this body of water, with readily movable silt on the bottom, is very high (82.9‰). In the extreme environmental conditions of this habitat, in addition to *L. papulosum,* the marine flowering plants *Ruppia spiralis* L. ex Dumort. and *Zannichelia major* (Hartman) Boenn. ex Reichenb. have been found. Daniel (1975) reported that this species is an indicator of salinity from 10-30‰ and that it grows best at a salinity of 24-28‰, but that it also grows in water the salinity of which is below 8‰. In Denmark it was found at a salinity range of 8-18‰, while in Sweden at 9.5-25‰ (Andersson *et al.*, 2003). It is interesting to mention that in Australia this species is also noted as the most salt and drought-tolerant macroalga, tolerating salinities up to 210‰ (García & Chivas, 2004).

In mineral waters from the territory studied a relatively small number of charophytes (nearly 8 species), otherwise widely distributed and eutolerant species, were found. The mineral waters of hot springs and spas are mostly alkaline-earth and mildly radioactive. The most frequently recorded species in these waters are *C. vulgaris* and/or *C. vulgaris* var. *gymnophylla* A. Br., then *C. braunii*, *Nitella gracilis* and *N. flexilis* (L.) C. Ag. (Vouk, 1919; Tortić-Njegovan, 1956; Petrovska, 1963; Marinović & Krasnići, 1970; Blaženčić, 1980; Blaženčić, 1984). On the other hand, in sulfuric mildly radioactive mineral waters *C. braunii, C. globularis, C. vulgaris* var. *gymnophylla*, *Nitella gracilis* and *Nitellopsis* sp. thrive (Vouk, 1919; Petrovska, 1963; Blaženčić, 1980).

One of the most striking ecological features of the observed charophytes is the conspicuous morphological variability of their highly organized thallus. Certain charophytes exhibited adaptive modifications of their common growth form in response to changes in the ecological conditions. Thus, with increase in water depth, some charophytes elongate their thalli and internodes, reducing the intensity of their calcification (incrustation). The longest specimens recorded were those of *C. globularis* growing in the Lake Kozjak in The National Park Plitvička jezera. Likewise, the "land" individuals of some species, which occur in marshy sites or bathed with shallow water in the flooded fields adjacent to mineral springs, exhibit remarkable structural modifications in respect to those living in deeper brook waters downstream of the springs. These modifications in habit also arise periodically, dependent on the water level. Hence, in shallow water or on saturated ground, charophytes produce helophytic, shorter and dense turf like growth forms, and when developing in deeper water they develop a slender, elongated hydrophytic habit. For instance, the helophytic form of *C. vulgaris* var. *gymnophylla* from Zvonačka spa, had a shorter thallus (6-7 cm) than the hydrophytic one (20-24 cm). It was more branched and had shorter internodes and longer leaves, which are sometimes only partially covered with cortical structure. The helophytic form is distinguished by more intense incrustation and more robust stipulodes, compared to the hydrophytic habit (Fig. 3).

A similar pattern of adaptation was also reported for *Lamprothamnium papulosum* from brackish water, in which the upper, contracted parts of the stem give this plant a bushy appearance, which is the origin of its name, the foxtail stonewort. It is well known, from the different keys for identification, that this charophyte has thin, upright stems up to 40 cm in height. The individuals that we recorded in shallow water in the locality Malo Blato near the Adriatic coast are

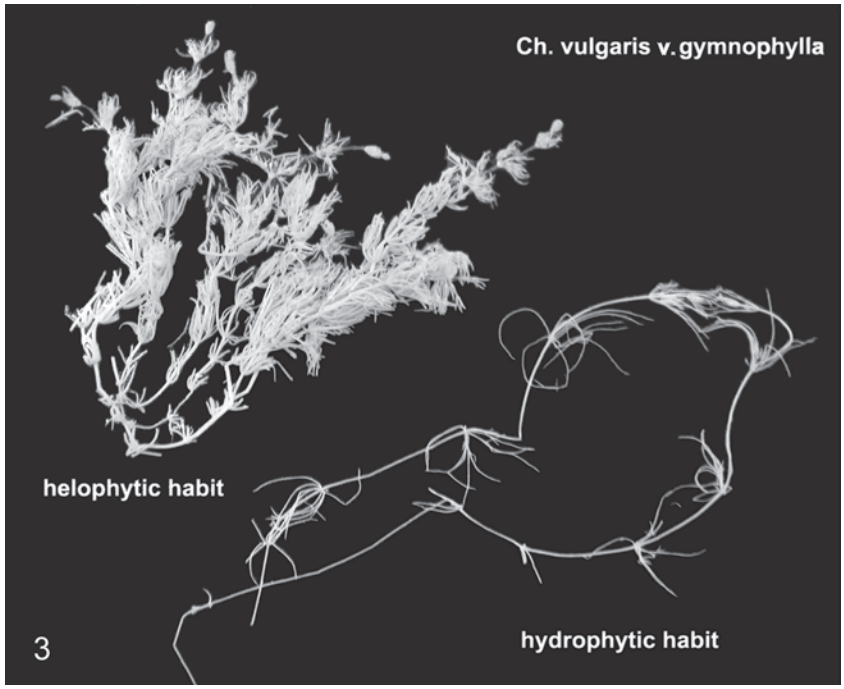

Fig. 3. Modification of charophyte thalli - helophytic and hydrophytic habit.

markedly short, up to 6 cm, dark green and densely packed. The plants are firm and "thorny" due to salt crystals deposited on the thallus. They are anchored to the soft, loose substratum by rhizoids on which white unicellular cluster-like tubers occur. All the mentioned modifications in the growth form of these species are actually plastic ecological responses to unstable environmental conditions. They suggest that these particularly advanced algae share some characteristics with land plants and indicate charophyte pre-adaptation for occupation of terrestrial habitats (Graham & Gray, 2001).

The distribution map of charophytes, presented on the UTM grid map, squares 50×50 km, indicate their richness and diversity in the west and central Balkans. Thus, the richest biodiversity centers, where 10-16 charophyte species were recorded to one square, of 100 km^2, are: Ljubljansko Barje marsh and its wider surroundings, the lower reaches of the River Krka with cascades and lakes, the estuary of the Neretva river with lakes, the complex of high-mountain lakes of Mts Durmitor, Volujak and Zelengora and lakes of Skadar and Ohrid (Fig. 4).

As might be supposed, among the least "choosy" regarding ecological demands and environmental conditions are *C. vulgaris, C. contraria, C. globularis*, otherwise widespread, cosmopolitan species. At the lowest altitudes, i.e. in coastal ponds, shallow pools and small lakes, *C. intermedia, C. baltica, C. canescens* and *Tolypella nidifica* were recorded, whereas *C. muscosa, C. rohlenae* Vilhelm and *C. strigosa* most frequently inhabit lakes located between 1200-1500 m in altitude.

Charophytes are among a small number of aquatic macrophytes that occur at the highest altitudes, e.g. *C. contraria* f. *capillacea* Mig., which grows in the glacial lake Zeleno in Mt Triglav at a height of 1983 m.

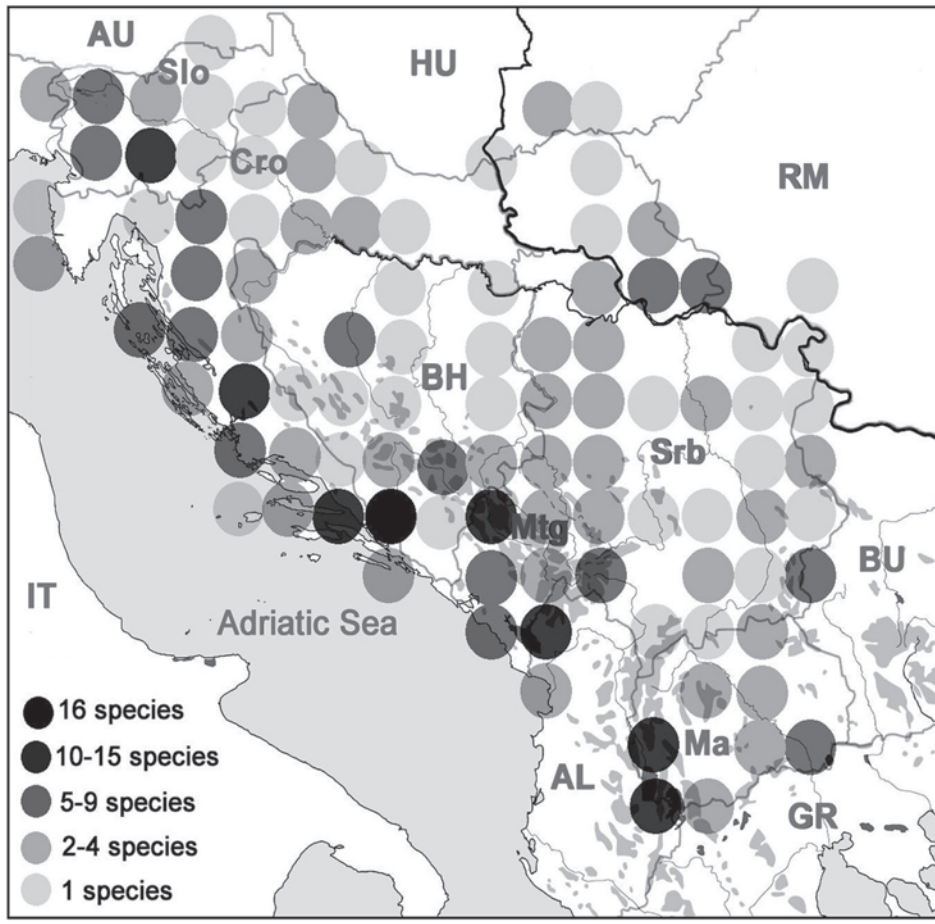

Fig. 4. Centres of richness and diversity of charophytes in the West and Central Balkans represented by number of charophyte species in UTM squares 50 × 50 km.

Endemism is an extremely interesting and relatively rare phytogeographic phenomenon among aquatic plants. Among the charophytes of the West and Central Balkans, several endemic species were recorded, with distribution restricted to some lakes or isolated watercourses. Such species are *C. visianii* J. Blaz. *et* V. Randj. in the Krka River (Blaženčić & Randjelović, 1994), *C. ohridana* in the Ohrid Lake (Krause, 1997) and Dojran Lake (Blaženčić & Blaženčić, 1999). The species *C. corfuensis* found only in the Baćinska lakes near the Neretva estuary could be considered as adriatic-ionian endemic since it also occurs on the Corfu Island, as cited by Wood & Imahori (1964, 1965). The species *C. rohlenae* was found in the stream Mratinje (Mt Maglić, Montenegro) by Vilhelm (1912), where it has not been observed recently and is thought to have disappeared (Fig. 5). These species deserve particular attention and protection owing to their restricted distribution and small populations in specific habitats.

Although the phytocenology of charophytes has only been sporadically studied in the area, more than 30 charophyte communities or communities in

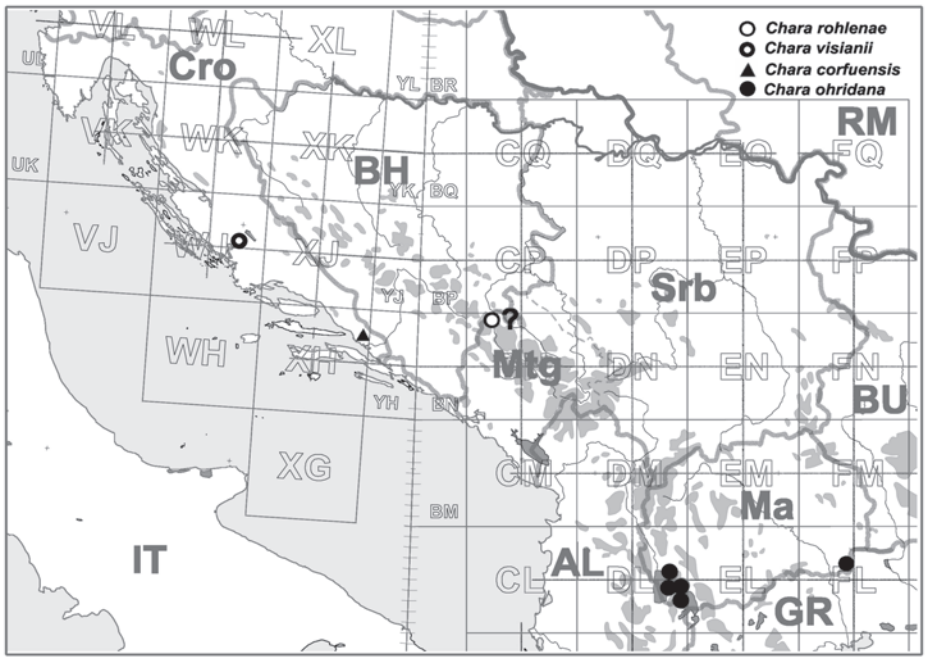

Fig. 5. Localities where the endemic charophytes were recorded - each dot corresponds to UTM square 10 × 10 km.

which these macroalgae are also cenobionts have been recorded (Lovrić, 1976, 1983, 1989; Blaženčić & Blaženčić, 1983, 1986, 1992, 1994, 1996, 2004; Randjelović *et al.*, 1993; Randjelović & Blaženčić, 1997; Blaženčić *et al.*, 1998).

Charophytes form monodominant and/or polydominant communities. On the basis of the available data it was established that these communities belong to the class *Charetea* Fukarek 1961 ex Krausch 1964, order *Charetalia* Sauer 1937 and the alliance *Charion fragilis* (Sauer 1937) Krausch 1964 as well as *Nitellion* prov. Dambska 1966. The mixed communities where higher aquatic plants and charophytes coexist belong to the class *Potametea* Tx. *et* Preising 1942, order *Potametalia* W. Koch 1926 and alliance *Eu-Potamion* (W. Koch 1926) Oberd. 1957, or to the class *Phragmitetea* Tx. *et* Preising 1942, order *Phragmitetalia* W. Koch 1926 and alliance *Phragmition australis* W. Koch 1926, as well as to the class *Fontinaletea antipyreticae* Hub. 1957, order *Fontinaletalia* Hub. 1957 and alliance *Fontinalion antipyreticae* Hub. 1957 (Randjelović *et al.*, 1993; Randjelović & Blaženčić, 1997).

Monodominant communities, built by *C. globularis* or *Nitella opaca*, occur in deeper and transparent lakes at the lower limit of distribution of macrophytic vegetation. Such communities are *Charetum globularis* (Corillion, 1957) J. Blaž. and *Nitelletum opacae* Corillion 1957 in the lakes of Plitvice (Croatia), at the depth of 13-20 m, as well as in the lake of Plav (Montenegro) at the depth of 5-6 m (Blaženčić & Blaženčić, 1986, 1994).

Communities formed by several species of charophytes are relatively rare and floristically determined as oligodominant. Most often they are distributed in

overlapping zones of populations of two or more species of charophytes, e.g. *C. globularis* and *Nitella opaca* or *C. vulgaris* and *C. contraria* or *C. globularis, C. virgata* and *Nitella opaca*, etc. Such communities are common in the lakes of Plitivice.

Mixed communities of charophytes and different vascular aquatic plants are more common, with charophytes being the dominant species in some of them. That is the case of *Charetum asperae* Corillion 1957, *Chareto-Lychnothamnetum barbati* V. Randj. & J. & Ž. Blaž. 1993 or *Chareto-Nitellopsidetum obtusae* J. & Ž. Blaž. 1983. In other cases, charophytes are sub-dominant, as in the communities *Myriophyllo verticillati* Soo 1927 or *Potametum perfoliati* W. Koch 1927 em. Passarge 1964 (Blaženčić & Blaženčić, 1983, 1986; Randjelović *et al.*, 1993).

The floristic diversity of charophyte communities decreases with water depth so that in deep aquatic ecosystems entirely uniform charophyte communities occur (monodominant), at the lower limit of distribution of macrophytic vegetation.

On the basis of recent data, more than a half of the charophytes, recorded in the West and Central Balkans are threatened to various degrees (Blaženčić *et al.*, 2006). In the threatened categories, under the revised IUCN criteria, there are 2 extinct (EX?), 12 critically endangered (CR), 13 endangered (EN), 8 vulnerable (VU), whereas only 7 charophyte species in the whole area studied are classified as low risk species (LR). Given that the charophytes are in such endangered state, we are willing to protect not only the species but also the undisturbed areas where they grow.

CONCLUSIONS

The heterogeneity of the West and Central Balkan aquatic environments has produced a great diversity and richness of vegetation, including charophytes. Forty two species from all the 6 extant charophyte genera were found in the fresh, brackish, salty and mineral waters of the region. Of particular interest is the species *Lamprothamnium papulosum* present in a salt marsh-like pond near the Adriatic coast where the salinity was 82.9‰.

Charophytes build monodominant communities usually at the lower depth limit in water, while in the shallower areas of aquatic environments they form either oligodominant algal communities or mixed communities with vascular aquatic plants. The floristic diversity of charophyte vegetation decreased with water depth and, in a similar manner, with the altitude of their aquatic habitats.

The establishment of centres of diversity and localities at which endemic species of charophytes survive is of particular importance, owing to the fact that in the entire area studied, the impact of human activity is severe. Bearing this in mind, it is clear that such sites deserve special protection, because this will provide for the conservation of a unique charophyte gene pool.

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