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# Distribution and habitat characteristics of *Vallisneria spiralis* L. in Croatia

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**Key words:** Butoniga Reservoir, Kupa River, macrophytes, *Potamogetonion, Nymphaeion albae*, Southeastern Europe, river ecology.

**Ključne besede:** umetno jezero Butoniga, reka Kolpa, makrofiti, *Potamogetonion*, *Nymphaeion albae*, jugovzhodna Evropa, rečna ekologija.

## Abstract

Eight new localities of the rare, strictly protected macrophyte *Vallisneria spiralis* have been recorded during a comprehensive survey of water bodies in Croatia. One record is located in the Mediterranean Region, in Butoniga Reservoir, while the remaining records are from the Continental Region, the majority of them situated along the Kupa River. *Vallisneria spiralis* occurred in slightly basic and alkaline environments and in a wide range of nutrient availability. In Continental localities, the species was present within *Myriophyllo-Nupharetum luteae* of the alliance *Nymphaeion-albae*, while in Butoniga, it occurred within the vegetation of the alliance *Potamogetonion*. Prior to our research, the species was recorded in Croatia only twice, with the older record dating back to the 19th century and the second from recent years, but neither one was confirmed in later surveys. The low number of records of *V. spiralis,* in spite of a wide-ranging search through hundreds of localities, confirmed the rare status of this species, although the reasons for such limited distribution remain unclear, especially bearing in mind that suitable aquatic habitats are quite widespread in the country.

## Izvleček

Med obširnimi raziskavami vodnih teles na Hrvaškem smo odkrili osem novih lokalitet redke, strogo zavarovane makrofitske vrste *Vallisneria spiralis*. Ena je v mediteranski regiji v umetnem jezeru Butoniga, preostale pa so v kontinentalni regiji, večina od njih se nahaja ob reki Kolpi. *Vallisneria spiralis* se pojavlja v zmerno bazičnih in kislih okoljih in v širokem razponu razpoložljivosti hranil. V kontinentalnem območju je vrsta prisotna predvsem v asociaciji *Myriophyllo-Nupharetum luteae* zveze *Nymphaeion-albae*, medtem ko v Butonigi uspeva v vegetaciji, ki jo uvrščamo v zvezo *Potamogetonion*. Pred našo raziskavo so vrsto zabeležili na Hrvaškem le dvakrat. Starejši podatek je iz 19. stoletja, novejša najdba pa je recentna, vendar v kasnejših pregledih ni bila nikoli potrjena. Majhno število najdb vrste *V. spiralis,* kljub intenzivnemu pregledu stotin lokalitet, potrjuje status redke vrste, čeprav razlogi za njeno redko pojavljanje ostajajo neznani, še posebej ker so primerni vodni habitati za njeno uspevanje v državi splošno razširjeni.

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# Introduction

*Vallisneria spiralis* L., commonly known as eelweed, eelgrass or tape grass, is a dioecious perennial freshwater plant native to southern Europe, northern Africa, the Middle East and southwest Asia (Les et al. 2008, Hussner 2012). The species is a submerged hydrophyte with fibrous roots, horizontal runners, short stem and linear leaves arranged in a basal rosette. Leaves are up to 100 cm long and 1 cm wide, with parallel veins and finely toothed margins (Casper & Krausch 1980). Male plants bear numerous minute flowers enclosed in a dehiscing two-valved spathe. When mature, the flowers completely detach from the spathe and rise to the surface, where they are dispersed by wind and currents. Pistillate flowers are singular, enclosed in a spathe and carried to the water surface on a long stalk. Following fertilization, the stalks coil spirally and fruits are withdrawn underwater where they mature (Les et al. 2008). In addition to dispersal by seeds, *V. spiralis* efficiently spreads asexually by runners, and can colonize new areas through plant fragments (Hutorowicz & Hutorowicz 2008, Heidbüchel et al. 2016).

*Vallisneria spiralis* is a typical component of lowland meso- to eutrophic aquatic habitats, both lotic and lentic (Hussner & Lösch 2005, Mukhopadhyay & Dewanji 2005, Bolpagni et al. 2015, Anđelković et al. 2016). It was reported from oligotrophic conditions as well, however, displaying lower growth rates (Bolpagni et al. 2015). The species shows high ecological plasticity, it promotes sediment stability and affects water column and sediment biogeochemistry, while its dense stands provide habitats for aquatic fauna (Pinardi et al. 2009, Ejsmont – Karabin 2011, Ribaudo et al. 2011).

*Vallisneria spiralis* is a thermophilous species originating from warmer climates and as such has become widespread mainly in tropical and subtropical, as well as in other areas outside its native range with milder and thus favourable climatic conditions. Furthermore, its introduced range has expanded to colder regions, such as Central, Northern and Eastern Europe, as well as Siberia, where it has been reported mainly from artificially heated and geothermal water bodies (Hussner & Lösch 2005, Hutorowicz & Hutorowicz 2008, Katsman & Kuchkina 2010, Zarubina & Sokolova 2011, Wasowicz et al. 2014). Nevertheless, climate change and the associated increase in water temperature could lead to the further spread of the species in areas of higher latitudes and altitudes (Hussner & Lösch 2005, Wu & Ding 2019). Since the species is a widely used aquarium plant, ornamental trading and release from aquaria into the wild are recognized as the main ways by which it is introduced (Hussner & Lösch 2005, Thiébaut 2007, Martin & Coetzee 2011, Hussner 2012).

The species is considered native to Croatia (Nikolić 2020) and is strictly protected under the Nature Protection Act (Official Gazette 80/13, 15/18, 14/19, 127/19). However, its distribution is poorly known, with only a few localities reported. Therefore, it is classified as Data Deficient (DD) (Nikolić 2020) according to IUCN criteria, although Least Concern (LC) globally (Gupta 2017). Prior to our research, *V. spiralis* had been recorded in Croatia only twice. The first record from the Continental Region dates back to the 19$^{th}$ century (Herbarium specimen ID - ZA12062, ZA Herbarium Croaticum), while the second is relatively recent and reported from the Mediterranean Region (Glasnović et al. 2015).

The aim of the present study was to determine the status of *V. spiralis* in Croatia. To accomplish this objective we (a) determined the current distribution of *V. spiralis* in Croatia, (b), identified its habitat preferences regarding the physico-chemical and chemical parameters, as well as water velocity and substrate type and (c) examined its preferences regarding the phytosociological characteristics.

# Materials and methods

## Study area

Data on the distribution of *Vallisneria spiralis*, as well as physicochemical and phytosociological characteristics of its habitats were collected mostly within the national system for monitoring of surface waters, and partly through an independent project focusing on the aquatic and riparian vegetation of the lowland watercourses. Over 600 locations were surveyed during the vegetation seasons from 2010 to 2019, ultimately covering most of the Croatian territory, including 277 rivers and 46 lakes (including reservoirs) distributed in three biogeographical regions – Continental, Alpine and Mediterranean (European Community 1992, European Environmental Agency 2020). Localities at which *V. spiralis* occurred are situated in the Continental Region (the Kupa and the Rečica rivers), as well as in the Mediterranean Region (Butoniga Reservoir) of Croatia (Figure 1).

Butoniga is a reservoir in the Istrian Peninsula constructed on the Butoniga River for the purpose of water supply and flood protection. The total reservoir area is 2.45 km$^2$, with an average depth of 9.1 m and is situated at 41 m a.s.l. The water body is classified as heavily modified (Vučković et al. 2019a), i.e. a body of surface water which is substantially changed in character as a result of physical alterations by human activity (European Community 2000).

The Kupa River is a right tributary of the Sava River, belonging to the Black Sea Hydrological Catchment. It rises

in the mountains of the Gorski Kotar Region, flows a few kilometres eastwards before forming a natural, 118 km long border between north-west Croatia and southeast Slovenia and then continues to flow through Croatia, reaching its confluence in the town of Sisak. The total length of the river is 279.4 km, with a catchment area of 10.226 km$^2$. In the upper and middle reaches, the river has a karstic character, while the lower reach, where *V. spiralis* was recorded, is classified as a large lowland river (European Community 2000, Mihaljević 2011). The Rečica River is a small lowland river with clayey-sandy substrate (European Community 2000, Mihaljević 2011), a right tributary of the Kupa River, with its mouth near the settlement Donja Rečica.

## Macrophyte survey

Watercourses were surveyed for macrophytes along 100 m long transects, while multiple 6 m × 100 m transects were used when surveying macrophytes in lakes. In less-accessible areas, the bottom was raked to reach the macrophytes, using either a long pole or a rope with a rake. Cover and abundance of each plant species were estimated using the expanded nine-degree Braun-Blanquet scale: r = one individual; + = up to 5 individuals; 1 = up to 50 individuals; 2 m = over 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).

All macrophytes were identified to the species level. The identification of *Vallisneria spiralis* was based on the morphological characteristics with the use of several identification keys (Casper & Krausch 1980, Lowden 1982, van de Weyer et al. 2011, Nikolić 2019). Collected specimens of *V. spiralis* were preserved in 50% ethanol with 10% of glycerol added, or desiccated in a herbarium press and deposited in herbarium ZA (Thiers 2020) (ID numbers ZA56139 - ZA56159). The nomenclature follows EURO+MED (2020) for vascular plants and AlgaeBase (Guiry & Guiry 2020) for Charophytes, while the syntaxonomical system proposed by Mucina et al. (2016) and Škvorc et al. (2017) was applied for the vegetation types. The coordinates of the localities were recorded using an e*T*rex 30x GPS device and a distribution map was created using ArcGIS 10.5 software.

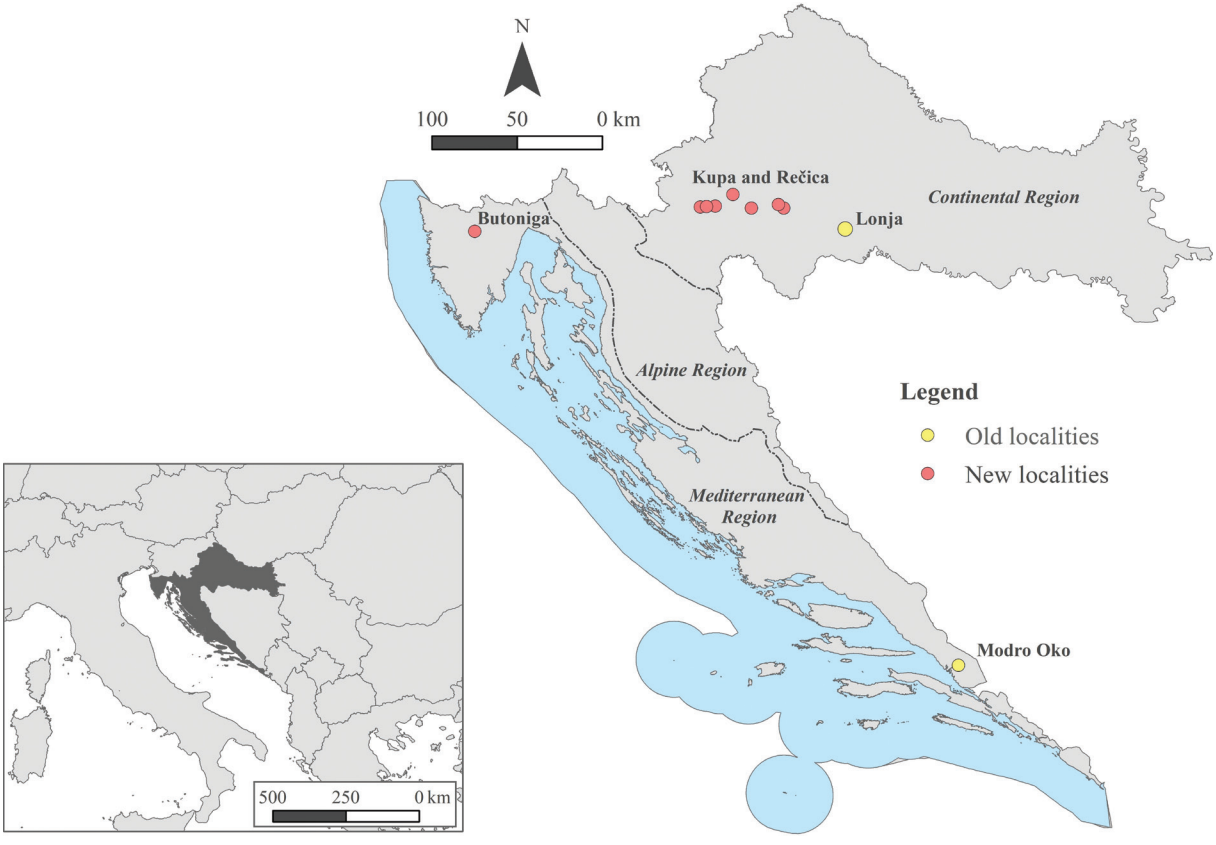


**Figure 1:** Distribution map of *Vallisneria spiralis* in Croatia.
**Slika 1:** Karta razširjenosti vrste *Vallisneria spiralis* na Hrvaškem.

Furthermore, all localities within the national surface water monitoring system were sampled for basic water physicochemical parameters and nutrients. Conductivity, salinity and pH were measured *in situ* with the SevenMulti Modular Meter System (Mettler Toledo) under standard conditions. Dissolved oxygen was measured *in situ* with a 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 of 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 collected in dark bottles, filled to the top, stored at 4–8 °C 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). The box-plot diagrams of physicochemical and chemical parameters were prepared using the program package Past 4.02 (Hammer et al. 2001). Water flow velocity was estimated according to Janauer (2003), who distinguishes four categories (1 = no flow, stagnant, 2 = low flow velocity, from just visible to ca. 30 cm/s, 3 = medium flow velocity, ca. 35–65 cm/s, 4 = high flow velocity, ca. > 65 cm/s).

# Results

Eight new localities of *Vallisneria spiralis* were recorded during the research of aquatic vegetation in Croatia (Figure 1). The majority of the localities are situated in the Continental part of Croatia. Six are distributed along 96.05 km of the lower course of the Kupa River (Figure 2), while a single locality is recorded in the Rečica River, near its confluence with the Kupa. The single locality in the Mediterranean part is from Butoniga Reservoir in Istria.

In the Kupa and Rečica rivers, *V. spiralis* was rooting in depths of up to 1.5 m, on muddy, sandy and gravelly substrate in medium and slow flowing water. During June and early July, flowering was not observed, while flowering individuals were recorded in early August. Male plants were found in Gradec Pokupski and Letovanić, while female plants were observed in Mala Gorica (Figure 2, Figure 3).

In the Kupa River, dense stands of *V. spiralis* were observed in Letovanić, where aquatic vegetation was abundantly developed and dominated by *Potamogeton nodosus*, *Myriophyllum spicatum* and *Najas marina* (Figure 3). *Vallisneria spiralis* abundance was lower in other localities in the Kupa, where it was associated with the more abundant *M. spicatum*, *Ceratophyllum demersum* and *Nuphar lutea* (Table 1). In the Rečica River, all macrophyte species were represented with only a small number of individuals. Surveyed aquatic vegetation at both rivers belongs mostly to the alliance *Nymphaeion-albae* Oberd. 1957 (class *Potamogetonetea* Klika in Klika et Novák 1941), i.e. vegetation of rooted floating-leaf macrophytes of sheltered nutrient-rich freshwaters of Western and Central Europe. The communities can be assigned to the association *Myriophyllo-Nupharetum luteae* (W. Koch 1926) Hueck 1931. However, elements of the alliance *Potamogetonion* Libbert 1931 are also present, since the communities gradually change depending on the water depth and velocity.

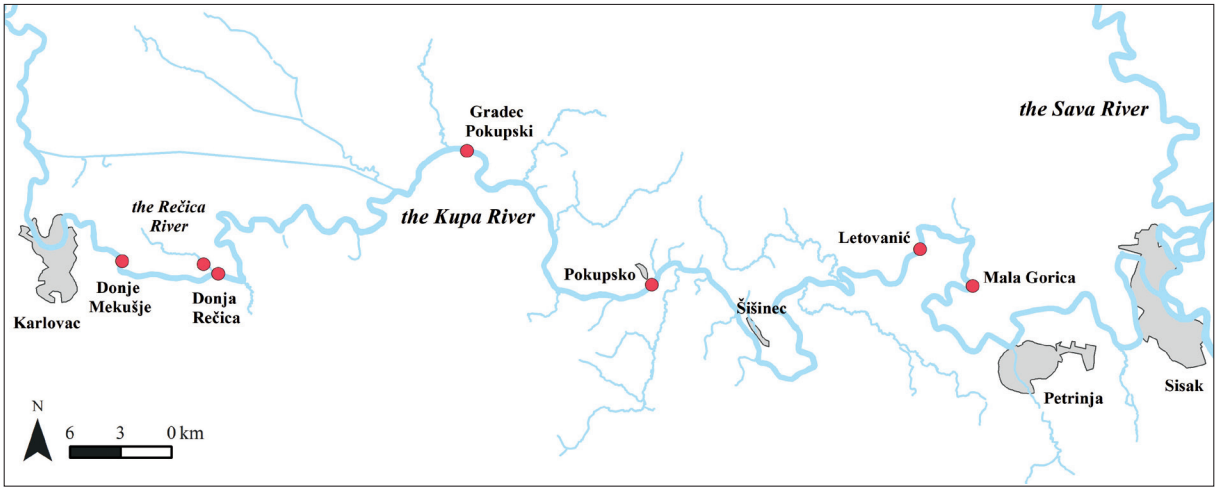


**Figure 2:** *Vallisneria spiralis* localities along the Kupa and Rečica rivers.
**Slika 2:** Lokalitete vrste *Vallisneria spiralis* vzdolž rek Kolpa in Rečica.


**Figure 3:** (A) *Vallisneria spiralis* habitus; (B, C) female plants at Butoniga Reservoir; (D) stand of *V. spiralis* in Letovanić; (E) Mala Gorica, typical habitat at the Kupa River.
**Slika 3:** (A) habitus vrste *Vallisneria spiralis*; (B, C) ženske rastline v umetnem jezeru Butoniga; (D) sestoj vrste *V. spiralis* pri kraju Letovanić; (E) Mala Gorica, značilno rastišče ob reki Kolpi.

The water in the Kupa River was slightly basic and alkaline, with conductivity not exceeding 428 µS/cm at any investigated locality. The measured concentration of reactive phosphorous was low, with annual average concentration ranging from <0.005 in the most upstream locality to 0.019 mgP/L in the most downstream locality. The measured concentration of ammonium was low at all investigated localities, while the concentration of nitrates was moderately elevated, but still indicating a good status regarding the eutrophication (Table 2, Figure 4). On the contrary, the measured concentration of nutrients in the Rečica River indicated eutrophication. In this locality, water was also slightly basic, alkaline, with conductivity ranging between 89.9 and 465 µS/cm (Table 2, Figure 4).

In Butoniga Reservoir, flowering female plants (Figure 3) were recorded in early August, rooted in the muddy substrate in up to 1 m deep, stagnant water. The species was here present within the vegetation of alliance *Potamogetonion*, codominant with *Potamogeton perfolatus* and *P. nodosus*, as well as *Chara vulgaris* (Table 1), a member of alliance *Charion vulgaris* (W. Krause et Lang 1977) W. Krause 1981 (class *Charetea intermediae* F. Fukarek 1961). Water in Butoniga Reservoir was clear, slightly basic and alkaline, with conductivity ranging from 286 to 428 µS/cm. Regarding the measured nutrient concentrations, the reservoir is oligotrophic (Table 2, Figure 4).

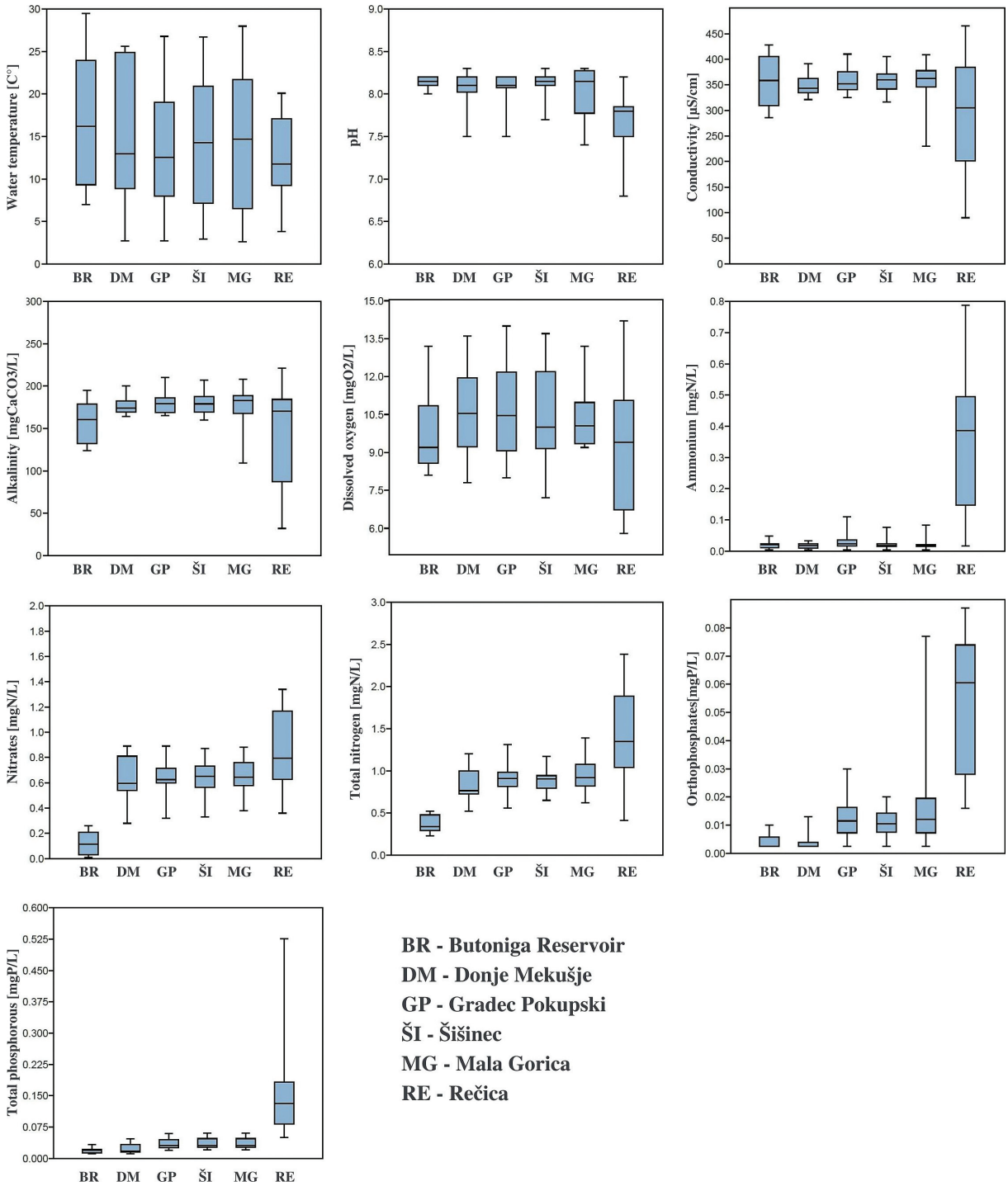


**Figure 4:** The box-plot diagrams of water physicochemical and chemical parameters at *Vallisneria spiralis* localities. * The values measured at Šišinec, situated between Pokupsko and Letovanić, were used as the measurements were not performed at the two latter sites.

**Slika 4:** Grafikoni kvantilov fizikalno - kemijske in kemijske lastnosti vode na lokalitetah vrste *Vallisneria spiralis*. * Vrednosti, merjene na lokacijah pri kraju Šišinec, med krajema Pokupsko in Letovanić, smo uporabili, ker nismo naredili meritev na slednjih dveh lokacijah.

**Table 1:** List of species at finding sites of *Vallisneria spiralis*, with the estimated cover and abundance according to expanded Braun-Blanquet scale and coordinates in WGS84 coordinate system, x = longitude, y = latitude. At locality Pokupsko (x =15.991128, y = 45.485593), visited in 2016, relevé was not made.

**Tabela 1:** Seznam vrst na rastišču vrste *Vallisneria spiralis* z ocenjeno pokrovnostjo in pogostnostjo v skladu z razširjeno Braun-Blanquetovo skalo in koordinate v WGS84 koordinatnem sistemu, x = zemljepisna dolžina, y = zemljepisna širina. Na lokaciji Pokupsko (x =15,991128, y = 45,485593), ki smo jo obiskali leta 2016, nismo naredili vegetacijskega popisa.

| **Finding site** | **Butoniga Reservoir** | **K u p a R i v e r** | | | | | **Rečica River** |
|---|---|---|---|---|---|---|---|
| | | **Donje Mekušje** | **Donja Rečica** | **Gradec Pokupski** | **Letovanić** | **Mala Gorica** | |
| **Year of sampling** | 2015 | 2016 | 2016 | 2016 | 2018 | 2016 | 2016 |
| **E** | 13.929641 | 15.597712 | 15.670385 | 15.857849 | 16.200952 | 16.240963 | 15.666359 |
| **N** | 45.326645 | 45.487189 | 45.481073 | 45.547518 | 45.496732 | 45.477213 | 45.481133 |
| *Vallisneria spiralis* L. | 2a | 2m | 2m | 1 | 2a | 2m | 1 |
| *Nuphar lutea* (L.) Sm. | . | 1 | 4 | 2m | 2a | 2a | 1 |
| *Myriophyllum spicatum* L. | . | 1 | 4 | 2m | 2b | 2a | . |
| *Ceratophyllum demersum* L. | . | + | + | 2m | 2a | 2a | 1 |
| *Alisma plantago-aquatica* L. | . | + | + | r | + | . | 1 |
| *Potamogeton perfoliatus* L. | 2b | . | 1 | 1 | . | 1 | . |
| *Potamogeton nodosus* Poir. | 2a | . | . | 1 | 2b | . | 1 |
| *Stuckenia pectinata* (L.) Börner | . | 2m | . | 1 | 2m | . | . |
| *Najas marina* L. | . | . | + | 1 | 2b | 2a | . |
| *Najas minor* All. | . | . | . | 1 | 2a | + | . |
| *Potamogeton berchtoldii* Fieber | . | . | . | . | 2m | 2m | . |
| *Nitellopsis obtusa* (Desv.) J.Groves | . | . | . | . | + | 2m | . |
| *Elodea canadensis* Michx. | . | . | + | . | 1 | . | . |
| *Sparganium emersum* Rehmann | . | . | 1 | . | . | . | 1 |
| *Potamogeton pusillus* L. | . | . | . | + | . | . | 1 |
| *Chara vulgaris* L. | 2b | . | . | . | . | . | . |
| *Nitella opaca* (Bruz.) C.Agardh | . | + | . | . | . | . | . |
| *Persicaria dubia* (Stein) Fourr. | . | . | . | 1 | . | . | . |
| *Rorippa sylvestris* (L.) Besser | . | . | . | 1 | . | . | . |
| *Alisma lanceolatum* With. | . | . | . | r | . | . | . |
| *Ranunculus fluitans* Lam. | . | . | . | . | 2m | . | . |
| *Butomus umbellatus* L. | . | . | . | . | . | + | . |
| *Lemna minor* L. | . | . | . | . | . | 2m | . |

**Table 2:** Basic physicochemical and chemical parameters of water at *Vallisneria spiralis* localities, based on monthly measurements during the year of sampling. *The values measured at Šišinec, situated between Pokupsko and Letovanić, are given instead as the measurements were not performed at the two latter sites.

**Tabela 2:** Osnovne fizikalno - kemijske in kemijske lastnosti vode na lokalitetah vrste *Vallisneria spiralis* na osnovi mesečnih meritev v letu vzorčenja. * Vrednosti, merjene na lokacijah pri kraju Šišinec, med krajema Pokupsko in Letovanić, smo uporabili, ker nismo naredili meritev na slednjih dveh lokacijah.

| Parameter | Butoniga Reservoir | Kupa River | | | | Rečica River |
|---|---|---|---|---|---|---|
| | | Donje Mekušje | Gradec Pokupski | Šišinec* | Mala Gorica | |
| water temperature [°C] | | | | | | |
| range | 7–29.5 | 2.7–25.6 | 2.7–26.8 | 2.90–26.7 | 2.6–29 | 3.8–23.5 |
| avg. | 16.80 | 15.30 | 15.10 | 16.20 | 17.00 | 13.20 |
| pH | | | | | | |
| range | 8–8.2 | 7.52–8.3 | 7.48–8.2 | 7.72–8.3 | 7.36–8.3 | 6.8–8.2 |
| avg. | 8.10 | 8.10 | 8.00 | 8.10 | 8.00 | 7.70 |
| conductivity [µS/cm] | | | | | | |
| range | 286–428 | 321–391 | 325–410 | 316–405 | 230–409 | 89.9–465 |
| avg. | 359 | 350 | 358 | 357 | 354 | 297 |
| alkalinity [mg$CaCO_3$/L] | | | | | | |
| range | 124–195 | 164–200 | 165–210 | 160– 207 | 109–208 | 32–221 |
| avg. | 158 | 177 | 180 | 180 | 176 | 147 |
| dissolved oxygen [mg$O_2$/L] | | | | | | |
| range | 8.1–13.2 | 7.8–13.6 | 8–14 | 7.2–13.7 | 9.2–13.2 | 5.77–14.2 |
| avg. | 9.70 | 10.70 | 10.70 | 10.40 | 10.40 | 9.20 |
| ammonium [mgN/L] | | | | | | |
| range | — | <0.008–0.033 | <0.008–0.110 | <0.008–0.076 | <0.008–0.083 | 0.016–0.788 |
| avg. | — | 0.017 | 0.031 | 0.023 | 0.023 | 0.352 |
| nitrates [mgN/L] | | | | | | |
| range | 0.02–0.26 | 0.28–0.89 | 0.32–0.89 | 0.33–0.87 | 0.38–0.88 | 0.36–1.34 |
| avg. | 0.15 | 0.63 | 0.65 | 0.65 | 0.66 | 0.84 |
| total nitrogen [mgN/L] | | | | | | |
| range | 0.23–0.52 | 0.52–1.20 | 0.56–1.31 | 0.65–1.17 | 0.62–1.39 | 0.41–2.38 |
| avg. | 0.37 | 0.83 | 0.92 | 0.88 | 0.96 | 1.42 |
| orthophosphate [mgP/L] | | | | | | |
| range | <0.005–0.010 | <0.005–0.010 | <0.005–0.030 | <0.005–0.020 | <0.005–0.077 | 0.016–0.087 |
| avg. | 0.004 | <0.005 | 0.012 | 0.011 | 0.019 | 0.052 |
| total phosphorous [mgP/L] | | | | | | |
| range | 0.010–0.030 | 0.011–0.047 | 0.020–0.059 | 0.021–0.060 | 0.029–0.117 | 0.050–0.526 |
| avg. | 0.020 | 0.023 | 0.036 | 0.036 | 0.053 | 0.160 |

# Discussion

Prior to our research, *Vallisneria spiralis* has been reported only from two localities in Croatia, first from the Lonja River, a record dating back into the 19th century (Herbarium specimen ID - ZA12062, ZA Herbarium Croaticum) and second from the Modro Oko Spring, reported in 2011 (Glasnović et al. 2015). The Lonja River is a medium-sized, eutrophic river, situated in the Continental part of Croatia. During our research, several localities in the river have been surveyed, including the stretches near Sovoj and Trebež, where the first record was made, but *V. spiralis* was not recorded. Extensive hydromorphological modifications in the 1970s, including the construction of dykes and retention, significantly altered the river flow and certain parts of the river completely dried up. As a consequence of the abovementioned changes, it is possible that *V. spiralis* no longer persists in this watercourse. The Modro Oko Spring is an oligohaline limnocrene karst spring situated in the Neretva River Delta in the Mediterranean part of Croatia, with clear water of high alkalinity and conductivity (Rimac et al. 2018). The aquatic vegetation of the spring was surveyed several times, from 2014 to 2016 (Rimac et al. 2018) and in 2018 (Vuković et al. 2018, 2019), however, *V. spiralis* was never confirmed.

In all localities surveyed within this study, measured values of pH and alkalinity indicate slightly basic, alka-

line conditions and along with measured water temperature and electrical conductivity fall within the ranges previously reported for *V. spiralis* (Hussner & Lösch 2005, Mukhopadhyay & Dewanji 2005, Rai & Tripathi 2009, Ejsmont-Karabin & Hutorowicz 2011, Collas et al. 2012). The species was growing on various substrates, muddy, sandy and gravely, in up to 1.5 m deep water, as already observed in other studies (Hussner & Lösch 2005, Zarubina & Sokolova 2011). The species is a typical component of lowland, meso- to eutrophic aquatic systems (Hutorowicz 2006, Pyka et al. 2007, Gecheva et al. 2011, Landucci et al. 2011), but known to occur in oligotrophic conditions as well (Bolpagni et al. 2015). Regarding the nutrient level, localities in Croatia cover considerable range, with the lowest level in oligotrophic Reservoir Butoniga, where *V. spiralis* was accompanied with *Chara vulgaris* and *Potamogeton perfoliatus*, both good indicators of low phosphorous concentration (Stefanidis et al. 2018). A study conducted in Greece showed that lake vegetation types characterized by the dominance of *P. perfoliatus* and *V. spiralis* appear to be associated with high nitrate concentrations (Stefanidis et al. 2019), but this was not the case in Croatia. In the Rečica River, levels of nutrients were high and *V. spiralis* was growing with *Ceratophyllum demersum* and *Potamogeton pusillus*, species tolerant to eutrophication (Smolders et al. 2001). In this locality, aquatic vegetation was sparsely developed, with all species represented with only few individuals, presumably because of considerable hydromorphological alterations, i.e. channelization and riverbed deepening.

The majority of localities are situated along the Kupa River, which is in near-natural condition along its entire course (Urbanič et al. 2020). Measured levels of nutrients suggest satisfactory status for a lower course of a lowland river, with only moderately raised concentration of nitrates. Here, *V. spiralis* was associated with species characteristic for lowland medium- to slow-flowing watercourses, such as *Potamogeton nodosus, Myriophyllum spicatum, Najas marina*, *C. demersum* and *Nuphar lutea*, within assemblages generally associated with more eutrophic water. These communities, as well as the community from the Rečica River can be assigned to the association *Myriophyllo-Nupharetum luteae* (W. Koch 1926) Hueck 1931., where constant presence of *C. demersum* indicates nutrient enrichment, at least in the sediment. Such communities are known to develop near the banks, in slow flowing and shallow segments of the rivers. Regarding the hydromorphology, localities on the Kupa River were assessed as in near natural condition, with no or only minor alternations in hydrological regime, longitudinal connectivity and morphology (Vučković et al. 2019b).

Eight newly recorded localities resulting from a comprehensive study of the aquatic vegetation covering the majority of the country's territory provide a valuable new insight into the distribution of *V. spiralis* in Croatia, confirming its status of a rare species. The species is considered native to Croatia and as a very rare is strictly protected. However, the information on its origin in other Southeastern European countries is contradictory. According to European Floras, the species is native to the whole Southeastern Europe (Hayek 1932–1933, Tutin et al. 1964–1980), but on the contrary, it is reported as an alien from Serbia (Lansdown et al. 2010, Anačkov et al. 2011, 2013), Romania (Ciocârlan 2009) and European Turkey (Lansdown et al. 2010). Furthermore, according to Soó (1964–1980), *V. spiralis* is usually considered native to the Mediterranean parts of the Balkans, while occurrences in the Pannonian ecoregion are most likely alien. In the neighbouring Serbia, *V. spiralis* is a most frequently recorded aquatic alien plant (Anđelković et al. 2016), listed as invasive in the Province of Vojvodina (Anačkov et al. 2011, 2013). It mostly inhabits tidal rivers, as well as permanent non-tidal smooth flowing watercourses, with the majority of records from the waterbodies situated in the Danubian floodplain, belonging to the Pannonian ecoregion (Radulović et al. 2010, Anđelković et al. 2016). Similarly, it is reported from Danube Delta and Banat Region (Ciocârlan 2011, Otves et al. 2014) in Romania, where the species is naturalized according to Anastasiu et al. (2007) and Lansdown et al. (2010) and potential threat to aquatic ecosystems (Sîrbu 2007, Otves et al. 2014). On the other hand, records from its native range in the Mediterranean, mostly include stagnant water bodies of different ecological characteristics. In Greece, *V. spiralis* is dominant or common macrophyte in shallow littoral zone of deep oligotrophic lakes, as well as in shallow meso-eutrophic and eutrophic lakes on calcareous bedrock, situated both at low- and mid-altitudes (Koumpli-Sovantzi 1989, Danielidis 1996, Hollis & Stevenson 1997, Chalkia & Kehayias 2013a,b, Matzafleri et al. 2013, Stefanidis et al. 2018). Furthermore, it is present in both Macedonian and Greek part of Lake Megali Prespa, a large, deep, basic and mesotrophic lake, situated at 850 m a.s.l (Talevska et al. 2009, Stefanidis et al. 2018.). In Italy, the species was also recorded at mid-altitudes, in shallow and basic lakes – eutrophic Lake Chiusi (Stella 1988, Lastrucci et al. 2014) and meso- to eutrophic Lake Trasimeno (Landucci et al. 2011). In both lakes, it was present and dominant within association *Potamo perfoliati-Vallisnerietum spiralis* Losev et Golub in Gloub, Losev et Mirkin 1991 (Lastrucci et al. 2014), while in Lake Trasimeno, it occurred in several other communities of the classes *Potamogetonetea* and *Charetea intermediae* (Landucci et al. 2011).

Although the majority of populations of *V. spiralis* in Croatia are located in the Continental Region, which for the most part corresponds to the Pannonian ecoregion in terms of the Water Framework Directive (European Community 2000), there is no evidence that the species has been introduced at some point. Having in mind its ecological preferences, and the fact that suitable aquatic habitats are quite widespread in Croatia, such limited distribution is indeed interesting and unexpected, especially the absence from the waterbodies of the Danube floodplain in eastern Croatia, which were quite well investigated in recent times (e.g. Ozimec & Topić 2001, Kočić et al. 2008, Ozimec & Topić 2018, Rožac et al. 2018, Nikolić 2020).

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