

## Distribution and morphological variations of invasive macrophytes *Elodea nuttallii* (Planch.) H. St. John and *Elodea canadensis* Michx in Croatia

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**Abstract** – The invasive species *Elodea nuttallii* was recorded for the first time in the Croatian flora in 2006, in the drainage channels of Kopački rit (Baranja). After its establishment, *E. nuttallii* begins to spread to the eastern and northern part of the drainage channel network from 2006–2009. High water levels are responsible for the linear spreading direction of *E. nuttallii*, *E. nuttallii* and *E. canadensis* show a wide range of morphological variation, which is characteristic of successful invaders. To show morphological variations of two *Elodea* species, the most important characters indicated in the literature were measured on 24 fresh collected samples from the seven sites in Croatia. In spite of some overlap in leaf length and width between the two *Elodea* species, the differences of all morphological traits except internode length are statistically significant. In *E. nuttallii* leaf width, length and internode length show a higher morphological variability as a result of the higher adaptive strategy to environmental parameters. The most reliable morphological characters distinguishing *E. nuttallii* and *E. canadensis* are leaf width 0.5 mm below the tip and the angle at the apex. *E. nuttallii* can be expected to spread to other areas of Croatia.

**Keywords:** distribution, *Elodea canadensis*, *Elodea nuttallii*, invasive species, morphological variation

### Introduction

The genus *Elodea* Michx. (Hydrocharitaceae) consists of rooted, submerged, perennial, freshwater macrophytes, native to North America. Since the 19<sup>th</sup> century, several *Elodea* species have become adventive in Europe. The first record was *Elodea canadensis* Michx.

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From the first finding reported in 1836 in a pond in Ireland (SIMPSON 1984), it became widespread in north and central European countries. By the middle of the last century it seems to have reached the maximum extent of its distribution (SIMPSON 1986, 1990, RODWELL 1995). In Croatia it was recorded for the first time in 1894 in Ješkovo pond in Gola, Podravina (NIKOLIĆ 2009).

The second species, *Elodea nuttallii*, has been known in Europe since 1939. The plant was first recorded in Belgium (WOLFF 1980, SIMPSON 1984) and it is spreading rapidly. It has displaced *E. canadensis* and has become as frequent in northern Europe as *E. canadensis* was before. So far, *E. nuttallii* has colonised most of Croatia's neighbouring countries: Hungary (KIRÁLY et al. 2007b), Slovenia (KIRÁLY et al. 2007a, MAZEJ GRUDNIK and SAVINEK 2010, MAZEJ GRUDNIK and GERM 2013), Serbia (VUKOV et al. 2006, LJEVNAIĆ-MAŠIĆ 2010), as well as all national stretches of the Danube, upstream (Germany, Austria, Slovakia) and downstream (Serbia, Bulgaria, Romania) from Croatia. It is only in Croatia in the Danube River Basin that it has not been recorded (JANAUER et al. 2007).

Since only female plants of the two *Elodea* species were introduced in Europe, both propagate mainly by vegetative reproduction (SIMPSON 1986, JAMES et al. 1999). The rapid growth, the rapid spread provided by vegetative reproduction, and the phenotypic plasticity are all characteristics of successful invaders. Due to low genetic variability of invasive species in the introduced range (RIIS et al. 2010) phenotypic plasticity is an essential of *Elodea* invasiveness in aquatic environments (HÉRAULT et al. 2008). By producing a better phenotype-environment match (AGRAWAL 2001) it maximizes fitness in variable conditions. In *E. nuttallii*, the extremes of the morphological variation are such that they appear to be distinct taxa in Europe, one with long, flat leaves, and the other with shorter, broader, strongly reflexed leaves (SIMPSON 1988, THIÉBAUT and DI NINO 2009). SIMPSON (1988) described *E. nuttallii* as the most variable of the species in the British Isles.

However, as a result of its wide range of morphological variations there are some differences among authors about the main distinguishing characters of *Elodea* species. These are linked to leaf length and width and the curvature of the plants. Because of this variability, we measured the most important characters indicated in the literature on fresh material of *Elodea* species collected in the field surveys to identify morphological features that are the most important for quick determination in field surveys. Thus, the first aim of our study was to show the distribution of *E. nuttallii* as a new species and the new sites of *E. canadensis*, which is well established in Croatia. The second aim was to indicate the main differences between two *Elodea* species in Croatia and give an identification key with the most important morphological characters.

## Materials and methods

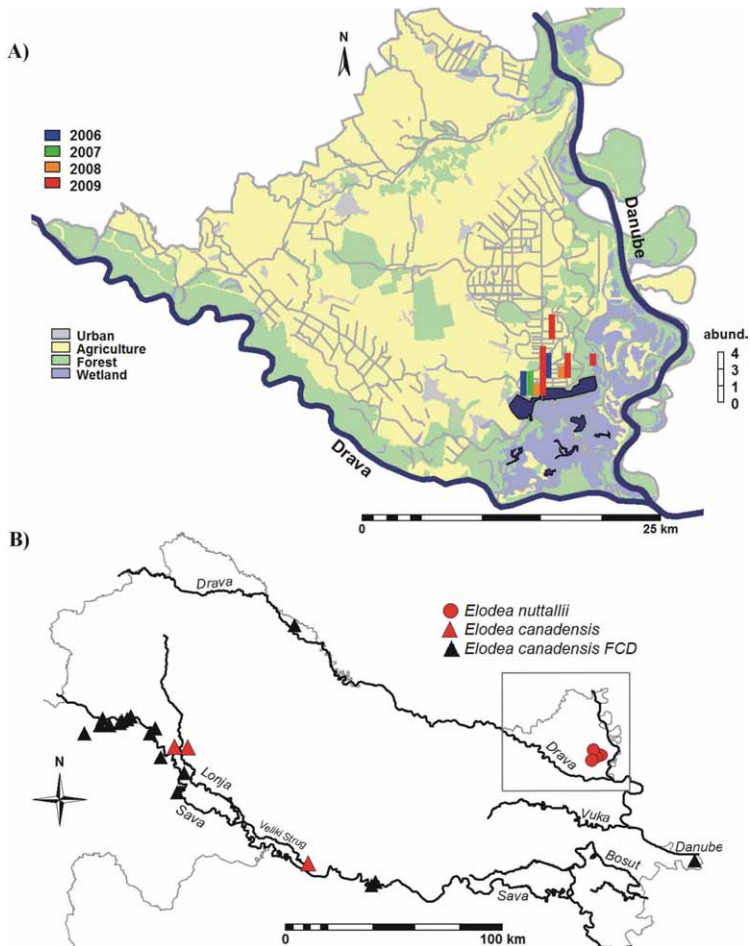
### Study area

One of the largest remaining floodplain areas in Central Europe stretches on territory belonging to Hungary, Croatia and Serbia and lies where the Danube and Drava rivers meet. In the Croatian part, the Croatian Baranja region (Fig. 1), the floodplain area lies within the triangle between the Danube and Drava rivers and is protected as the Kopački rit Nature Park. In the past, the area was covered by alluvial forests and marshes and was under the constant impact of flood water. The marshy and flooded area was considerably reduced dur-

ing the 19<sup>th</sup> century by reclamation activities. After the drainage network system was constructed, parts of the former floodplain were turned into large agricultural fields.

The water in the drainage channel system is controlled by the Danube and Drava rivers. High water levels in the channel system are most frequent in May and June and low water levels from August to October. Occasionally, water level can also be high in autumn, due to the high amount of precipitation.

The drainage network system of Baranja mainly drains the arable land. Resulting fertilizer run-off causes eutrophication, leads to channels being overgrown with vegetation and consequently, due to mud sedimentation, the channels become shallower. Thanks to finan-



**Fig. 1.** A) The spread of *E. nuttallii* in Baranja (Croatia) in the investigated period 2006–2009. Corine land cover map with classes: agriculture, forest, urban and wetland is used as background of Baranja. The height of the bar represents the adjusted Braun-Blanquet scale of abundance. B) Distribution map of *E. nuttallii* and *E. canadensis* in Croatia. Distribution map of *E. canadensis* represents the new sites in the Lonja River Basin (▲) and known distribution according to Flora Croatica Database – FCD (▲).

cial investment in the waterways of Baranja during the last few years, the drainage channels are subject to management with regular harvesting and maintaining.

## Methods

Field work was carried out at 75 sites that were monitored during 2006, 2007, 2008 and 2009 in the summer (July–August). *E. canadensis* and *E. nuttallii* were found at seven sites. The percentage cover occupied by the *Elodea* species was determined visually from the 50 m reference channel length (HAURY et al. 1996) and converted to a Braun-Blanquet scale (BRAUN-BLANQUET 1964) adjusted as follows; 1 = a few scattered specimens,  $2 \leq 5\%$ ,  $3 = 5\text{--}25\%$ ,  $4 = 25\text{--}50\%$ ,  $5 = 50\text{--}75\%$ ,  $6 > 75\%$ . The *Elodea* species were identified according to CASPER and KRAUSCH (1980) and SIMPSON (1986).

During the investigated period, *E. canadensis* was found at three sites and seven samples were collected at different times, while *E. nuttallii* was found at four sites and 17 samples were collected at different times. Voucher specimens were deposited in a private herbarium. A few plants of *Elodea* species were randomly collected to investigate the morphological variations of each *Elodea* sample. The average values of collected plants for each sample were used in further analysis for each measured morphological trait. All collected samples were analysed morphometrically in the laboratory. Samples of the fresh plants were captured by digital imaging equipment. Scion Image Beta 4.0.3 software (Scion Corporation 1997–2005) was used to measure the morphological traits of each *Elodea* plant: leaf length, leaf width at the mid-point of the fully developed upper leaves, leaf width 0.5 mm below the tip, the angle at the apex between leaf margin and midrib, as well as the internode length, obtained as the length of ten internodes below the three-cm long apex. Also, the width/length ratio was further calculated and the curvature of the leaves was visually determined. The t-test was used to compare the differences of the morphological traits between the two species.

## Results

During the period of investigation, *E. nuttallii* and *E. canadensis* had geographically separated distributions in two different River Basins (Fig. 1B): *E. canadensis* appears in the Lonjsko polje floodplain (Sava River Basin) and *E. nuttallii* in the Kopački rit floodplain (the mouth of the River Drava into the Danube). *E. nuttallii* was found at four sites in the drainage channel system of Baranja (Fig. 1B). After it was established in the period from 2006–2008 in Kopački rit floodplain, linear spreading along the drainage network channels was noted in 2009 (Fig. 1A). *E. canadensis* was found at three new sites in Lonja River Basin in the central part of Croatia during the investigation period. At all investigated sites, both *Elodea* species for the most part grew in relatively rich communities and never as the dominant species.

The most significant taxonomical character of the two *Elodea* species is connected to the male flowers (DANDY 1980) and the leaf curvature (BOWMER et al. 1995, SELL and MURRELL 2007). With an evident lack of petals, male flower of *E. nuttallii* breaks off and floats on the surface in anthesis. However, only female plants have been found in Europe (SIMPSON 1988). Furthermore, most of the samples collected in the research area of *E. nuttallii* had only straight and no recurved leaves (Fig. 2A). In both *Elodea* species the leaves are usually in whorls of three (rarely more, sometimes two). In *E. nuttallii* leaves were longer (7.62–14.9 mm) and

narrower at the mid-point (0.63–1.64 mm) and at 0.5 mm below the tip (0.33–0.72 mm). In *E. canadensis* leaves are shorter (5.62–9.61 mm) and wider at the mid-point (1.83–2.38 mm) and at 0.5 mm below the tip (1.03–1.51 mm). Internode length is similar in both *Elodea* species. The angle at the leaf apex in *E. nuttallii* did not exceed 34°, while in *E. canadensis* it is in a range between 50° and 60°. The width/length ratio is also higher in *E. canadensis* (0.24–0.40) than in *E. nuttallii* (0.09–0.17).

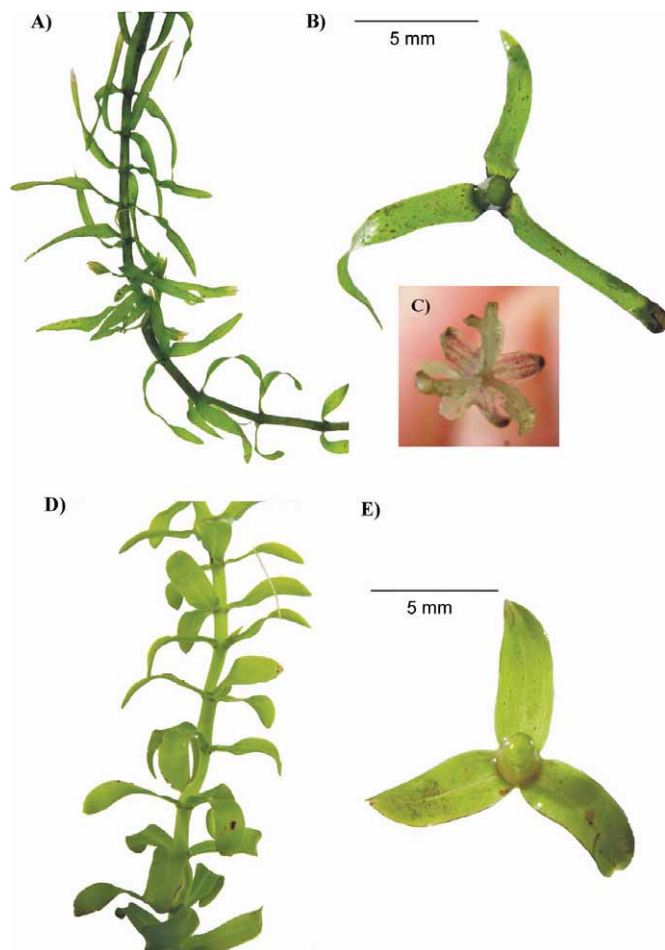
The most reliable characters for distinguishing *E. canadensis* from *E. nuttallii* were the width of a leaf measured at a point approximately 0.5 mm below the apex, the angle at the apex and the width/length ratio (Tab. 1). They were greater in *E. canadensis* and show the shape of the leaf apex, which is the most important taxonomical feature indicated in identification keys (SIMPSON 1984, DANDY 1980). In *E. nuttallii* leaf apices are acuminate or narrowly acute, while in *E. canadensis* they are obtuse or broadly acute (Fig. 2).

**Tab. 1.** Morphological differences between species *Elodea canadensis* and *E. nuttallii*. The mean  $\pm$  standard deviation and total range of parameter values (in parentheses) were given with sample sizes for *E. canadensis* (N = 7) and for *E. nuttallii* (N = 17). The morphological characters were compared by t-tests and the significance levels (P) are shown.

	<i>E. canadensis</i>	<i>E. nuttallii</i>	Statistics
Leaf width at the mid-point (mm)	2.05 $\pm$ 0.22 (1.83–2.38)	1.14 $\pm$ 0.27 (0.63–1.64)	t = 6.602 P < 0.001
Leaf length (mm)	7.22 $\pm$ 1.38 (5.64–9.61)	10.45 $\pm$ 2.11 (7.62–14.01)	t = -4.449 P < 0.001
Width/length ratio	0.29 $\pm$ 0.06 (0.24–0.40)	0.11 $\pm$ 0.03 (0.09–0.17)	t = 7.039 P < 0.001
Leaf width 0.5 mm below the tip (mm)	1.22 $\pm$ 0.17 (1.03–1.51)	0.50 $\pm$ 0.09 (0.33–0.72)	t = 8.152 P < 0.001
Angle at the leaf apex (°)	54.34 $\pm$ 3.49 (50.89–60.35)	28.31 $\pm$ 3.55 (19.92–33.97)	t = 10.814 P < 0.001
Internode length (mm)	58.65 $\pm$ 8.11 (38.35–81.78)	52.64 $\pm$ 18.64 (29.52–87.43)	t = 0.405 P = 0.692

Our investigation showed that leaf length and width can be used to separate species, although these characteristics exhibited some overlap between *E. canadensis* and *E. nuttallii* (Tab. 1). The variability of leaf length, width and internode length and some overlap between these characteristics in the investigated *Elodea* species show that they are more dependent on external environmental parameters. Leaf width, length and internode length (Tab. 1) of *E. nuttallii* show a higher morphological variability in relation to *E. canadensis*. In our investigation the invasive species *E. nuttallii* was found for the first time in Croatia. We propose an identification key for two *Elodea* species that exist at the moment in Croatia according to literature (DANDY 1980, SIMPSON 1986, BOWMER et al. 1995, ERHARD 2005) and our results:

*E. canadensis*: Leaves linear-oblong or ovate. Most of leaves are 1.75 mm wide or more. Leaf apices are broadly acute or obtuse. Angle at the leaf apex is greater than 45°. Towards the stem apex leaves usually overlapping in regular rows and lying along the stem, often oblong or ovate.



**Fig. 2.** (A–C) *Elodea nuttallii*: A) part of the plant, B) whorl with the leaves, C) female flower. (D–E) *Elodea canadensis*: D) part of the plant, E) whorl with the leaves. Photo by A. Kočić.

*E. nuttallii*: Leaves linear or linear-lanceolate. Most of leaves are less than 1.75 mm wide. Leaf apices are narrowly acute or acuminate. At least some leaves are strongly recurved. Leaf lamina is often strongly twisted. Angle at the leaf apex is less than 45°. Leaves are usually folded along the midrib, somewhat recurved, with undulate margins, rarely more than 10 mm long.

## Discussion

The main direction of the spread of *E. nuttallii* in the Danube River Basin is from Western Europe down the river into the Delta area (JANAUER et al. 2008), so the introduction pathway of the species in Croatia was probably from Hungary. In fact, *E. nuttallii* was noticed in the southern part of Hungary, along the Danubian River Basin near the Croatian-Hungarian border (KIRÁLY et al. 2007b).

Since the first finding of *E. nuttallii* in 2006 and its establishment in Baranja (Croatia), it has spread to the eastern and northern part of the drainage channel network within the investigated period (Fig. 1A). The spread and direction of the *E. nuttallii* pathways in Croatia are probably caused by high water levels, which are a key factor in the flooded research area that links the water of the main river with the drainage network system (Fig. 1A) and interconnects the stagnant waters in the channels.

Our results show that the direction in which *E. nuttallii* spreads is along the channels. The linear spread of *E. nuttallii* was also recorded in some neighbouring countries: in Hungary along the Danube River (KIRÁLY et al. 2007b), in Serbia along the Banatska Palanka-Novi Bečej canal, which is directly connected with the Danube River (LJEVNAIĆ-MAŠIĆ 2010). Linear spread was recorded in some other parts of Europe, for example in Poland along the Vistula River (KAMIŃSKI et al. 2010). *E. nuttallii* was recently also found in the rivers Drava and Lendava in Slovenia (KIRÁLY et al. 2007a, MAZEJ GRUDNIK and SAVINEK 2010, MAZEJ GRUDNIK and GERM 2013). Thus, the second possible direction of introduction that we can expect in Croatia is from Slovenia along the Drava River. However, since *E. nuttallii* is easily mistaken for *E. canadensis*, its present distribution in Croatia might be more extensive than has been actually established.

The cause of the linear spread of *E. nuttallii* in the Baranja drainage system could be the mechanical control conducted for several years. This method did not reduce the population of *E. nuttallii*, but, on the contrary, probably had an important role in increasing the rate of spread due to transportation of cut-off fragments in the water, as found by BARRAT-SEGRETAIN (2005). Moreover, regular cleaning reduces the biological resistance and favours the establishment of alien species. Thus, in Slovenia the spread of *E. canadensis* is described as the consequence of habitat change and destruction (KUCHAR et al. 2010).

Our investigation showed the spread of *E. canadensis* at some new sites in the Lonja River Basin. It was recorded along the Sava River in Croatia (NIKOLIĆ 2009) and it is well established in Croatia (Fig. 1B). During the period of investigation it grew in relatively rich communities without any remarkable increases in the cover at researched sites. SIMPSON (1984) describes increases in the cover of *E. canadensis* as rare events. A few months after *Elodea* has invaded habitats, its cover increases for a brief period and then declines (BARRAT-SEGRETAIN and CELLOT 2007). Stands of *E. canadensis* were noted in Croatia after establishment in a hydro-power plant reservoir (LODETA et al. 2009).

The measured morphological characters of two *Elodea* species in general confirm that *E. canadensis* is a species with broader and shorter leaves and *E. nuttallii* with longer and narrower leaves (SIMPSON 1986, 1988, DANDY 1980). However, results of morphometric comparison shows that in both species the leaves are smaller than stated by the above mentioned authors, and for *E. nuttallii* they are similar to the findings of ERHARD (2005) and THIÉBAUT and DI NINO (2009). As regarding leaf width, DANDY (1980) and SIMPSON (1986, 1988) take 2 mm as the limiting value (*E. canadensis* have leaves broader than 2 mm, and *E. nuttallii* have narrower leaves). Our research shows that some specimens of *E. canadensis* have narrower leaves (below 2 mm), which is in accordance with research of BOWMER et al. (1995) who indicate that if the majority of leaves are 1.75 mm wide or more the species is *E. canadensis* and if the majority of leaves are < 1.75 mm wide then it is *E. nuttallii*. In relation to identification keys in both species the leaves were smaller in size. The morphological differences can be explained by ecological parameters and shallow water of floodplains (down to 2 m) in our research. As THIÉBAUT and DI NINO (2009) explained for *E. nuttallii*, the

shorter and broader-leaved phenotype typically occurs in shallow streams, whereas the longer and narrower-leaved phenotype occurs in deeper waters. High morphological variations in leaf length and its dependency to environmental parameters exclude it as a relevant distinguishing character of *Elodea* species.

We found that the angle at the leaf apex proved to be a good character for distinguishing *E. canadensis* and *E. nuttallii* with a limit around 45°, which is similar to the finding of ERHARD (2005). The width of a leaf measured at a point approximately 0.5 mm below the apex, the angle at the apex and the width/length ratio were greater in *E. canadensis*, and show the shape of the leaf apex that is the most important taxonomical feature indicated in identification keys (DANDY 1980, SIMPSON 1984). SIMPSON (1986) and SELL and MURRELL (2007) describe the leaf apices as important distinguishing leaf characters. In *E. canadensis* they are obtuse or broadly acute and in *E. nuttallii* acuminate to narrowly acute. Moreover, according to ERHARD (2005) in *E. nuttallii* the lateral leaf margins are more or less parallel, and in *E. canadensis* are convex.

Leaf size characters and internode length showed high morphological variability especially in *E. nuttallii*, which SIMPSON (1988) describes as the most variable of the species in the British Isles. The higher morphological variability of *E. nuttallii* is explained by TRÉ-MOLIÈRES et al. (2007) as a higher adaptation strategy in relation to *E. canadensis*.

The impact of *E. nuttallii* at the established sites in the channel drainage network of Baranja is currently negligible; however, because of the interactions between species and new habitats a spread of the taxon is expected (LARSON and WILLEN 2007).

In many countries, the introduction of *E. nuttallii* has resulted in displacement of *E. canadensis* from many waters (SIMPSON 1990). BARRAT-SEGRETAIN (2005) explains that after *E. nuttallii* fragments colonize the area with *E. canadensis*, the two *Elodea* species will probably coexist at least for a time, but displacement often occurs over a relatively short time, in one or two years (JAMES et al. 1999). In Croatia, there are no overlaps at the moment between the distributions of the two species. Considering that *E. nuttallii* is in its expansionary phase in Europe and that it spreads much more rapidly than *E. canadensis*, we can expect the spread of *E. nuttallii* to the new areas. As a new invasive species in Croatia, its current invasions and spreading need to be carefully monitored.

The first record in Croatia of the invasive species *E. nuttallii* was in 2006 in the drainage network channels of Kopački rit (Baranja). High water levels are responsible for *E. nuttallii* spreading in a linear direction in the investigated period in the flooded area. As regards the species *E. canadensis* already established in Croatia, we have located some new sites. A higher morphological variability of leaf width, length and internode length in *E. nuttallii* in relation to *E. canadensis* is a result of higher adaptivity to environmental parameters. To distinguish *E. nuttallii* and *E. canadensis* the most reliable parameters are leaf width 0.5 mm below the tip and the angle at the apex. Because of the rapid spread of *E. nuttallii* in Europe we can expect its spread to new areas in Croatia.

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