Rapid Communication

First records and molecular confirmation of invasive species *Elodea nuttallii* (Planch.) H.St.John, 1920 in Lithuania

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Abstract

Alien aquatic plant species have significant ecological and economic impacts on freshwater ecosystems and are mentioned amongst the major threats to aquatic biodiversity. Detection of alien species at the beginning of invasion can help avoid their spread and prevent negative impacts on aquatic ecosystems. This study presents the first records of invasive species *Elodea nuttallii* in Lithuania. This species was found in three localities of the Nemunas River (southern Lithuania) in 2020 and 2021. The localities were mapped using system grid cells arranged based on geographical coordinates with sides of 6′ latitude and 10′ longitude. The habitat characteristic and associated species were presented. The identity of *Elodea nuttallii* was confirmed using a molecular method (sequencing of two regions *ITS* and *psb*E-*pet*L). The molecular analysis was effective in supporting the identification of individuals collected in the early stages of growth. We suppose that *Elodea nuttallii* may be more widespread in the region of north-eastern Poland, north-eastern Belarus and southern Lithuania and will continue to spread north and northeast directions. The expansion of *Elodea nuttallii* can have a negative impact on valuable natural habitats, particularly on protected throughout Europe water courses with the *Ranunculion* vegetation.

Key words: aquatic plants, molecular identification, rivers, alien species

Introduction

Increasing human populations and expanded global commercial relations together with human activities and climate change-induced environmental changes cause spreading and often the establishment of species worldwide (Rodríguez-Merino et al. 2018; Pyšek et al. 2020). Despite a relatively low number of alien aquatic plant species reported for Europe (almost 100) (Hussner 2012), they make a significant ecological and economic impact on freshwater ecosystems and are mentioned amongst the major threats to aquatic biodiversity (Havel et al. 2015). Early detection of alien species at the beginning of invasion and rapid response can help avoid their widespread and prevent negative impact on aquatic ecosystems (Reaser et al. 2020). Most alien aquatic plants naturalised in Europe are native to

Northern America. The genus *Elodea* (Hydrocharitaceae) is presented by six species: *E. bifoliata* H.St.John 1920, *E. callitrichoides* (Rich.) Casp., 1857, *E. canadensis* Michx., 1803, *E. granatensis* Humb. & Bonpl. 1810, *E. nuttallii* (Planch.) H.St.John, 1920, *E. potamogeton* (Bertero) Espinosa 1928 (Cook and Urmi-König 1985; WFO 2021). Three species of *Elodea* (*E. candensis*, *E. nuttallii*, *E. callitrichoides*) are still known in Europe (Josefsson 2011). *Elodea canadensis* is the most widespread alien aquatic plant in Europe. It has been reported in 41 countries (Hussner 2012), however, *E. nuttallii* is becoming more frequent and is replacing *E. canadensis* in many regions (e.g., Cook and Urmi-König 1985; Simpson 1990; Barrat-Segretain 2001). Like other *Elodea* species, *E. nuttallii* is a perennial submerged aquatic plant. It has been found growing in very different water bodies, but usually in quiet water at shorelines of lakes, reservoirs, and ponds or in slowly flowing water along rivers and streams, in canals and ditches and wetlands such as cranberry plantations (Uotila 2009; Josefsson 2011; Dzhus 2014). Although both male and female plants have been introduced into European botanical gardens, only female plants appear to be spreading in nature (Cook and Urmi-König 1985). Since the first wild record in 1914 (Cook and Urmi-König 1985), *E. nuttallii* has spread to many European countries. According to the latest summaries (Steen et al. 2019; Dembowska et al. 2021), *E. nuttallii* (with an indication of the first record) occurs in 26 European countries: England (1914), Belgium (1939), the Netherlands (1941), France (1950), Germany (1953), Ireland (1960), Belarus (1964), Austria (1970), Denmark (1974), Luxemburg (1980), Switzerland (1980), Slovakia (1986), Czech Republic (1988), Hungary and Sweden (1991), Italy and Poland (1994), Romania (1997), Ukraine (2001), Bulgaria (2002), Serbia (2005), Croatia and Norway (2006), Slovenia (2007), Albania and Russia (2017). The last record of occurrence in Russia (Panasenko and Shcherbakov 2018) has indicted the easternmost distribution point. As the plant species widely distributed in many European freshwaters, *E. nuttallii* has been included into the List of invasive alien species of Union concern (European Union (EU) Regulation 1143/2014).

In many cases, the spread of *E. nuttallii* is related to river basins, for example, the Rhône River (Barrat-Segretain 2001) and the Alsatian Rhine (Greulich and Trémolières 2006) in France, the Danube River crossing Germany, Austria (Janauer et al. 2007), Slovakia (Hrivnák et al. 2019; Bubíková et al. 2021), Serbia (Vukov et al. 2008), Bulgaria (Georgiev et al. 2019), Romania (Ciocârlan et al. 1998; Sârbu et al. 2006), Croatia (Kočić et al. 2014) and its tributary Drava (Grudnik et al. 2014) in Slovenia, the Dniper in Ukraine (Prokopuk and Zub 2019; Davydova et al. 2021), the Pripet River in Belarus (Dubovik 2013), the Vistula in Poland (Kamiński 2010).

According to GBIF data (GBIF Secretariat 2021) and literature sources cited above *E. nuttallii* is rare in Eastern European and Scandinavian Peninsula countries. Of the Eastern Baltic countries, this species has so far

Table 1. Physico-chemical characteristics (average annual values) of the Nemunas River near Druskininkai.

pH	8.2
Flow velocity m/s	0.64
Temperature °C	10.6
Conductivity µS/cm	447.8
Dissolved oxygen (O_2 mg/l)	10.6
Biochemical oxygen demand BOD7 (O_2 mg/l)	3.91
Ca^{2+} mg/l	225.8
NH_4^+ (N mg/l)	0.128
NO_2 (N mg/l)	0.015
NO_3 (N mg/l)	1.014
TN mg/l	1.43
$PO4^{3-}$ (P mg/l)	0.051
TP mg/l	0.102

only been recorded in Estonia (Kukk et al. 2020), however it has never been found in Latvia and Lithuania. In this paper we described the first sites of invasive *E. nuttallii* in Lithuanian fresh waters (Nemunas River). Identification of the specimens collected in the early stage of growth was supported by molecular analysis.

Materials and methods

Study area

Field surveys were conducted in southern Lithuania in June 2020 and August–September 2021. The approximately 60 km long section of the middle Nemunas River from the state border of Belarus to Merkinė village in Lithuania was investigated. Upstream of the state border, the Nemunas River via the Čarna Hančia River and Augustow Canal is connected with different water bodies in the territory of Belarus and further in Poland. The studied part of the Nemunas River crosses the Dainava plain characterized by a low precipitation (600–650 mm per year) and weak density of the hydrographic network (0.7 km/km^2) (Česnulevičius 2008). The data on water physicochemical parameters of the monitored Nemunas River section were acquired from the Environmental Protection Agency (AAA 2020) and presented in Table 1.

Species identification and distribution

During the field surveys of the Nemunas River, *Elodea* sp., having a different appearance from *E. canadensis*, was found in three locations. In the field these plants differed from the known *E. canadensis* by relatively long, almost linear leaves and incompact plat apices, however without the obvious characteristic to *E. nuttallii* leaves recurving and twisting. Phytosociological relevés of the plant communities with *Elodea* spp. were conducted applying the Braun-Blanquet (1964) approach.

Identification and morphological measurement of dried specimens tentatively recognised as *E. nuttallii* were performed in the laboratory.

Measurement of the stems and internodes length, counting of the branches was made for 4 well-developed and preserved plants collected in August 2021. Morphology of 30 moistened verticillate leaves from main stem of plants collected in August and September 2021 was studied. The most questionable specimens collected at the beginning of the vegetation period (April 2020) were used for genetic testing.

Herbarium specimens were deposited at the Herbarium of the Institute of Botany of the Nature Research Centre (BILAS 92710, 92711 leg./det. Liucija Kamaitytė-Bukelskienė and Zofija Sinkevičienė) and the Herbarium of Vilnius University (WI P33612, leg./det. Jurgita Butkuvienė). Nomenclature and taxonomy follow WFO (2021). The map with the first localities of *E. nuttallii* in Lithuania was compiled by applying a system of grid cells arranged based on geographical coordinates with sides of 6′ latitude and 10′ longitude.

Molecular analyses

Six specimens collected in April 2020 that tentatively identified as *E. nuttallii* and six specimens of *E. canadensis* from the Nemunas River above Merkinė were used for genetic testing. The 10–15 mg of green plant material was used for DNA isolation. The total genomic DNA was extracted using Plant Mini Kit (Qiagen), following the manufacturer's protocol. The quality of DNA extractions was verified by electrophoresis on 1% agarose gel.

The *psb*E-*pet*L region (primers *psb*E (5'-AGTAGAAAACCGAAATAA CTAGTTA-3') and *pet*L (5'-AGTAGAAAACCGAAATAACTAGTTA-3') of the *cp*DNA and additional analysis using *ITS* sequences of nuclear DNA (primers *ITS*1 (5'-TCCGTAGGTGAACCTGCGG-3') and *ITS*4 (5'-TCCT CCGCTTATTGATATGC-3') were analysed in 12 *Elodea* plants presumably representing different species – *E. canadensis* and *E. nuttallii*. The amplification reactions of *psb*E-*pet*L region were performed as described by Bobrov et al. (2018), and the amplification of the *ITS*1-*ITS*2 region was performed according to Butkuvienė et al. (2020). All polymorphic variants of the *ITS* and *cp*DNA sequences were submitted to GenBank (ON584397–ON584398, ON600780–ON600781).

The MEGA X ver.10.1.8. programme (Tamura et al. 2021) was employed to align sequencing results with *Elodea ITS* region and *psb*E-*pet*L sequences from the NCBI GenBank database, which was extracted using the BLAST® (Madden et al. 1996) tool.

Results

Habitats and associated species

Elodea nuttallii was recorded in three localities of the Nemunas River (southern Lithuania) in 2020 and 2021. The species was mapped in two grid cells (Figure 1). The first time *E. nuttallii* was found in the Nemunas River upstream of Merkinė village (Y 54.149192, X 24.180477) on 22 June

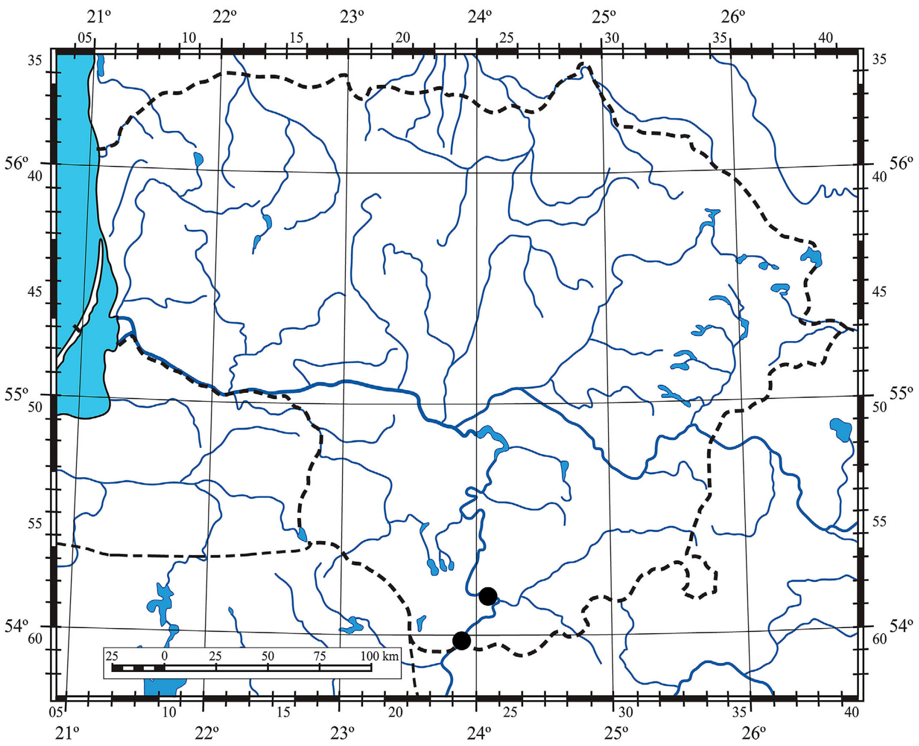

Figure 1. Distribution of *Elodea nuttallii* in Lithuania (upper point includes one location, lower – two locations).

2020 (Figure 1, upper point). The growing site is on the edge of a rapid covered with boulders of various sizes and patches of silty sand deposits and with typical vegetation of the *Ranunculion fluitantis.* Several young shoots grew at a depth of 0.65 m, on a sandy and gravelly bottom with dominant *Myriophyllum spicatum* L. and other submerged plants (Table 2, 1st relevé).

The second location of *E. nuttallii* was discovered in the Nemunas River downstream of the Baltoji Ančia River mouth situated along the left shore (Y 53.954161; X 23.835154) on 31 August 2021 (Figure 1, lower point). Both species *E. nuttallii* and *E. canadensis* were found growing together in this location. The plants of both species were well-developed and had clearly different appearance (Figure 2).

The third location was detected about 10 km downstream from the second (Y 53.987915; X 23.914747) on 16 September 2021 (Figure 1, lower point). In these two locations, *E. nuttallii* was found growing under similar conditions. Shallow depressions (0.1–0.5 m) formed on the edges of the riverbed with abundant boulders were typical habitats of *E. nuttallii*. The bottom of the depressions mainly consisted of nutrient-rich silt or silty sand. *Elodea nuttallii* was not very abundant because the water surface was covered by *Lemnaceae* species (Table 2, 2nd–4th relevés, Figure 3). Plants in the third location were very fragile with many formed turions. The species composition of relevés 3rd and 4th with dominant helophytes can be attributed to the helophyte communities.

Table 2. Phytosociological relevés with occurrences of *Elodea nuttalli* in the Nemunas River (South Lithuania).

Species	Relevé			
	1	2	3	4
***Elodea nuttallii* (Planch.) H.St.John**	+	**1**	**1**	+
Elodea canadensis Michx.		1		
Ch. Potamogetonetea				
Ceratophyllum demersum L.	+			
Najas major All.			+	+
Myriophyllum spicatum L.	4	1		
Potamogeton nodosus Poir.	1	1	1	
Potamogeton perfoliatus L.		1		
Ranunculus cf. *fluitans* Lam.	1		+	
Stuckenia pectinata (L.) Börner	2	+		
Ch. Lemnetea				
Lemna turionifera Landolt		1	3	
Lemna gibba L.			2	3
Spirodela polyrhiza (L.) Schleid.	+	3	2	3
Ch. Phragmito-Magno-Caricetea				
Agrostis stolonifera L.		1		1
Alisma plantago-aquatica L.		+		1
Butomus umbellatus L.	1	1	+	3
Carex sp.		+		1
Glyceria maxima (Hartm.) Holmb.		1	+	+
Leersia orhyzoides (L.) Sw.		+		+
Mentha aquatica L.		1		+
Phalaris arundinacea L.				1
Rorippa amphibia (L.) Besser		+		
Sparganium erectum L.		+	4	1
Depth m	0.65	0.1–0.3	0.1–0.2	0.2
Substrates	Sand/gravel	Silt/sand/boulders	Silt/sand/boulders	Silt/sand/boulders

Morphological features of collected specimens

Suspected *E. nuttallii* collected in the Nemunas River downstream of the Baltoji Ančia River mouth was non-flowering but well-developed and suitable for morphological examination and final identification. The length of 4 measured stems varied from 34.7–42.1 cm. They have 3–8 lateral branches, several of them were repeatedly branched. The length of internodes (N = 20) was 3.2–22 cm, with an average 6.5 ± 4.1 cm.

Leaves morphology was studied from plants of locality near Baltoji Ančia as well as from the site located downstream. The leaves were in whorls of three, linear to linear lanceolate, straight, or slightly recurved, with weakly undulate margins and acuminate apices. Lamina of some leaves was only slightly twisted. According to Simpson (1986, 1988) morphological differences of *E. nuttallii* and *E. canadensis* are based on a combination of leaf shape, leaf apex shape and leaf width 0.5 mm below the apex-tip. Leaf shape and character of apex in collected plants fully corresponded with features of *E. nuttallii* indicated by Simpson (1986, 1988). Moreover, leaf width 0.5 mm below the apex in both populations ranged from 0.5 to 0.7 mm, with an average 0.6 ± 0.07 mm and not exceeded maximal value 0.7(0.8) mm recorded by Simpson (1986, 1988) for *E. nuttallii*. Plant leaves from first site were longer (16.49 ± 1.4) and wider (2.4 ± 0.3) than in plant collected

Figure 2. Habitus of *Elodea canadensis* and *E. nuttallii* from a location downstream of the Baltoji Ančia River mouth. Photograph by dr. Z. Sinkevičienė.

downstream (respectively 15.5 ± 1.8, 1.7 ± 0.3). In general *E. nuttallii* from the Nemunas River had significantly longer leaves than indicated in many literature sources (Table 3). Similar long-leaved morphotypes have been also described by Simpson (1988).

Molecular analysis

The sequences of nuclear DNA *ITS* region obtained from the samples by direct sequencing were 806 bp (aligned sequences). Analysis of *ITS* sequencing data revealed 26 variables and 780 conserved sites. Only one site was identified as a six bases insertion/deletion (Table 4 – from 705 bp position to 710 bp position). In addition, single nucleotide polymorphism (SNP) was detected in twenty positions (Table 4).

Figure 3. Typical habitat of *Elodea nuttallii* in the Nemunas River – shallow depression along the helophyte belt at the shore covered by *Lemnaceae* species. Photograph by dr. Z. Sinkevičienė.

The alignment of the sequences of *psb*E-*pet*L region included 1109 base pairs. However, the *psb*E-*pet*L region turned out to be less variable. Here, in total 12 variables and 1097 conserved sites. Predominantly single nucleotide substitutions (SNP) (eight positions) and one four base pairs insertion/deletion (from 355 nt position to 358 nt position) (Table 5).

Discussion

Elodea nuttallii has been detected in Lithuania accidentally, however this finding was not a complete surprise considering the rapid spread of *E. nuttallii* in the last two decades. The closest countries where *E. nuttallii* was recorded are Belarus (Dubovik 2013; Dzhus 2014) and Poland (Kamiński 2010), located south of Lithuania. *Elodea nuttallii* has not been found in Latvia (Grinberga

Table 3. Morphological characters of *Elodea nuttalli* leaves. Mean ± standard deviation and range of parameter values for the Nemunas River plants (N = 30) comparing with literature (Thiébaut and Di Nino 2009; Prokopuk and Zub 2019).

	Nemunas	West Europe	Ukraina	Croatia
Leaf length, mm	15.9 ± 1.6 12.0–18.5	11.7 ± 3.1	10.2 ± 3.0	10.45 ± 2.11 7.62–14.01
Leaf width at the mid-point, mm	1.9 ± 0.3 1.1–2.4	2.2 ± 0.9	1.9 ± 0.4	1.14 ± 0.23 0.63–1.64

Table 4. Sequences variation in the *ITS* region of *Elodea nuttallii* and *Elodea canadensis*.

ITS	139	140	178	253	255	256	257	258	433	481	485	517	520	591	621	690	705	706	707	708	709	710	712	714	719	723
Elodea nuttallii	G	T	T	C	G	C	T	C	G	C	A	T	A	A	T	T	G	T	G	T	A	A	C	T	C	C
Elodea canadensis	C	C	C	T	T	G	C	T	A	T	T	C	G	G	C	C	–	–	–	–	–	–	T	C	T	T

Table 5. Sequences variation in the *psb*E-*pet*L region of *Elodea nuttallii* and *Elodea canadensis*.

*psb*E-*pet*L	324	346	355	356	357	358	424	480	615	722	725	816
Elodea nuttallii	G	T	T	T	T	G	G	C	C	G	T	G
Elodea canadensis	T	G	–	–	–	–	T	T	T	A	G	C

and Priede 2010), situated to the north of Lithuania. The findings in the southern part of Lithuania suggest that *E. nuttallii* has spread from Belarus. The Nemunas River in the west northern part of Belarus via the Čarna Hanča River and Augustow Canal connects with many water bodies in Poland and the Vistula River Basin, where *E. nuttallii* occurs (Kamiński 2010). The first possible finding of *E. nuttallii* in Poland mentioned by Kamiński 2010) is in the Biebrza River, having a connection with Augustow Canal. The location is about 80 km away from the Lithuanian border. The other close to Lithuania locality of *E. nuttallii* known since 1964 in Belarus is Lake Svityaz (Dubovik 2013). It is possible that *E. nuttallii* has been neglected in north-eastern Poland and north-eastern Belarus as well as in Lithuania. We suppose that *E. nuttallii* may be more widespread in this region. The species has all the typical traits of successful invaders: rapid growth, vegetative reproduction and easy dispersion by waterfowl and currents (Cook and Urmi-König 1985). The rapid spread and climate change with higher temperatures during the vegetation season suggest that *E. nuttallii* will continue to spread to north and northeast directions. The species has also been recorded in Northern countries such as Sweden, Finland and Norway, however, it has not been recorded in Latvia (GBIF Secretariat 2021). Moreover, the specimens identified as *E. nuttalli* are already included in the Estonian herbarium base (https://elurikkus.ee/generic-hub/occurrences; https://elurikkus.ee/plant-atlas/taxon) and its distribution is mapped (GBIF Secretariat 2021; Kukk et al. 2020). The detection of this species in Latvia – the last Eastern Baltic country is very likely in the nearest future. The occurrence of *E. nuttallii* is highly undesirable in valuable protected areas, especially in such as Natura 2000 (Steen et al. 2019). Bearing in mind the spread of *E. nuttallii* through the river systems this expansion can have a negative impact on river biota and especially on protected throughout Europe habitat of water courses with *Ranunculion* vegetation.

Both *Elodea* species (*E. canadensis* and *E. nuttallii*) exhibit wide morphological variation and morphological features may overlap, especially when plants grow under extreme conditions (Simpson 1988; Kočić et al. 2014; Prokopuk and Zub 2019; Thiébaut and Di Nino 2009). That can be a significant problem of species identification. Typical *E. nuttallii* have narrower and more elongated linear or linear-lanceolate leaves, while leaves of *E. canadensis* are linear oblong to ovate. Other distinguishing features of *E. nuttallii* are strongly recurved and twisted leaves (Simpson 1986). *Elodea nuttallii* from the Nemunas River had typical elongated linear to linear-lanceolate leaves, however without clear expressed recurving and twisting. Absence of these features was observed also in plants from the Iberian Peninsula (Simpson 1986). Leaf shape, leaf apex shape and leaf width 0.5 mm below the apex-tip in our collected plants fully corresponded with features combination indicated by Simpson (1986, 1988) as typical for *E. nuttallii.* This species from the Nemunas River distinguished by significantly longer leaves than indicated in literature cited. Thiébaut and Di Nino (2009) found that the shorter and broader-leaved phenotype of *E. nuttallii* typically occurs in shallow streams, whereas the longer and narrower-leaved phenotype occurs in deeper waters. The plants we observed were growing in shallows, so they may have been affected by several factors such as water level fluctuations, current, shading, abundance of free-floating plants and other.

We had no major problem identifying well-developed plants collected in August and even these collected in September. The specimens collected in June at an early stage of development were questionable. In this case, molecular identification was helpful in distinguishing two morphologically similar species. The regions of nuclear and chloroplast sequencing confirmed morphological species identification. The identical genotypes of *E. canadensis* and *E. nuttallii* have been described in other countries (Huotari and Korpelainen 2012; Rybicky and Voytek 2013).

Conclusions

The occurrence of invasive species *E. nuttallii* in the Nemunas River at the border crossing of Lithuania, Poland and Belarus suggest that this species could be more widely distributed in this region. Extensive research is needed in the nearest future to assess the true extent of the invasion and the potential impact on native species communities and habitats of water bodies in the region. Ongoing climate change is favourable for the species expansion to the northeast.

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Authors' contribution

All authors performed the field research, analyzed the data, and contributed to editorial changes in the manuscript. JB, DN, JP carried out genetic testing. ZS and JB wrote the manuscript.

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