

Rapid Communication

The beginnings of *Pistia stratiotes* L. invasion in the lower Danube delta: the first record for the Province of Vojvodina (Serbia)

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Abstract

Pistia stratiotes L. (water lettuce) is known as one of the most troublesome aquatic macrophytes, strongly affecting the environment and human activities in slow-moving and stagnant inland water systems. It was introduced into Europe in the 20th century and has since spread to 15 European countries. This paper presents data on the first record of this invasive aquatic plant in natural inland waters of Vojvodina (northern lowland part of Serbia) and discusses the possible pathways of its introduction. Field research was conducted in October and November 2017 on the Begej River in Vojvodina Province (Serbia), where populations of *P. stratiotes* were documented for the first time near Srpski Itebej, in close proximity (1.2 km downstream) of the Romanian border. The plant samples were collected, photographed *in situ* and deposited in the BUNS Herbarium. The relevant environmental data were also recorded *in situ* and water samples were collected for chemical analysis. This new record presents the first documented case of this invasive alien in rivers in Serbia, and expands upon the previous findings of this species in natural thermal waters of south-eastern and eastern Serbia. Taking into account the distance from the Romanian border and the fact that it has already been present in this country for several years, this introduction could be attributed to the spontaneous spreading of *P. stratiotes* from this neighbouring country. Nevertheless, despite this possibility, discarded aquarium plants cannot be entirely discounted as a potential source of this new introduction. Although it is hard to predict the exact consequences of this new introduction at such an early stage, bearing in mind the detrimental impacts *P. stratiotes* can have on the environment and excellent lateral connectivity of the Begej River with the canal and river network of Serbia, this finding highlights the need to take urgent action to control and instate regular monitoring measures, especially on the rivers and canals located close to the state borders.

Key words: water lettuce, invasive, alien, aquatic plant, Begej River

Introduction

An extensive network of rivers and canals crisscrossing the European continent acts as a major invasion corridor facilitating the introduction and spread of a significant number of aquatic alien species (Bij de Vaate et

al. 2002; Galil et al. 2007). Nunes et al. (2015) have performed a comprehensive assessment of aquatic alien species introductions on the European level and documented a continuous rise in the number of new introductions of alien species in European inland waters over the last 60 years. Likewise, Hussner (2009) has shown that the number of alien aquatic plants has increased significantly since the 1980s in the territory of central Europe, with a total of 96 alien aquatic plants, belonging to 30 families, being documented in the European freshwater systems (Hussner 2012). Out of these 96 species listed by Hussner (2012), six alien aquatic plants have been reported so far for the territory of Serbia (Anđelković et al. 2016).

Pistia stratiotes L. (Araceae), commonly known as water lettuce, is a free-floating perennial monocotyledonous freshwater macrophyte. Although its native range is most often cited as South America, recent molecular data (Renner and Zhang 2004) have reinvigorated the debate over the plants provenance (Sculthorpe 1967; Howard and Harley 1997). It has a worldwide distribution in the areas of tropical and subtropical climate (DeWald and Lounibos 1990; EPPO 2017a) and is considered to be one of the most widely distributed aquatic plants (Holm et al. 1977). It causes major problems in the freshwaters of tropical and subtropical regions of the world (Howard and Harley 1997; Champion et al. 2014; Lozano and Brundu 2016; EPPO 2017b), where it has been noted as a troublesome water weed ever since the 1960s (Sculthorpe 1967). *Pistia stratiotes* grows in slow-moving rivers and reservoirs, irrigation channels, ponds, lakes, canals and ditches (Cilliers 1991; Venema 2001; Adebayo et al. 2011; Hussner et al. 2014), where it often forms dense floating mats, which spread from the marginal areas of the waterbody over open water (Sculthorpe 1967; Howard and Harley 1997; Brunel et al. 2010b; Chamier et al. 2012). Such rigorous growth often leads to drastic reductions in the diversity of native aquatic plant and animal communities (Ercolini 2008; Brunel et al. 2010b). Despite its invasiveness and potential detrimental effects on aquatic ecosystems, this plant is still widely sold for aquaria and ornamental use (Brunel et al. 2010b).

Pistia stratiotes was first introduced into Europe in the 19th century (Loudon et al. 1850). The first published record of its presence in the wild dates to 1973 in the water canals of the Netherlands (Mennema 1977 in Šajna et al. 2007). In Italy the species was first recorded in 1998, in the Province of Cremona, followed by subsequent records in the Italian regions of Tuscany, Lombardy, Veneto, Emilia Romagna and Campania (see Brundu et al. 2012). Over the years it has been introduced to a number of European countries, e.g. Czech Republic, Spain and Russia (Pyšek et al. 2002; García Murillo et al. 2005; Shapovalov and Saprykin 2016), but without forming self-replacing populations. Meanwhile, overwintering populations of this invasive species have been reported in geothermal

waters in Europe: in Germany (river Erft, North-Rhine Westphalia; Hussner and Heiligtag 2013; Hussner 2014) and Slovenia (thermal stream Topla; Šajna et al. 2007). Although Hussner (2012) has documented its presence in 11 European countries, more recent records of the EPPO (European Plant Protection Organization) have confirmed its presence in a total of 15 countries (EPPO 2017b).

Brunel et al. (2010a) and Tanner et al. (2017) have marked *P. stratiotes* as a high priority species for conducting a PRA (Pest Risk Assessment) on the European level, which has led to the publication of an express PRA (EPPO 2017b). Since 2012 *P. stratiotes* has been on the EPPO List of Invasive Alien Plants (EPPO 2017a), while in 2017 it has been placed on the EPPO A2 List of pests recommended for regulation as quarantine pests “locally present in the EPPO region” (<https://www.eppo.int/QUARANTINE/list A2.htm>). Its invasiveness score range according to the USAqWRA impact score scheme was between 54 and 67, making it one of the highest scored invasive species of South American origin and consequently one of the major risks (Lozano and Brundu 2016). In Germany it has been listed on the Grey List (potentially invasive species; Hussner et al. 2014), as it is still only present as a rare occurrence, and has not naturalized in natural waters, only in geothermal waters (Hussner and Heiligtag 2013), while in Spain and Portugal it is regarded as a prohibited species (EPPO 2017a).

Although no official records of *P. stratiotes* have been reported in natural waters of Serbia (IASV 2011; Lazarević et al. 2012; Hussner 2012; Anđelković et al. 2016; EPPO 2017a, b), this species was occasionally documented in the thermally abnormal waters, i.e. natural thermal springs of southeastern and eastern Serbia (Randelović et al. 1995; Bogosavljević et al. 2007; Lansdown et al. 2016). Therefore, given the importance of this new record, the aims of this paper are to expand the existing knowledge on the distribution of *P. stratiotes* in Serbia, by collating the prior literature data on its presence with the first records of its presence in natural waterways of the Vojvodina Province (Northern lowland Serbia), and to discuss the possible pathways for this new introduction.

Materials and methods

Study area

The Begej River (Bega in Romanian) belongs to the Danube drainage basin (Black Sea catchment area) and is the biggest tributary of the Tisa River. Begej is an international river, which springs in Romania and subsequently flows through Romania and Serbia (Bukurov 1984). Begej consists of two river sections, which are known as the Stari Begej (i.e. the Old Begej) and Begej Canal, *aka* the Plovni Begej (i.e. the Navigable Begej; Figure 1). These two sections join near the village of Klek, thus forming the Begej River. From Timișoara (Romania) to its mouth, the length of the Plovni Begej is

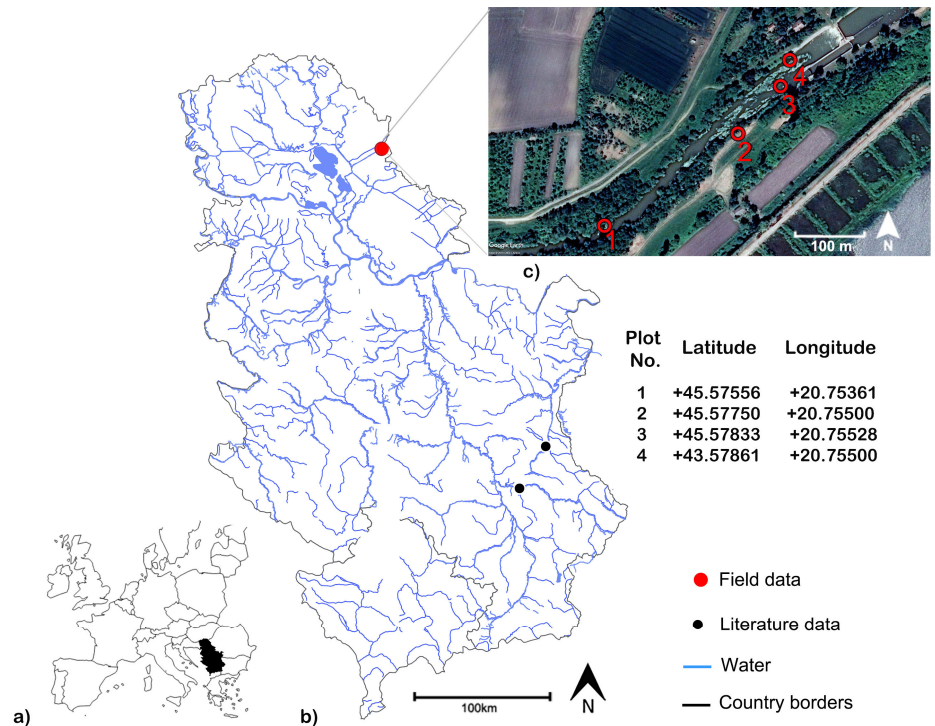


Figure 1. The maps show: a) the country of study at the European level; b) a detailed map of Serbia showing previous records of *Pistia stratiotes* in the literature (black dots) and the new records (red dot) documented in this study; c) a detailed area of the field site, with four points where *Pistia stratiotes* was recorded, along with their GPS coordinates (for details see Supplementary material Table S1).

118 km, 88 km of which are situated in Serbia (Bogdanović and Marković 2005). In its middle and lower reaches water levels of Plovni Begej are regulated by dams, weirs and other hydrotechnical control activities and its riverbed is channelized. Flow velocities in the canal are low enough for sedimentation, with the depth of the canal now too small for navigation. Currently, the maximum allowed flow through the canal is 83.5 m³/s, and the minimum is maintained at 5 m³/s (Dubovina et al. 2018).

Field sampling

Field research was done in October 2017 as part of a regular monitoring survey on the Plovni Begej River in the Banat region, Vojvodina Province, Serbia (45.57839N; 20.76350E, alt. 87 m). The presence of *P. stratiotes* was first documented on this occasion and first plant samples were collected near the village of Srpski Itebej. The previous records of *P. stratiotes* in Serbia were georeferenced using OziExplorer (OziExplorer 2009), and plotted on the map (Figure 1) along with the new field records, which were obtained using a handheld Garmin GPSMAP 60CSx navigator. The occurrence map (Figure 1) in the UTM projection (WGS84 ellipsoid) was created using the DIVA-GIS 7.5 software (Hijmans et al. 2012).

The sampling site was located in close proximity and downstream of the state border with Romania (Figure 1). Given the importance of this new



Figure 2. *Pistia stratiotes* in the water of Plovni Begej river in Srpski Itebej on November 8, 2017. Photographed by: Šipoš Š. Šandor.

finding, field research was repeated in November 2017, with the aim of reassessing the situation in the field and collecting additional samples of *P. stratiotes*. Research was conducted upstream of a weir located in Srpski Itebej, on a 500 m long river transect, along which the environmental parameters were also recorded, following River Habitat Survey (RHS) methodology (Raven et al. 1997). Sampling of *P. stratiotes* was conducted from the boat. The collected samples were photographed (Figure 2) with a Sony NEX 5R camera and their exact GPS coordinates recorded (Supplementary material Table S1). The samples were stored in 70% ethanol and transferred to the laboratory of the Department of Biology and Ecology of the Faculty of Sciences, University of Novi Sad. One sample of *P. stratiotes* was deposited in the collection of the Herbarium of the Department of Biology and Ecology Faculty of Sciences, University of Novi Sad (BUNS Herbarium, accession number: 2-1515), while the others have been preserved in 70% ethanol. The identity of *P. stratiotes* (Figure 3) was subsequently confirmed using the standard plant identification keys (Cook 1990).

Water was sampled at three points along the 500 m long river transect, and the values presented in Table 1 represent the average values of the measured parameters. Environmental parameters (Table 1) were assessed *in situ*, in order to obtain accurate data on the habitat characteristics. Water

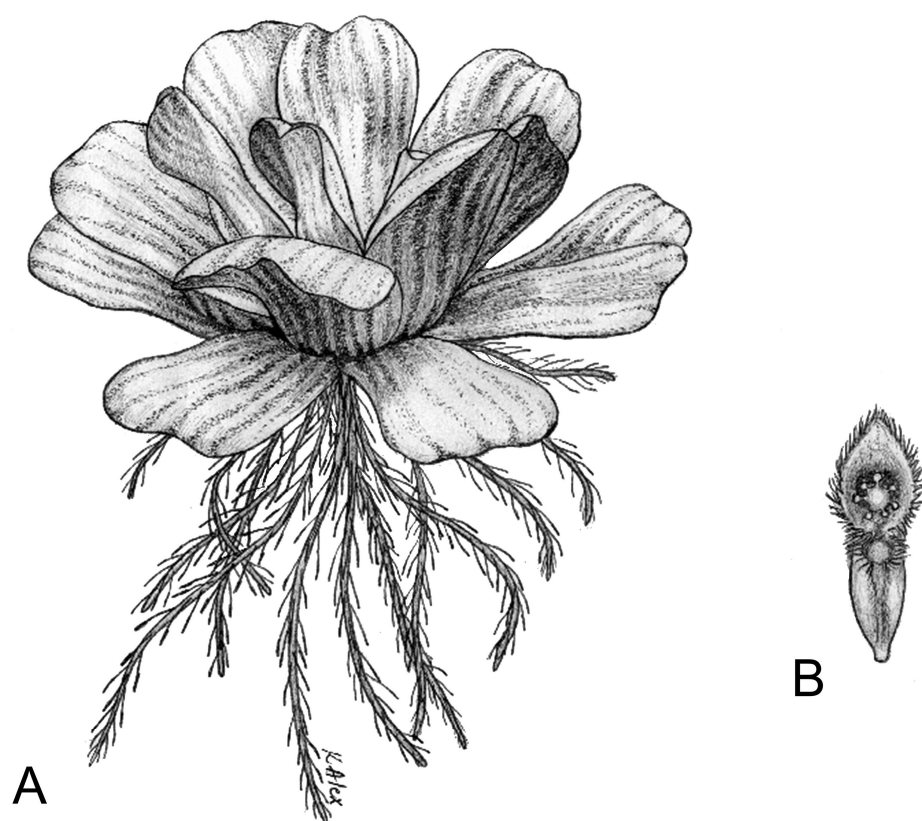


Figure 3. *Pistia stratiotes* L. 1753: A – lateral view of the plant; B – frontal view of the inflorescence. Drawings by Aleksandra Kovačević.

Table 1. Environmental parameters at the site of the Plovni Begej river (Srpski Itebej, Banat, Vojvodina, Serbia) where *Pistia stratiotes* was recorded. sat = dissolved oxygen saturation; WTemp = Water Temperature; DO = Dissolved Oxygen; EC = Electrical Conductivity; TSS = Total Suspended Solids; COD = Chemical Oxygen Demand; BOD = Biochemical Oxygen Demand; TOC = Total Organic Carbon; NO₃ = Nitrates; SUR = Surfactants

Sampling date	Environmental measurements										
	sat (%)	WTemp (°C)	DO (mg/l)	pH	EC (μS/m)	TSS (mg/l)	COD (mg/l)	BOD (mg/l)	TOC (mg/l)	NO ₃ (mg/l)	SUR (mg/l)
26.10.2017	82.2	12.5	8.62	7.6	333	8.0	5.0	3.4	2.6	4.7	2.3
08.11.2017	86.6	9.2	9.84	7.4	335	11.3	7.1	4.4	3.2	4.2	2.4

transparency was assessed using the Secchi disk. Surface water temperature, dissolved oxygen concentration and saturation were measured using a portable dissolved oxygen meter Hanna HI9146-10, conductivity using a conductivity portable meter WTW COND 3110 and pH with a portable WTW pH 330i meter. Water samples were collected in order to obtain data on the chemical parameters (Total Suspended Solids - TSS, Total Organic Carbon - TOC, Nitrates - NO₃, Surfactants - SUR, Chemical Oxygen Demand - COD, Biochemical Oxygen Demand - BOD), which were measured using a SECOMAM Pastel-UV Field/lab analyzer.

Results

Georeferenced field occurrences of *P. stratiotes* were recorded in natural surface waters of Serbia, along with relevant environmental data. *Pistia stratiotes* was recorded in four points within the studied section of the river

(Figure 1; Table S1). During the first field trip, (October 26, 2017), *P. stratiotes* was recorded in three spots (Figure 1). In the first one, six mature rosettes of 12–17 cm were documented, with a vast number of young plants. Four individuals each of 10–15 cm were recorded in both the second and third spot (Figure 1). The second field trip (November 8, 2017) confirmed all three previously recorded findings, with the same number of individuals of *P. stratiotes* present. Moreover, one additional spot (Spot 4, Figure 1) was documented in November, where a single mature rosette of 13 cm was documented, with a number of young plants. Points 1 and 4 are situated on the left bank of the Plovni Begej River, while spots 2 and 3 are on the right bank (Figure 1).

When analyzing the environmental data, Secchi disk measurements have shown that the transparency was to the bottom of the river on both dates, with a water depth of 1–1.5 m. The predominant river bottom substrate was silt. At the time of the sampling the water temperature was 12.5 °C in October and 9.2 °C in November, with the saturation levels being 82.2% and 86.6% and dissolved oxygen 8.62 mg/l and 9.84 mg/l, respectively. BOD and COD (as proxies for the organic load content) were used as a rough estimate for the trophic status of the river. According to the BOD (3.4 mg/l and 4.4 mg/l) and COD (5.0 mg/l and 7.1 mg/l) values, this part of the Begej River is clearly above the national environmental quality standards. TOC (total organic carbon) concentrations were 2.6 mg/l in October and 3.2 mg/l in November and TSS (total suspended solids) concentrations were 8.0 mg/l in October and 11.3 mg/l in November (Table 1).

Discussion

In Serbia, the occurrence of *P. stratiotes* was previously recorded in natural thermal springs, first in 1994 in a thermal spring “Banjica” in the Sićevačka klisura gorge (Randelović et al. 1995) and later on in a new site near Knjaževac city, in Rgošte (Rgoška Banja spa; Bogosavljević et al. 2007). Furthermore, there is also data indicating that this invasive alien plant species has been planted intentionally with the purpose of creating an artificial wetland in the Sokobanja spa, as an experimental part of a waste water treatment facility (Nikolić et al. 2009). Therefore, even though the specimens of *P. stratiotes* have previously been reported in thermally abnormal waters of eastern and southeastern Serbia, these new findings represent the first records of the occurrence of *P. stratiotes* in natural running waters of Serbia and are the first overall records for the region of Northern lowland Serbia (Vojvodina Province). The Begej River, like most of rivers in Vojvodina, belongs to permanent non-tidal, smooth-flowing watercourses (C2.3) following the national updated system for the classification of habitats (Lakušić and Medarević 2010), which complies with the EUNIS system (European Union Nature Information System, Davies et al. 2004).

Pistia stratiotes is an aquatic plant species able to grow under varying environmental conditions. Temperatures between 22 and 30 °C and high-nutrient conditions are optimal for its growth and development (Pieterse et al. 1981; Henry-Silva et al. 2008). Although at the first time of sampling the water temperature was 12.5 °C (in October) and 9.2 °C (in November, Table 1), according to Pieterse et al. (1981) and Hussner et al. (2014) *P. stratiotes* can still develop at temperatures as low as 10 °C, and withstand freezing air temperatures as a small floating form, provided that its leaves are in direct contact with the water surface and the water temperature is above 10 °C (Hussner et al. 2014). Despite this, it is susceptible to low temperatures and frost, being prone to dieback when enclosed in ice and exposed to temperatures slightly above 0 °C (MacIsaac et al. 2016).

Pistia stratiotes was found to be tolerant to salt, withstanding 200 mM NaCl in the water (6 PSU; Upadhyay and Panda 2005). The inorganic nutrient content in the analysed samples was high (4.7 mg/l in October and 4.2 mg/l in November) and these values were higher than those recorded by Šajna et al. (2007). The documented pH values at the study site were close to neutrality (7.6 and 7.4), which is in line with the values recorded by DeWald and Lounibos (1990) and Šajna et al. (2007). Meanwhile, the EC values were slightly lower (333 and 335 vs. 457–461), when compared to those measured by Šajna et al. (2007) in the winter period. Inorganic nutrients, nitrates, were high (4.7 mg/l in October and 4.2 mg/l in November) and these values were higher than recorded in Šajna et al. (2007). If the populations of *P. stratiotes* prove to be adaptable to such environmental conditions, its potential for further spread within the region will encompass many surface standing and slow-moving inland waters. Furthermore, all of the four spots (Figure 1, Table S1) where *P. stratiotes* plants were observed are located close to the shore, where the river flow is very slow, due to various fallen tree logs and reed vegetation overgrowing the riverbanks. This is in agreement with literature on the establishment of *P. stratiotes* in the marginal areas of slow-moving inland waters (Sculthorpe 1967; Howard and Harley 1997; Šajna et al. 2007; Brunel et al. 2010b; Chamier et al. 2012; Hussner and Heiligttag 2013).

Given that the new population was discovered very close to the Romanian border (the distance between the weir in Srpski Itebej and the border being 1.2 km), where the species is known to occur (Hussner 2012; Lansdown et al. 2016; EPPO 2017a, b), the newly described records could have been dispersed from the neighbouring populations. Similar hypothesis was made following the arrival of another invasive alien macrophyte *Cabomba caroliniana* A. Gray, which has most likely spread into Serbian waters from the neighboring Hungary (Vukov et al. 2013). However, despite that this is the most likely introduction pathway for *P. stratiotes* and that the area surrounding the field site where it was

recorded is not urban, but used primarily for agricultural production (Živković MM, *pers. observation*, November 8 2017), discarded aquarium plants cannot be completely discounted as a possible source of introduction, especially as this has been cited as the major pathway of entry into the countries of the EPPO region (EPPO 2017a, b).

Given that at this stage not enough is known on the state of establishment of *P. stratiotes* in Serbian rivers, this early warning should provide an incentive to take urgent action, promote control and instate regular monitoring measures. Furthermore, due to the significant lateral connectivity of the Begej River with the canals of the Danube-Tisa-Danube hydrosystem and rivers in the northern low-lying part of Serbia, the potential for further expansion of this species via these aquatic corridors is alarming. Consequently, regular monitoring of these surface waters is clearly a necessity, which is further supported by the recommendations provided by the EPPO PRA (EPPO 2017b), the EPPO Standard on aquatic invasive alien plants (EPPO 2014) and the IPPC (1997) guidelines for the surveillance (ISPM No. 6). All of these abovementioned documents state that it is advisable to inform National Plant Protection Organizations (NPPOs) of the need to conduct monitoring surveys on the affected waterways.

Conclusion

Given that the exact population range and density of *P. stratiotes* are as of yet unknown, as well as that the species was not found during the winter period, it is hard to predict the future environmental consequences of this new introduction in the territory of Serbia. Nevertheless, this new record of *P. stratiotes* raises the concern that this invasive plant could also be present in other rivers and canals in the territory of Serbia, and that the current lack of distribution records is simply an artifact of undersampling. Given the current paucity of distribution information and the potential negative impacts of its presence (Brunel et al. 2010a, b; Lozano and Brundu 2016; EPPO 2017a, b; Tanner et al. 2017) broad surveys and updated distribution records for this invasive plant should be a priority. Furthermore, this first record of *P. stratiotes* in surface running waters of Serbia highlights the need for implementing early control measures and rigorous monitoring (IPPC 1997; EPPO 2014, 2017b) and research actions in the years to come, on the national level, but with a special emphasis on this river and other border waterways.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Coordinates of the first *Pistia stratiotes* records in the Province of Vojvodina (Serbia).

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_Zivkovic_etal_Table_S1.xlsx