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'Only introduced' or 'invasive': spread of the alga *Aulacoseira ambigua* f. *japonica* from Asia to Africa and Europe

„Zgolj preseljena" ali „invazivna": širjenje alge *Aulacoseira ambigua* f. *japonica* iz Azije v Afriko in Evropo

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Abstract: Many non-native algal species are found in waters all over the world. Many terms are used to describe such organisms that have expanded their distribution. However, a unified model or concept remains to be defined, as how biological 'invasions' are seen depends on the perspective. Understanding invasive organisms is important for biodiversity, science policy and water management. During monitoring sampling at Slivniško Lake (Slovenia) in 2016, the curved diatom *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams was identified. This species originates from Japan, from where it was described more than 100 years ago. The chronology of the published distributions of *A. ambigua* f. *japonica* defines its spread from Japan to Asia, then to western Russia and South Africa, and now to central Europe. This study provides further evidence that *A. ambigua* f. *japonica* has become established in Europe (Slovenia), as supported by light microscopy and scanning electron microscopy. To the best of our knowledge, this is the first qualitative and quantitative description of *A. ambigua* f. *japonica* in Europe. It has been suggested that the shape of these algal colonies is significant for the interpretation of ecological information, and indeed, also in the present case, spiral colonies were found in this eutrophic water body. Although curved cells and colonies can also be interpreted as (sub)populations or morphological variants, only detailed molecular studies can reveal if these also have taxonomic significance.

Keywords: algae, *Aulacoseira ambigua* f. *japonica,* introduced species, invasive species, scanning electron microscopy

Izvleček: V vodah po vsem svetu najdemo veliko tujerodnih vrst. Za opis organizmov, ki se širijo, je v uporabi veliko različnih pojmov. Kaže se potreba po opredeliti enotnega modela ali koncepta, saj je videnje bioloških "invazij" odvisno od perspektive. Razumevanje invazivnih organizmov je pomembno za biotsko raznovrstnost, znanstveno politiko in upravljanje z vodami. Med spremljanjem vzorčenja na Slivniškem jezeru (Slovenija) leta 2016 smo med drugim določili tudi ukrivljeno diatomejo *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams. Ta vrsta izvira iz Japonske, kjer je bila opisana pred več kot 100 leti. Kronologija objavljenih pojavljanj *A. ambigua* f. *japonica* opredeljuje njeno širjenje iz Japonske v Azijo, nato v zahodno Rusijo in Južno

Afriko, zdaj pa v osrednjo Evropo. Ta raziskava vsebuje dodatne dokaze, da se je *A. ambigua* f. *japonica* uveljavila v Evropi (v Sloveniji), podprte s svetlobno mikroskopijo in vrstično elektronsko mikroskopijo. Gre za prvi kvalitativni in kvantitativni opis *A. ambigua* f. *japonica* v Evropi. Oblika algnih kolonij je lahko pomembna za razlago ekoloških informacij; v danem primeru so bile v evtrofnem vodnem telesu najdene spiralne kolonije. Čeprav so ukrivljene celice in kolonije lahko interpretirane tudi kot (sub)populacije ali morfološke različice, lahko le natančne molekularne študije razkrijejo, če imajo ti morfotipi taksonomski pomen.

Ključne besede: alge, *Aulacoseira ambigua* f. *japonica,* vnesene vrste, invazivne vrste, vrstična elektronska mikroskopija

Introduction

Many terms are used to describe organisms that expand their distribution, including 'introduced', 'non-indigenous', 'non-native', 'invasive', 'exotic', and even 'alien'. This has made studies associated with biological invasion often difficult and confusing to follow (Kokocinski et al. 2017). After 20 years of studies of such organisms, a unified model or concept remains to be defined, particularly because how biological 'invasions' are seen is a matter of perspective (Heger et al. 2013). The term 'invasive' usually relates to a new species that spreads rapidly through a new environment, and this has a negative connotation. Thus an invasive organism is seen to have a tendency to spread to a degree that is believed to cause damage to the environment, or ecosystem services such as human economy or health. Indeed, the International Union for Conservation of Nature defines an invasive species as a widespread non-indigenous species that has adverse effects on the invaded habitat. Usually, man is commonly believed to be responsible for invasive organisms that have been introduced into places away from their natural range of distribution.

The conceptual model of the ecological impact of the invasion process can be divided into three stages: transport, establishment, and spread (Kokocinski et al. 2017). Many species are transported and become established, but their spread and any associated negative impact also rely on favourable natural conditions in the particular ecosystem. Nevertheless, it is difficult to define the spatial and temporal scale of such an expansion, and also the negative impact that is might have, as every invasive species has a different interaction with the ecosystem and its members. It is common sense that non-stressed ecosystems can more easily cope with changes, in comparison to ecosystems that are exploited by humans.

There are many published lists of local invasive algal species according to countries or regions, and these represent mostly cyanobacteria and other potentially toxic species (Kokocinski et al. 2017). For cyanobacteria, it is understandable that special attention must be paid, as some cyanobacterial species can produce potent toxins. Instead, it is rare that diatoms are considered as invasive. The best described case is the diatom *Didymospehnia geminata* (Taylor and Bothwell 2014), for which blooms have been reported in rivers worldwide that have been somewhat hastily attributed to their introduction. Here, the evidence indicates that such *D. geminata* blooms are probably not caused by their specific introduction, but rather by the environmental conditions in connection with the phosphorous concentrations, which promote excessive stalk production by this historically rare species (Taylor and Bothwell 2014). This emphasises how important it is to understand whether the successful dominance of a species is a result of its introduction or of changing environmental conditions that have facilitated what was an already existing population, or whether both processes apply. The origins of such invasive microorganisms are importance for science policy and management (Taylor and Bothwell 2014).

To date, few data have been published relating to the freshwater diatom *Aulacoseira ambigua* f. *japonica* (F. Meister) Tuji & D.M. Williams.

The very first description of this species was by Meister (1913), more than 100 years ago in Japan, who named it as *Melosira japonica*. The first record of the species with the current name was also in Japan, by Tuji and Williams (2007). According to AlgeBase (2017), the accepted basionym and homotypic synonym of *A. ambigua* f. *japonica* Tuji & D.M. Williams is *M. japonica* F. Meister. This species has also been referred to as *Melosira ambigua* f. *curvata* (Skabichevskii 1960), *Melosira ambigua* f. *spiroides* (Chalfina 1966), and *Melosira granulata* var. *tenuissima* f. *spiralis* (Wakabayaschi and Ichise 1982). In 2010 this species was reported for Korea (Joh 2010), and 6 years later, for South Africa (van Vuuren and Taylor 2016). Then only one year after that it was identified in France (Anon. 2017). Following this chronology and movement, the path that *A. ambigua* f. *japonica* has followed from its origins in Japan to initial spread to Asia, then western Russia and South Africa, and now central Europe.

Here we provide further evidence that *A. ambigua* f. *japonica* has become established in Europe (Slovenia). To the best of our knowledge, this is the first qualitative and quantitative description of *A. ambigua* f. *japonica* in Europe.

Study area, materials and methods

Study area

Slivniško Lake (Slivniško jezero) is located in the eastern part of Slovenia, near to Šentjur, which is 20 km from the Croatian–Slovenian border (GKY 534340, GKX 116230; 46°11′17″N, 15°26′48″E). Slivniško Lake has a surface area 840,000 m^2, a length of 5,000 m, and a width of 500 m. It is 292 m above sea level, and has a mean depth of 5 m, with maximum depth of 14 m. This accumulation is based on a carbonate geological base, with inflow from the Ločnica Stream, and outflow of Voglajna River. A reservoir was created in 1976 for industrial use, but due to technological development, for many years it has only been used as a floodwater retention buffer, and as a lake for fishing and tourism activities. Indeed, Slivniško Lake is famous for its fishing competitions, with a recent winning catfish of 255 cm in length, and 95 kg in weight.

The ecological characteristics of Slivniško Lake have been followed through the National Freshwater Monitoring Programme, with the data included here originally from national reports (Remec-Rekar 2011-2016). Slivniško Lake is described as a lowland accumulation with moderate ecological potential, with it included in the ecotype of shallow Pannonian plain with Alpine influence. The ecological status of Slivniško Lake has remained moderate, and it has never shown any cyanobacterial blooming. From 2011 to 2016, it had good chemical status. Over the period from 2011 to 2016, the mean total nitrogen and phosphorous through the water column was 519 $\mu g\ L^{-1}$ and 113 $\mu g\ L^{-1}$, respectively. It had a mean biovolume of 4 $mm^3\ L^{-1}$, mean annual chlorophyll *a* of 7 $\mu g\ L^{-1}$, and maximum chlorophyll *a* of 21 $\mu g\ L^{-1}$ (in 2014). The mean Secci depth was 1 m. According to the Organisation for Economic Cooperation and Development criteria, from 2011 to 2016, this classification defined Slivniško Lake as a eutrophic water body.

Sampling and laboratory sample preparation

Sampling of the phytoplankton of Slivniško Lake was carried out in accordance with national methodologies, from a boat with an automated sampler, and for the euphotic zone in its deepest part. Immediately after sampling, the contents of a 100 mL tube was fixed with 4% formalin and transferred to the laboratory. The fixed samples were stored at 4 °C. Light microscopy analyse was carried out up to 5 months after the sampling, at 1,000× magnification (Microscope Nikon Eclipse TE300, Japan). A subsample for the examination of diatom frustules only was cleaned of organic material by addition of HNO_3, with heating leading to degradation of the organic matter, with only silicate (silica) frustules of the diatom species remaining.

Scanning electron microscopy

The cleaned samples were centrifuged and washed three times with acetone, and placed directly on a metal holder. After sputter coating with platinum, the algae were analysed using a scanning electron microscope (JSM-7500 F; JEOL, Japan). The sample was not as rich in *A.*

ambigua f. *japonica* as samples described by van Vuuren and Taylor (2016), and they were more fragmented due to the protocol with acid cleaning of the organic material, so only short chains were seen with the scanning electron microscopy. Nevertheless, the observation of fresh material also revealed long spiral colonies, although these were unfortunately not captured in the light microscopy images.

The species

Aulacoseira ambigua f. *japonica* was found in Slivniško Lake in 2016 during National Freshwater Monitoring. During this year, this diatom alga was found in phytoplankton, in March, July and October, with a mean concentration of 372 cells mL^{-1}, and with mean biovolume of 0.14 mm^3 L^{-1}, which represented 21% of total Bacillariophyta biovolume, and 2.3% of total phytoplankton biovolume. The lengths were measured using computer software (Nikon NIS Elements 3.22.15), with the arithmetic mean for diameter and length calculated from 20 separate cells.

These studies of Slivniško Lake during the National Freshwater Monitoring from 2011 to 2016 revealed a different dominant species each year. Relative annual mean abundance >2% was seen for: *Aphanocapsa* sp. (Nägeli 1849), *Aphanothece* sp. (Nägeli 1849), *Coelastrum* sp. (Nägeli 1849), *Cyanobium* sp. (Rippka & Cohen-Bazire 1983), *Eutetramorus planktonicus* (Korshnikov 1953), *Merismopedia tenuissima* (Lemmermann 1898), *Navicula minima* (Grunow 1880), *Oocystis lacustris* (Chodat 1897), *Pediastrum simplex* (Meyen 1829), *Peridinium umbonatum* (Stein 1883), *Phacotus lenticularis* (Diesing 1866), *Tetrastrum komarekii* (Hindák 1977) and *Woronichinia naegeliana* (Elenkin 1933). Relative annual mean biovolume >2% was seen for: *Aulacoseira granulate* (Simonsen 1979), *Ceratium hirundinella* (Dujardin 1841), *Chlamydomonas rigensis* (Skuja 1927), *Coelastrum* sp. (Nägeli 1849), *Cosmarium* sp. (Corda ex Ralfs 1848), *Cryptomonas obovata* (Czosnowski 1948), *Cyclotella* sp. (Brébisson 1838), *Dinobryon divergens* (Imhof 1887), *Euglena ehrenbergii* (Klebs 1883), *Euglena* sp. (Ehrenberg 1833), *Fragilaria ulna* (Lange-Bertalot 1980), *Koliella* sp. (Hindák 1963), *Lepocinclis ovum* (Lemmermann 1901), *Pandorina morum* (Bory 1824), *Pediastrum duplex* (Meyen 1829), *Pediastrum duplex* var. *gracillimum* (West & G.S.West 1895), *Pediastrum simplex* (Meyen 1829), *Phacotus lenticularis* (Diesing 1866), *Phacus longicauda* (Dujardin 1841), *Sphaerocystis schroeteri* (Chodat 189) and *Trachelomonas* sp. (Ehrenberg 1833).

Observations

Centric diatoms rarely form spiral colonies (van Vuuren and Taylor 2016), and therefore the spiral shaped diatom colonies with distinctly curved cells were relatively easy to determine. After investigation under light microscopy (Fig. 1) and scanning electron microscope (Fig. 2), the taxon from Slivniško Lake was identified as a special form of *A. ambigua*, defined as *A. ambigua* f. *japonica* (F. Meister) Tuji & D.M. Williams. The mantle of the cells of the *Aulacoseira* genus is much deeper than for other centric taxa, which gives the cells a cylindrical or tube-like structure. The cells are linked as valve face to valve face by short spines (van Vuuren and Taylor 2016). With mean diameter of 5 μm and length of 10 μm (Figs. 1, 2, 3), these matched the previously reported ranges of 3 μm to 12 μm in diameter, and 5 μm to 15 μm in length, with their characteristic hollow ringleiste and specific external rimoportulae (Potapova and English 2010).

Discussion

Aulacoseira ambigua f. *japonica* has never before been recorded for the locality of Slivniško Lake. In 2016, it was well established, but it had not become dominant. Such establishment of this species represents the second stage of the conceptual ecological model for an invasive species, with expansion of the invasion as the final stage (Kokocinski et al. 2017). Nevertheless, biological invasions are important parts of the functioning of aquatic and terrestrial ecosystems (Sukenik et al. 2015).

Previous studies have revealed decreases in species diversity after invasion by alien species, along with alterations to ecosystem functioning due to the reproduction and feeding behaviours

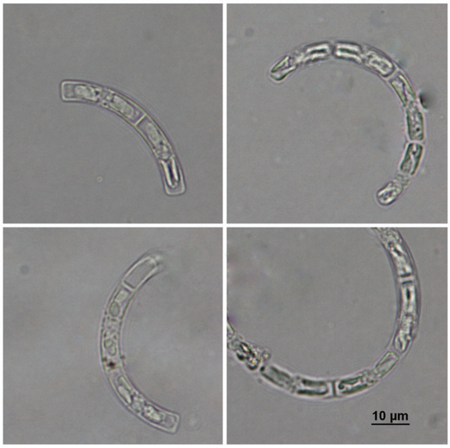

Figure 1: *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams from Slivniško Lake (Slovenia), light microscopy with phase contrast. Scale bar of 10 µm is in the bottom right corner.
Slika 1: *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams iz Slivniškega jezera (Slovenija), posnetek svetlobnega mikroskopa s faznim kontrastom. V spodnjem desnem kotu je merilce dolžine 10 µm.

of such organisms, and because of the release of new chemical compounds (e.g., cyanotoxins) after expanded invasion (Sukenik et al. 2015). Further studies are needed to enable us to recognize better and mitigate the potential threats associated with such invasive processes. Here the path followed in the travels of a species can also help in the determination of its origin, as the origins of such invasive microorganisms can be important for science policy and management (Taylor and Bothwell 2014).

Although curved cells and colonies of *A. ambigua* can be interpreted as a potential (sub) population (Potapova and English 2010) or a morphological variant (Tremarin et al. 2013), only detailed molecular studies can reveal if this truly has taxonomic significance or not. The morphological variations and the distributions of *A. ambigua* in Brazilian environments were described by Tremarin et al. (2013), although they only indicated long, slightly curved chains (with no curved individual cells). Tuji and Williams (2007) reported that the shape of such colonies is significant for the interpreting of ecological information. Indeed, ecological differences for *A. ambigua* and *A. japonica* (*M. japonica*) have been reported, whereby Tanaka (2002) defined straight colonies in mesotrophic waters, and Tuji and Williams (2007) defined spiral colonies with curved cells in eutrophic waters. This latter is confirmed also in the present case, as Slivniško Lake has been described as a eutrophic water body since 2011, and it showed only spiral colonies of *A. ambigua* f. *japonica* in 2016. However, it is important to bear in mind that such monitoring studies just provide a snap-shot in time, and that you cannot stop the evolution while studying the ecology.

This demonstrates that *A. ambigua* f. *japonica* has become established in Europe (Slovenia), with the support of light microscopy and scanning electron microscopy images. To the best of our knowledge, this is the first qualitative and quantitative description of *A. ambigua f. japonica* in Europe.

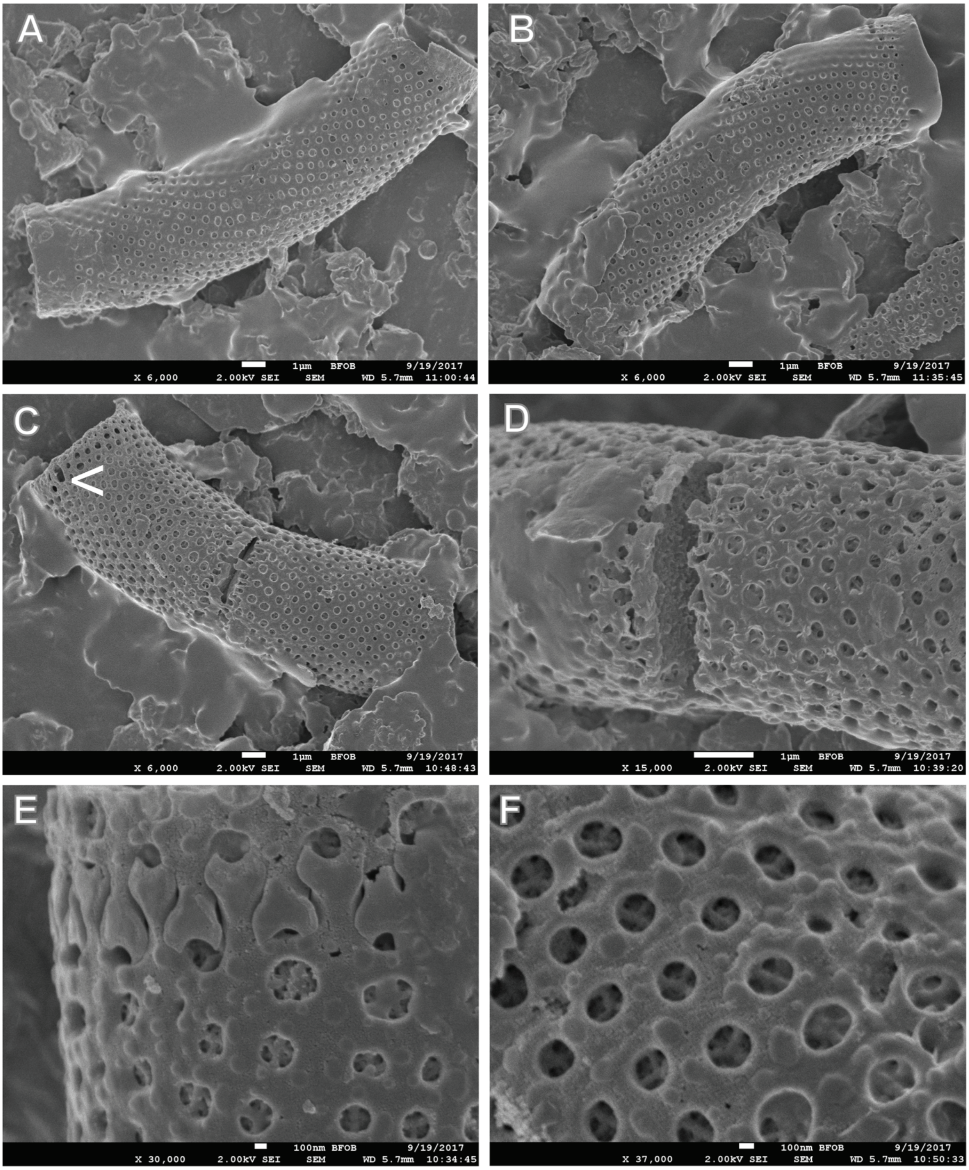

Figure 2: *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams from Slivniško Lake (Slovenia), scanning electron microscopy. A, B - The curvature of the individual cells. B, C - Position and structure of the external rimoportulae (white arrow). D - Separation of the valves. E - Linking valve showing complex shape of the linking spines on the valve face. F - Detail of the mantle areolae. Scale bar is positioned at the bottom of every picture; A-D: 1 µm, E-F: 100 nm.

Slika 2: *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams iz Slivniškega jezera (Slovenija), posnetek vrstičnega elektronskega mikroskopa. (A, B) Ukrivljenost posameznih celic. (B, C) Položaj in struktura zunanjih rimoportul (bela puščica). (D) Razmejitev silikatne lupinice. (E) Povezovalna struktura, ki prikazuje kompleksno obliko povezovalnih zobcev na čelni strani silikatne lupinice. (F) Podrobnosti ploskve. Merilce se nahaja na spodnjem robu vsake slike; A-D: 1 µm, E-F: 100 nm.ploskve. Merilce se nahaja na spodnjem robu vsake slike; A-D: 1 µm, E-F: 100 nm.

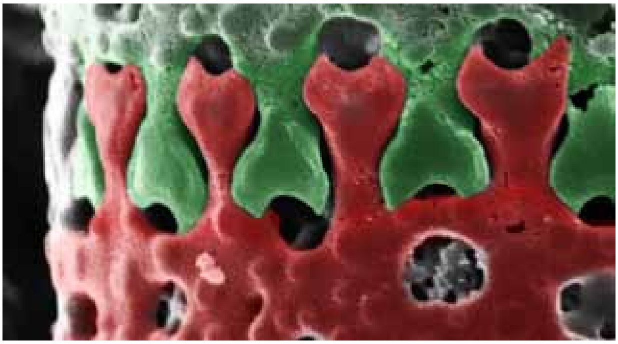

Figure 3: A detail of the cell joint structure - linking valve of alga *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams from Slivniško Lake (Slovenia), scanning electron microscopy. Scale bar as on Figure 2E.

Slika 3: Detajl povezovalne strukture dveh lupinic alge *Aulacoseira ambigua* f. *japonica* Tuji & D.M. Williams iz Slivniškega jezera (Slovenija), posnetek vrstičnega elektronskega mikroskopa. Merilce kot na sliki 2E.

Summary

Biological migrations are important parts of the functioning of aquatic and terrestrial ecosystems. Alga *Aulacoseira ambigua* f. *japonica* has never before been recorded for the locality of Slivniško Lake. In 2016 this species at this location became well established, but it had not become dominant in the algal community. Only detailed molecular studies will reveal if this species with its morphological variation can be interpreted as a potential (sub)population or a morphological, or maybe even as ecological variant, with a link to the eutrophication of water body. The migration path of this species revealed some information, but further studies are needed to enable us to better recognize and mitigate the potential threats associated with invasive processes.

Povzetek

Biološke migracije so pomembni deli delovanja vodnih in kopenskih ekosistemov. Alga *Aulacoseira ambigua* f. *japonica* še nikoli prej ni bila zaznana na lokaciji Slivniškega jezera. Leta 2016 pa je ta vrsta na tej lokaciji postala dobro uveljavljena, vendar v algni združbi ni prevladala. Le podrobne molekularne študije bodo razkrile, ali je ta vrsta s svojimi morfološkimi različicami potencialna (sub)populacija ali morfološka oz. celo ekološka varianta, ki je povezana z evtrofikacijo vodnega telesa. Selitvena pot te vrste je razkrila nekatere informacije, vendar so potrebne nadaljnje študije, ki nam omogočajo boljše prepoznavanje in zmanjševanje potencialnih groženj, povezanih z invazivnimi procesi.

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