

## Rapid Communication

## First records of American *Wolffia columbiana* in Europe – Clandestine replacement of native *Wolffia arrhiza*?

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### Abstract

*Wolffia columbiana* is a member of the family Lemnaceae and native to the Americas. In 2013, the first two occurrences of *Wolffia columbiana* in Europe were found in Germany and the Netherlands. Differentiation between *W. columbiana* and the rare native *W. arrhiza* is difficult and a scanning electron microscope (SEM) was used to confirm plant identification. A brief description of both populations of *W. columbiana* and some identifying characteristics are given. Due to their small size, *Wolffia* species are easily overlooked in the field and/or misidentified, and thus it seems likely, that even more unknown occurrences of alien *Wolffia* species might occur in Europe. The remaining known occurrences of putative *W. arrhiza* should be inspected to check their identity. We hypothesize, that alien *W. columbiana* might be sometimes overlooked or misidentified as the native *W. arrhiza*, and thus it may have a more widespread distribution within Europe than thought.

**Key words:** alien aquatic plant, dispersal, duckweed, plant invasion, Lemnaceae, watermeal

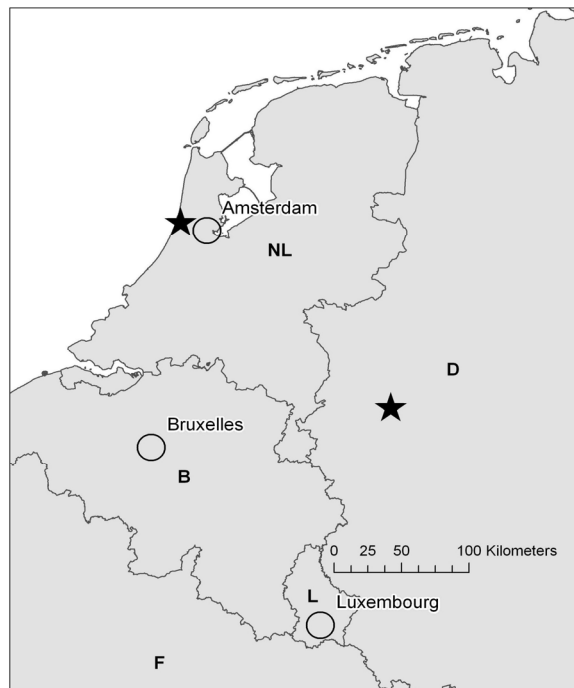
### Introduction

Introduced invasive plants can cause a serious threat to biodiversity, and the percentage of introduced aquatic plants causing ecological or economic impacts upon their habitats is much higher than for terrestrial plant species (Vilà et al. 2010; Hussner 2012). During the last decades, an increase in the number and upcoming problems associated with spreading alien aquatic plant species has been reported (Sheppard et al. 2006; Hussner 2012).

Ornamental trade is considered as the most important pathway of introduction of alien species (Brunel 2009; Champion et al. 2010; Hussner et al. 2010). Most reports relate to those species that have become serious pests in Europe, like *Ludwigia grandiflora* Greuter and Burdet (Thouvenot et al. 2013) or *Hydrocotyle ranunculoides* L. fil. (Newman and Dawson 1999). It is documented, that about 50 % of the introduced alien aquatic plant species show the potential to become pests

in their introduced countries (Champion and Clayton 2000; Hussner et al. 2014). Amongst the 25 known alien aquatic plant species in Germany, two *Lemna* species, *Lemna minuta* Humb., Bonpl. and Kunth and *Lemna turionifera* Landolt are reported, and the former species has become widespread in numerous European water bodies (Hussner 2012).

*Wolffia* species, also members of the family Lemnaceae (or Araceae in the broader sense), are known to be the tiniest flowering plants on earth (Daubs 1965; Fintha 1979). In Europe, *Wolffia arrhiza* (L.) Horkel ex Wimm. is the only native species of the genus, which comprises 11 species worldwide (Crawford and Landolt 1995). Populations of *W. arrhiza* have declined during the last few decades in Central Europe and this species has become rare and is already extinct in many regions (NetPhyD and BfN 2014). *Wolffia arrhiza* is listed as endangered in the Red List of Threatened Species of Germany (Korneck et al. 1996). In the federal state North Rhine-Westphalia, *W. arrhiza* is



**Figure 1.** The localities of the two found *W. columbiana* populations in Germany and The Netherlands (asterisks).

listed as near extinct (Raabe et al. 2010), the last records were made in 1994 near Wesel (Abts 1994) and in 2008 north of Aachen (Raabe et al. 2010). However, there are still many records of *W. arrhiza* in the Netherlands, listed in the Atlas of the Dutch flora (FLORON 2014).

Here we describe the first reports of American *Wolffia columbiana* Karsten in Europe. We argue that, due to the difficulty with identification of *Wolffia* species and the fact that there was no previous report of this species in Europe, the alien *W. columbiana* might be often misidentified as *W. arrhiza* and thus the recent distribution of *W. arrhiza* in Europe is uncertain.

## Locality of the samples and methods

### Localities

In summer 2013, the first author found two populations of a *Wolffia* species, which was hitherto unknown to Europe (Figure 1). The first population was found in July 2013 in a ditch within the protected area Urdenbacher Kämpe in the south of Düsseldorf, Germany, (51°08'39"N, 6°52'35"E). There, *Wolffia* grew together with other floating Lemnaceae (*Lemna gibba* L.,

*Lemna minor* L., *Lemna minuta* Kunth, *Spirodela polyrhiza* Schleiden) and submersed *Elodea nuttallii* (Planch.) H. St. John. The floating plants formed a dense mat of more than 50 m<sup>2</sup>, in which *Wolffia* grew in mixture with the larger Lemnaceae.

The second population was found in a coastal dune waterbody of the Zuid-Kennemerland National Park in the south of IJmuiden, Netherlands, (52°26'32"N, 4°36'32"E) in August 2013. There, *Wolffia* formed a loose mat of ca. 30 m<sup>2</sup> with single specimens of *Hydrocharis morsus-ranae* L., floating above a dense stand of submersed *Elodea canadensis* Michx. Samples from both localities were collected for further investigation and identification.

### Identification

We used the identification key and species descriptions in the Lemnaceae monograph by Landolt (1980; 1986) for plant identification and for taxonomic reference. The differentiation between the different *Wolffia* species is based on stomatal number and general plant morphology. Due to the frond thickness, a light microscope provided only low contrast between stomata and epidermal cells and made stomata counting inaccurate. Therefore we used a scanning electron microscope (SEM) to enable accurate counts.

### Preparation and Scanning electron microscope (SEM) technique

Following a modified method of White and Wise (1998), the samples were fixed for 1 h in glutaraldehyde and then rinsed three times with phosphate buffered saline (PBS). After fixing the samples were dehydrated through a graded ethanol series (50% – 70% – 80% – 90% – 96% – 100%) for 10 min respectively. Pure ethanol was finally replaced by pure acetone. The samples were then dried by the critical point method, glued on SEM specimen mounts by conductive adhesive tabs (Plano) and coated with gold. They were viewed under a Zeiss scanning electron microscope Leo 1430 VP at an accelerating voltage of 18.00 kV.

## Results

### Species description

SEM allowed non-ambiguous counting of stomata (Table 1). The fronds of the German samples contained 3–14 stomata per frond (mean 5.5), the Dutch samples contained 3–11 stomata per frond

**Table 1.** Number of stomata per frond (x = specimen with flowers).

Germany		
Nr.	Stomata/frond	flowering
1	14	x
2	3	-
3	4	-
4	4	-
5	5	-
6	3	-
	5.5	Mean
Netherlands		
Nr.	Stomata/frond	flowering
1	7	-
2	9	-
3	11	-
4	4	-
5	4	-
6	8	-
7	4	-
8	8	-
9	3	-
	6.4	Mean

(mean 6.4). Landolt (1980; 1986), distinguished *Wolffia* species based on stomatal number with 1–15 stomata per frond for *W. columbiana*, with 10–100 stomata per frond for *W. arrhiza*. Thus *W. columbiana* was determined for both provenances.

Additionally, the convex and only slightly flattened upper frond surface provided further evidence for this identification, as the frond surface of *W. arrhiza* is more flattened. Moreover, the frond margins are transparent, which is typical for *W. columbiana*, rather than the more opaque margins in *W. arrhiza* (Daubs 1965; Landolt 1980; 1986; Armstrong personal communication).

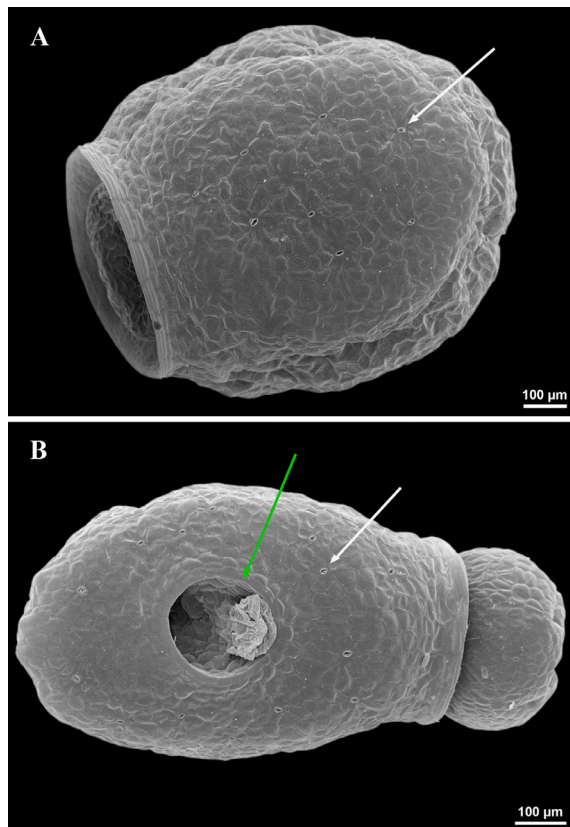
Both samples contained several flowering specimens. Figure 2A shows a vegetative frond with typical stomatal number. Figure 2B shows a flowering frond with 14 stomata on the mother frond, which is above the average of typical vegetative fronds.

The SEM photos of the *Wolffia* fronds were sent to Prof. Armstrong, Palomar College, USA, for determination, and he confirmed their identity as *W. columbiana*.

## Discussion

The documented two populations of *W. columbiana* are the first two known occurrences of this species in Germany and the Netherlands (van de Weyer pers. comm. 2014; van Valkenburg and Pot pers. comm. 2014) respectively. Even though *Wolffia columbiana* is listed in the GBIF database (<http://data.gbif.org/occurrences/239833788>) for the area of Zurich by W. Koch for the year 1950, there is reason to suspect that this is an erroneous entry, as this alleged record neither occurs in the Flora of Zurich (Landolt 2001) nor in the monograph of Lemnaceae by Elias Landolt (1980, 1986). Landolt (1986) states a similar percentage of flowering plants between 1.5 and 3 % in nature for both the Old World species *W. arrhiza* and the New World species *W. columbiana*. However, in Europe *W. arrhiza* has never been observed flowering, with the only exception being a single record from the northern forelands of the Caucasus (Benková 1957; Hegi 1980). As both sampled occurrences comprised abundant flowering specimens, this may give an additional clue to their identity as *W. columbiana*.

Long-distance dispersal of *Wolffia* species is often mediated by water birds along major bird migration routes (Jäger 1964), but the transatlantic introduction of *W. columbiana* from the Americas is more likely a result of aquarist trade and



**Figure 2.** Typical vegetative frond of *Wolffia columbiana* with eight stomata (A); flowering frond of *Wolffia columbiana* with fourteen stomata (B). White arrow: stoma, green arrow: flower.

aquarium waste, a common pathway of introduction of alien aquatic plant species globally (Brunel 2009; Champion et al. 2010). As tiny *Wolffia* plants are easily overlooked, we argue that there are most likely more occurrences of *W. columbiana* than the two described localities within Germany, the Netherlands or neighbouring countries within Europe. Despite the lack of data on the competitive behaviour of *Wolffia columbiana* in its introduced range, we hypothesize that there is a possibility of its subsequent spread leading to substitution of European native *Wolffia arrhiza*. The remaining occurrences of assumed *W. arrhiza* in Europe should be critically assessed with regard to the possibility they may be *W. columbiana* or other exotic watermeal species.

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