Wolffia columbiana and W. globosa (Araceae) new to Britain

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Abstract
In the course of surveys of ditches in the Pevensey Levels in October 2021, a large population of the non-native Wolffia columbiana H. Karst. was found, new to Britain. Subsequent investigation showed W. columbiana to be widespread on the Pevensey and Gwent Levels and present in other grazing marsh ditch complexes in Somerset, West Sussex and Kent, while W. globosa (Roxb.) Hartog & Plas was also found in the Gwent Levels. This paper describes the known distribution of W. columbiana in Britain, provides information on identification of different Wolffia species and considers the implications of this discovery for conservation of the native W. arrhiza (L.) Horkel ex Wimm.

Keywords: non-native; invasive; Duckweed; distribution; identification

Introduction
Wolffia L. is a genus of duckweed which includes the smallest flowering plant in the world and is distinguished from other duckweeds by a combination of swollen lower parts and the lack of roots. The genus includes 11 species worldwide (POWO, 2021) and, due to their small size, identification typically relies in part on microscopic characters. Since 2013, three non-native species have been recorded in Europe: W. columbiana H. Karst. (Schmitz et al. 2014), W. globosa (Roxb.) Hartog & Plas (Kirjakov & Velichkova 2013) and W. australiana (Benth.) Hartog & Plas (FLoron, 2021) and it has always been likely that they would appear in the UK (e.g. Adams, 2019).

In September 2021 whilst surveying parts of the Pevensey Levels in East Sussex (v.c.14) to update records of the status of Potamogeton acutifolius, RVL and EJ found a population of Wolffia in one of the larger drains. Initially thought to be the native W. arrhiza (L.) Horkel ex Wimm., closer inspection revealed a number of characters suggesting that it might, in fact, be one of the alien species. Microscopic examination then confirmed its identity as W. columbiana (subsequently also confirmed by W. van der Ven), a first record of this non-native species in Britain. Subsequent investigation showed W. columbiana to be present in a number of areas, with photographic evidence of its earlier occurrence; and W. globosa was found at a single site in the Gwent Levels (v.c.35).
This paper describes the discovery of *W. columbiana* in Britain with information on its current known distribution, as well as the discovery of *W. globosa*, provides guidance on identification of *Wolffia* species and considers the potential period for which *W. columbiana* has been established in the country.

**Recording *Wolffia* in Britain in 2021**
Following initial discovery of *W. columbiana* in the ditch in the Pevensey Levels, the levels were re-visited in early October 2021, to attempt to locate populations of *W. arrhiza*. Sites with past records of *W. arrhiza* were visited but most were overgrown and shaded by reeds and willow scrub. *Wolffia* populations were only found at three sites and all involved *W. columbiana* (Table 1; Fig. 1).

A few days later a visit was made by RVL to the Gwent Levels and a total of six sites surveyed, representing all the main areas within the levels from which *W. arrhiza* had been reported. All sites supported populations of *W. columbiana* (Fig. 2) and one included plants subsequently identified as *W. globosa*. A visit was then made to the Somerset Levels (v.c.6) to look for *Wolffia* populations. Most of the ditches surveyed with past records were in a late stage of succession with no *Wolffia* found; however, Brock’s Pill Rhyne which had no previous records of *Wolffia* had a complete carpet of *W. columbiana*. A request to A.J. Lockton and S. Buckingham for material from Kent then provided records of *W. columbiana* from seven sites, while a visit by RVL to an area of the Rother Valley on the East Sussex – Kent border failed to turn up any *Wolffia* at all, with some sites in too far advanced a stage of succession and others simply with *Lemna* and *Spirodela* species but no *Wolffia*. Edgcumbe Park in Devon, from which there was an incongruous record of *W. arrhiza* in 1974, was visited in early November 2021 but the only duckweed present was *Lemna minuta*.

![Figure 1. Floating groups of *Wolffia columbiana* fronds, showing variety in size, frond outline and the degree to which the differentiated margin is visible, as well as the very pale green colour (images: R.V. Lansdown).](image)

In November 2021 a *Wolffia* population was sampled in a pond in east Surrey (v.c.17), this did not survive postage particularly well but appeared to include *W. arrhiza*, possibly as well as *W. columbiana* and *W. globosa* however identification is not absolutely certain (see also Discussion). *W. arrhiza* was also tentatively identified in a sample (also including *W. columbiana*) from ditch on the Dowells in sheep pasture near Kenardington (TQ9784.3126) in East Kent (v.c.15).
| v.c. | Grid Reference | Locality | Collector               | Date       |
|------|----------------|----------|-------------------------|------------|
| 5    | ST3645.5216    | Brock's Pill Rhyne, Somerset Levels | R.V. Lansdown | 14.10.2021 |
| 13   | TQ0213         | Amberley Wild Brooks               | M. Cousins, J. Whitman | 2017       |
| 14   | TQ6079.0751    | ditch, Pevensey Levels             | E. Jones (image)       | 2018       |
| 14   | TQ6731.0837    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 22.9.2021 |
| 14   | TQ6889.0710    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 22.9.2021 |
| 14   | TQ6769.0764    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 22.9.2021 |
| 14   | TQ6079.0751    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 23.9.2021 |
| 14   | TQ6459.0419    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 7.10.2021 |
| 14   | TQ6612.0606    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 7.10.2021 |
| 14   | TQ6257.1058    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 7.10.2021 |
| 14   | TQ6731.0837    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 21.10.2021|
| 14   | TQ6769.0764    | ditch, Pevensey Levels             | R.V. Lansdown, E. Jones | 21.10.2021|
| 15   | TR276631       | fishing lake by the River Stour below Pluck's Gutter | A.J. Lockton | 14.10.2021 |
| 15   | TQ9784.3126    | ditches in sheep pasture near Kenardington | S. Buckingham | 16.10.2021 |
| 15   | TQ9792.3128    | ditches in sheep pasture near Kenardington | S. Buckingham | 16.10.2021 |
| 15   | TQ9211.3010    | recently cleaned roadside ditch near Reading Street | S. Buckingham | 16.10.2021 |
| 15   | TR0105.2844    | roadside pond near Brenzett Corner | S. Buckingham | 16.10.2021 |
| 15   | TR0432.3188    | ditches in arable near Newchurch | S. Buckingham | 16.10.2021 |
| 15   | TR0763.2285    | ditch on edge of arable, Greatstone | S. Buckingham | 16.10.2021 |
| 35   | ST3968.8569    | Middle Road Reen, Gwent Levels     | R.V. Lansdown   | 12.10.2021 |
| 35   | ST3878.8512    | Elver Pill Reen, Gwent Levels      | R.V. Lansdown   | 12.10.2021 |
| 35   | ST3824.8486    | Parish Reen, Gwent Levels          | R.V. Lansdown   | 12.10.2021 |
| 35   | ST3770.8550    | Parish Reen, Gwent Levels          | R.V. Lansdown   | 12.10.2021 |
| 35   | ST3522.8337    | Saltmarsh Reen, Gwent Levels       | R.V. Lansdown   | 12.10.2021 |
| 35   | ST3727.8534    | Parish Reen, Gwent Levels          | R.V. Lansdown   | 12.10.2021 |
Figure 2. *Wolffia columbiana* in the Gwent Levels with *Lemna gibba, L. minor, L. minuta* and *Spirodela polyrhiza* (image: R.V. Lansdown).

**Identification**
All *Wolffia* species are very small; the largest is *W. arrhiza*, which is typically 0.7-1.3 × 0.6-1.0 mm but is only slightly larger than *W. australiana* and *W. columbiana*; the smallest, which is also the smallest known flowering plant, is *W. globosa* which is typically 0.5-0.8 × 0.2-0.4 mm (Table 2). They are composed of a photosynthetic upper part which is green and opaque, below which there is an expanded lower layer which is firm and may be green or colourless.

**Table 2. Summary of identification characters of *Wolffia* species known from Europe** (from Landolt, 1986; Landolt, 1994; Landolt, 2000; Bog et al., 2020)

| Character       | *W. arrhiza* | *W. australiana* | *W. columbiana* | *W. globosa*       |
|-----------------|--------------|------------------|-----------------|--------------------|
| Greatest width  | just below surface | at surface       | well below surface | well below surface |
| Length (mm)     | (0.5) 0.7-1.3 (1.5) | (0.5)1.0-1.3(1.5) | (0.5)0.7-1.2(1.4) | (0.4)0.5-0.8(0.9)  |
| Width (mm)      | (0.4)0.6-1.0(1.2) | (0.3)0.5-0.7(0.8) | (0.5)0.6-1.1(1.2) | (0.3)0.4-0.6       |
| Length : width  | 1-1.33       | 1.33-2           | 1-1.33          | 1.33-2.33          |
| Depth : width   | 1-1.33       | 2-3              | 1-1.33          | 0.75-1.33          |
| No. of stomata  | (0)30-100    | 50-80            | 1-10(-30)       | 8-25(35)           |
| Surface colour  | bright green | bright green     | pale green      | pale green         |
| Surface         | opaque       | opaque           | translucent     | translucent        |
| Lower colour    | translucent  | ?                | green           | green              |
Species of *Wolffia* can be very difficult to identify, particularly as counting stomata appears to be unreliable without recourse to Scanning Electron Microscopy (SEM) (Schmitz *et al.*, 2014; W. van der Wen pers. comm.). In spite of this, many species have been recorded outside their native range (Table 3), with species originating in the Americas (*W. columbiana*), Asia (*W. globosa*) and Australasia (*W. australiana*) (Armstrong & Thorne 1984, Landolt 1986, Mazzeo *et al*. 1993, Landolt 1994) all occurring in Europe (Kirjakov & Velichkova 2013, Schmitz *et al*. 2014, FLoron 2021, Armstrong n.d.). An attempt has therefore been made here to provide guidance on all known species worldwide, while details are provided (Table 2) for the species known to occur in Europe.

**Table 3. Summary of the native and non-native distribution of *Wolffia* species**

| Species    | native range                                      | non-native range                                      |
|------------|---------------------------------------------------|------------------------------------------------------|
| *W. angusta* | Australia, Malaysia, Singapore (Landolt, 1994, POWO, 2021) |                                                      |
| *W. arrhiza* | UK east to European Russia and south to South Africa (POWO, 2021) | Brazil, Japan, USA (California) (POWO, 2021)         |
| *W. australiana* | Australia, New Zealand (POWO, 2021) | Netherlands (FLoron, 2021) |
| *W. borealis* | Canada (POWO, 2021), USA                          |                                                      |
| *W. brasiliensis* | Canada to Paraguay, Uruguay, northern Chile (POWO, 2021) |                                                      |
| *W. columbiana* | Canada to Argentina (POWO, 2021)                  | UK, Netherlands, Belgium, France, Germany, India, Italy, Japan (Kax *et al.*, 1978; Kadono, 2004; Ardenghi *et al.*, 2017; FLoron, 2021, Niebler *et al.*, 2021; J. Geslin pers. comm.) |
| *W. cylindracea* | Kenya to South Africa (POWO, 2021)               |                                                      |
| *W. elongata* | Colombia (POWO, 2021)                            |                                                      |
| *W. globosa* | Pakistan to Japan, Malaysia (POWO, 2021)         | Bulgaria, Colombia, France, Germany, Japan, Peru, USA (Kirjakov & Velichkova, 2013; Niebler *et al.*, 2021, POWO, 2021) |
| *W. microscopica* | Pakistan, India (Sree *et al.*, 2014; POWO, 2021) |                                                      |
| *W. neglecta* | Sri Lanka, India and Pakistan (POWO, 2021)        |                                                      |
### Key to the identification of *Wolffia* species
(from Landolt, 1986; Landolt, 1994; Sree *et al.*, 2014; Landolt, 2000; Ward *et al.* 2021; Bog *et al.* 2013):

1. Fronds with a long appendage below, resembling a root but tapering into the frond
   - *W. microscopica*
   - Fronds without an appendage below
   - 2

2. At least larger fronds with a prominent dorsal papule
   - *W. brasiliensis*
   - Fronds without a prominent dorsal papule
   - 3

3. Fronds 0.3-1 x as deep as wide; vegetative apex of frond pointed and slightly upturned
   - *W. borealis*
   - Fronds 0.7-3 x as deep as wide; vegetative apex of frond ± flat
   - 4

4. Fronds widest in centre of cross-section, well below the water surface, showing as a clearly differentiated margin c. one-fifth of width of frond seen from above (Fig. 3)
   - *W. columbiana*
   - Fronds either of even width in cross-section or widest in upper part without clearly differentiated margin from above, or differentiated margin very narrow (roughly <one-tenth of width of frond seen from above)
   - 5

5. Fronds 2-3 x as deep as wide
   - *W. australiana*
   - Fronds <1.5 x as deep as wide
   - 6

6. Fronds 0.3-0.8 mm wide; 1.3-2 × as long as wide; deep green on upper surface
   - *W. neglecta*
   - Fronds 0.2-0.5 mm wide; 1.7-2.5 × as long as wide; whitish green on upper surface with more intense green margins
   - 7

7. Darker green upper part of fronds 1.33-2.5 x as long as wide, 1.5-3 x as deep as wide with the greatest width at the water surface (almost no translucent margin visible from above)
   - *W. angusta*
   - Darker green upper part of fronds 1-2.33 x as long as wide, 0.75-1.5 x as deep as wide with the greatest width below the water surface (at least laterally a translucent margin visible from above)
   - 8

8. Fronds intensely green and mostly shiny at the surface with mostly >30 stomata
   - *W. arrhiza*
   - Fronds not shiny, light green to rather intensely green with <30 stomata
   - 9

9. Fronds with no translucent margin at the tip, with 15-30 stomata
   - *W. cylindracea*
   - Fronds with a distinct translucent margin at the tip, mostly <20 stomata
   - *W. globosa*
Of the species which have been recorded in Europe, *W. columbiana* is relatively easily recognised by the pale green, translucent upper part surrounded by the clearly differentiated lower layer which is distinctly visible from above due to the almost globose cross-section of fronds (Figs. 1 and 3). *W. australiana* is typically larger than the other species and narrower than all except *W. globosa* but is best recognised by the fact that fronds are parallel-sided in cross-section and can be much deeper than wide. *W. globosa* is typically much smaller than the other species, forming populations of uniformly tiny fronds which are narrower than *W. arrhiza* and *W. columbiana*. *W. arrhiza* (Fig. 4) is then recognised by the fact that it lacks the diagnostic characters of the other species. However, identification of plants can be far from straightforward.

Figure 3. *Wolffia columbiana* showing the broad differentiated margin visible from above (left) and almost globose fronds (right: left-hand plant from the side, right hand from one end), material from the Pevensey Levels in October 2021 (images: R.V. Lansdown).

Figure 4. *Wolffia arrhiza* (with *Lemna gibba*) in a pond at the Hameau du Bouchet, Parc Naturel Régional de la Brenne, France showing no differentiated margin (image: R.V. Lansdown).
**Discussion**

There has been an increase in new regional records of non-native duckweed species in recent years (e.g. Lansdown 2008, Lansdown *et al.* 2015, Rumsey & Lansdown 2012) (Table 2). It is clear that these species are being moved around the world to an increasing extent and there is a need for increasing scrutiny to recognise new invasions when they occur. *Wolffia columbiana* has clearly become well-established in Europe, where it is now known from the UK (this study), Belgium, France (J. Geslin pers. comm.), Germany (Schou *et al.* in prep.), Italy (Ardenghi *et al.* 2017) and the Netherlands (FLORON 2021). Random sampling of *Wolffia* demonstrated that in Germany and the Netherlands *W. columbiana* now is much more frequent than the native *W. arrhiza* (Schmitz *et al.* 2016, W. van der Wen pers. comm.). *W. australiana* has also been recorded from the Netherlands where it is known from only a few sites (FLORON 2021), while *W. globosa* is known from single sites in France (Niebler *et al.* 2021), Germany (Schou *et al.* in prep.) and Bulgaria (Kirjakov & Velichkova 2013).

The first published record of *Wolffia* in Britain was given by Trimen (1866), in relation to a population in a pond near Staines, Middlesex, although Gray (1866) mentioned having been shown specimens about 50 years earlier which were believed to have been collected from Putney Common. Until 2021, all populations were assumed to be *W. arrhiza*. However, the widespread occurrence of *W. columbiana* in 2021 strongly suggests that it has been established for some time; two sets of records are of particular note. The first record of *Wolffia* from the Pevensey Levels is from 1994 (BSBI Distribution Database); it would be surprising in an area so intensively botanised if it had been present in any abundance prior to that date. *W. columbiana* has certainly been established since 2018 (image EJ) and is now abundant (Fig. 5). It is credible that *Wolffia arrhiza* was never in the Pevensey Levels but that *W. columbiana* arrived at least as long ago as the 1990s. Similarly, the first record of *Wolffia* from the Gwent Levels was in 1982 and it seems credible that *W. arrhiza* was never present in the area, but that records involved *W. columbiana* or even *W. globosa*. In other areas, the Somerset Levels (v.c.5 and v.c.6), East Kent (v.c.15) and the Thames floodplain (in v.c.17), *Wolffia* populations were all first recorded in the 19th or the early part of the 20th century and are likely to have involved *W. arrhiza*; however, all except v.c.17 have been shown since to support *W. columbiana*. Photographs of material from the Lewes area show that *W. columbiana* was present there at least in 2011 (Lyons, 2011).

When the decade in which *Wolffia* populations were recorded for the first time in a hectad is compared, there is a striking increase in the middle of the 20th century and a second in the early 1990s (Fig. 6). It is tempting to think that at least part of one of these increases could be due to the arrival and spread of *W. columbiana*, although the 1950 peak is likely to be at least partly linked to fieldwork for the first atlas (Perring & Walters, 1962).

Duckweeds show a remarkable capacity for dispersal, and *W. columbiana* has even been shown to remain viable when transported through ingestion by birds (Silva *et al.*, 2018). Whether by such means or by transfer by attachment to waterfowl, it is feasible that avian transmission may account for the Pevensey Levels and Romney Marsh introductions. The occurrence of *W. columbiana* in Italy in 2016 was believed to have derived from a neighbouring fish farm, where importation of fish cultures may carry *Wolffia* as contaminants (Ardenghi *et al.*, 2017). Inspection
of the bases of plants of *Pistia stratiotes* and other non-native plant species sold in garden centres shows that many include non-native duckweeds such as *Lemna aequinoctialis*, *L. minuta*, *L. valdiviana* and *Spirodea oligorrhiza* (Rumsey & Lansdown, 2012; RVL unpublished data). It is highly likely that the inadvertent or deliberate introduction of cultivated aquatic plants to the wild brings with it some of these non-native duckweeds.

**Figure 5.** A ditch in the Pevensey Levels with a monospecific population of *Wolffia columbiana*, showing the characteristic pale green colour of populations (image: E. Jones).

**Figure 6.** Number of new hectad records of *Wolffia arrhiza* per decade in Britain.
Images taken from the side of plants of *W. columbiana*, which have a globose cross-section, a differentiated margin when viewed from above and translucent upper cells, from the Gwent Levels (Fig. 3) more closely resemble those of *W. arrhiza* given by Landolt (1994, Fig. 2h) than those of *W. columbiana* (Landolt 1994, Fig. 2o), particularly in the extent of the differentiated upper part and the degree to which the upper part is domed. This character therefore appears to be unreliable.

*Wolffia* species are very small and there are only a very small number of features which can serve for morphological identification, particularly as counts of the number of stomata appear to be unreliable without recourse to SEM. Counts of stomata using a dissecting microscope suggest that populations of *W. columbiana* recorded in north-western France had <10 stomata per frond, whereas counts from material from the Gwent, Pevensey and Somerset Levels typically had (8-10-20(-25). Schmitz *et al.* (2014) using SEM noted that counts from German populations found 3–14 stomata per frond (mean 5.5) and Dutch populations 3–11 per frond (mean 6.4). Landolt (1986) notes that “In Florida, there are clones of *W. columbiana* which have up to 30 stomata and therefore resemble *W. arrhiza*. The difference in counts in Europe could be because *W. columbiana* has spread within each country from one or only a few introductions, with most reproduction being vegetative and as a result, the populations involve a small number of clones. Apart from gross and typically unequivocal morphological structures, such as presence of a dorsal papule in *W. brasiliensis* or tapering into a root-like structure below in *W. microscopica*, most identification is based on poorly-defined descriptive terms or relative characters, such as “spherical”, “ellipsoid” or “boat-shaped” (Landolt 1986), “Fronds intensely green and mostly shiny at the surface” (Landolt 1994), relative length to width, width to depth etc. There is also typically an overlap in characters presented and as the plants are so small, even small overlaps can render characters almost useless. It is therefore very difficult to achieve confident and, above all, reliable identification of *Wolffia* species other than those with particularly distinctive characters, such as *W. brasiliensis*, *W. columbiana* and *W. microscopica*. As noted by Landolt (1994) there is a need for more research.

The most striking aspect of the survey of *Wolffia* populations following the discovery of *W. columbiana* in the Pevensey Levels in 2021 was the very limited number of populations of *W. arrhiza* recorded. There is an urgent need to re-visit *Wolffia* populations to confirm the identity of the species involved, document all non-native species present and as a high priority, confirm the distribution and conservation status of *W. arrhiza*.

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