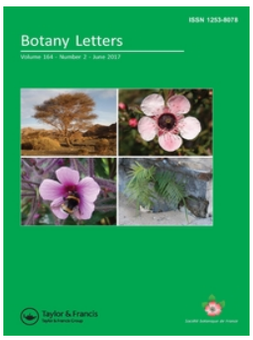

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Wolffia columbiana (Araceae, Lemnoideae): first record of the smallest alien flowering plant in southern Europe and Italy

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ABSTRACT

Wolffia columbiana (Colombia watermeal), a naturalized American neophyte, is recorded for the first time from southern Europe. Identification, distribution, invasion status, ecology, and pathways of introduction are presented. Furthermore, the potential invasive behaviour and impacts in the new growing site have been assessed with the EPPO prioritization process scheme for invasive alien plants.

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Alien species; aquatic plants; biological invasion; duckweeds; Europe; vectors

Introduction

The genus *Wolffia* Horkel ex Schleid. (Araceae, Lemnoideae; watermeal) includes 11 rootless aquatic species, distributed worldwide, especially in the warm temperate and tropical regions (Landolt 1994, 1998, 2000). In the European continent, a single species is considered native, *W. arrhiza* (L.) Horkel ex Wimm., which occurs in most countries (Euro+Med 2006–2017; Landolt 1986, 1994). Three neophytes, palaeotropical *W. globosa* (Roxb.) Hartog & Plas, American *W. columbiana* H.Karst., and Oceanian *W. australiana* (Benth.) Hartog & Plas, have recently been recorded, respectively, from Bulgaria (Kirjakov and Velichkova 2013), Germany and the Netherlands (Schmitz, Köhler, and Hussner 2014), and the Netherlands (Achterkamp 2014).

In March 2016, an extended population of *Wolffia* was discovered in a pond in Pieve Porto Morone (Lombardia, Italy). A closer microscopic examination of live specimens revealed a clear dissimilarity from *W. arrhiza*, the only species known in Italy (Celesti-Grapow et al. 2009), leading eventually to American *W. columbiana*. The species represents a novelty to the flora of southern Europe and Italy as well as the smallest alien flowering plant in this part of the continent (0.77–0.90 mm in length). Its finding augments the local contingent of non-native aquatic plants, which, in all of Europe, has the highest concentration in Italy and France (Hussner 2012). The remarkable presence of alien macrophytes in this area is historically associated with rice cultivation (especially in northern Italy), yet an increasingly significant role is being played by the trade of aquarium and aquatic ornamental plants (Ardenghi, Galasso, and Banfi 2015; Ardenghi et al. 2016; Mazza et al. 2015; Van Valkenburg and Pot 2008), regarded as a probable source of accidental introduction of alien *Wolffia* species both in Europe and North America (Armstrong 1985, 1989; Armstrong and Thorne 1984; Schmitz, Köhler, and Hussner 2014).

In this paper, identification aspects, invasion status, and pathways of introduction concerning *Wolffia columbiana* in Italy are discussed. Additionally, the potential impacts and invasive behaviour have been assessed by means of the EPPO prioritization process scheme for invasive alien plants (EPPO/OEPP 2012).

Material and methods

Plant sampling and species identification

The present work is based on the study of plant material collected on field trips conducted by the authors in Pieve Porto Morone (province of Pavia, Lombardia, Italy), between March 2016 and January 2017. Live plants were cultivated at the Botanical Garden of the University of Pavia and voucher specimens are stored at BR, FI, MSNM (herbarium codes according to Index Herbariorum: Thiers 2017+), and the private herbarium of the first author.

Besides the works by Landolt (1986, 1994, 2000) and Armstrong (2017), the identification of the specimens was also allowed by comparison with live individuals of *Wolffia arrhiza* collected in Arena Po (WGS84: 45.09772°N, 09.36526°E) in late September 2016.

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Physical and chemical water analyses

In September 2016 and January 2017, water temperature, percentage of oxygen saturation, pH, and water conductibility were recorded in the growing site, by means of a multiparametric probe HACH-HQ40. Moreover, 500 ml of the pond water was sampled and, once in the laboratory, the level of nitrate ($N\text{-}NO_3$), ammonia ($N\text{-}NH_4$), and total phosphorus (tot P) was determined. The principal chemical data were analysed by means of Merck's spectroquant chemical kit and a photometer NOVA 60 (Millipore, Billerica, MA, USA). The concentration of some parameters was lower than method sensitivity; thus, they were not considered in subsequent analyses (e.g. nitrate data).

Potential impacts and invasive behaviour

Potential impacts and invasive behaviour of the Italian population of *Wolffia columbiana* were assessed following the "EPPO prioritization process for invasive alien plants" introduced by the European and Mediterranean Plant Protection Organization (EPPO/OEPP 2012).

Results and discussion

Nomenclature

Wolffia columbiana H.Karst., Bot. Untersuch. (Berlin) 1: 103 (1865) ≡ *Grantia columbiana* (H.Karst.) MacMill., Metasp. Minnesota Valley: 135 (1892) ≡ *Bruniera columbiana* (H.Karst.) Nieuwl., Amer. Midl. Naturalist 2 (11–12): 306 (1912).

Lectotype (designated by Landolt 1986, 454): Colombia, Santa Marta, [without date], *H.Karst* (STU).

Description and identity of the specimens

Aquatic herb, perennial. *Roots* absent. *Fronds* floating on the water surface, single or two joined together, with the greatest width below the water surface; shape from nearly spherical to widely ellipsoid; size: 0.77–0.90 mm long, 0.67–0.73 mm wide, 1.05–1.29 times as long as wide, about 1.30 times as deep as wide; upper surface bright pale green, transparent, especially at the edge (which appears distinctly wide with substage lighting) (Figure 1b); *stomata* in number of 4–9(–12) per frond. *Turions* present, similar to the floating fronds but smaller. *Flowers*, *fruits*, and *seeds* not observed.

Using keys and descriptions provided by Landolt (1986, 1994, 2000) and Armstrong (2017), the identity of *Wolffia columbiana* was confirmed thanks to the low number of stomata per frond (less than 10, with the exception of one individual with up to 12), which ruled out *W. arrhiza*, bearing 10–100 ([20–]30–120 according to Landolt 1994); in addition, *W. arrhiza* has dark green upper surface (non-transparent), without a distinct translucent edge at substage illumination. Eventually, the almost isodiametric shape of the fronds (1.00–1.30 times as long as wide) (Figure 1b) led to the exclusion of *W. globosa* and *W. cylindracea* Hegelm., characterized by more elongated fronds, respectively, 1.30–2.00 (1.30–1.70 according to Landolt 1994) and 1.30–1.50 times as long as wide; the low number of stomata (less than 50), along with fronds' depth/width ratio (about 1.30 vs. 2.00–3.00) and length (up to 0.90 mm vs. [0.50–]1.00–1.30[–1.50] mm), allowed the exclusion of *W. australiana* too.

Figure 1. The pond "Lanca degli Occhiali" in Pieve Porto Morone (September 2016) (a), where *Wolffia columbiana* forms a uniform green layer over the surface together with *Spirodela polyrhiza* and sporadic individuals of *Lemna minor* (May 2016) (b). Photographs by R. Sconfietti (a) and N. M. G. Ardenghi (b).

Worldwide distribution and ecology

The native range of *Wolffia columbiana* extends through the temperate and tropical regions of North and South America, from Canada southwards to Argentina (Landolt 1986, 1994). Outside the Americas, it is known only from central and western Europe, in over 80 localities in Germany and the Netherlands (FLORON 2014; Schmitz, Köhler, and Hussner 2014; Schmitz, Köhler, and Nesemann 2016). Previous records from Kashmir (Kak, Bakaya, and Javeid 1978) and Switzerland (Schmitz, Köhler, and Hussner 2014) proved to be wrong (Landolt 1986; Schmitz, Köhler, and Hussner 2014).

In its primary range, *Wolffia columbiana* grows in mesotrophic to eutrophic still to slowly flowing waters (ponds, streams), with pH 9 or higher, at low to high (up to 2600 m) elevations, often associated with other pleustophytes; it does not tolerate winter temperatures lower than −8°C and summer temperatures higher than +28°C (Armstrong 2017; Landolt 1986, 1994, 2000).

The new Italian population: location and invasion status

Wolffia columbiana was first discovered in early March 2016, in the southern part of Lombardia (north-western Italy), within a small pond (or "bodri", which is the vernacular term employed in some parts of the lower Po Plain to indicate a deep water body, shaped as an upside-down cone and connected to the superficial aquifer, originated from the drilling of the ground by the river overflowing the banks; see Brignani and Ferrari 2002) locally known as "Lanca degli Occhiali" for its glasses shape (WGS84: 45.10153°N, 09.41822°E) (Figure 1a), along the northern bank of the river Po, in the town of Pieve Porto Morone (province of Pavia) (Figure 2).

The waterbody is occupied by still water; temperature ranged from 16.5°C (September 2016) to 3.5°C (January 2017), oxygen saturation from 2.2% (September 2016) to 103.9% (January 2017), and pH from 8.4 (September 2016) to 10.6 (January 2017). The level of nitrogen compounds is very low: $N\text{-}NH_4$ varied from 0.04 mg/l (September 2016) to 0.007 mg/l (January 2017), while $N\text{-}NO_3$ is always lower than method sensitivity (< 0.5 mg/l); the level of P total is quite high, fluctuating from 0.38 mg/l (September 2016) to 0.17 mg/l (January 2017). The same consideration can be done for the water conductibility: the high value recorded in September 2016 (779 µS/cm) is similar to the value in January 2017 (831 µS/cm).

In the growing site, *Wolffia columbiana* is co-dominant in a community of eutrophic pleustophytes (*Lemnion minoris* O. de Bolòs & Masclans 1955); it occupies the entire surface of the pond (2300 m^2), with a cover of 90%, together with *Spirodela polyrhiza* (L.) Schleid. (95%) and *Lemna minor* L. (1%) (dates of relevés: 6 May 2016, 29 September 2016; cover values were obtained by considering both emerged and submerged layers of plants in the water column) (Figure 1). The relevant size of *W. columbiana* mass is the result of the high clonal proliferation: young smaller fronds were constantly observed during all our field surveys, still connected to the mother plants or freely floating among them. The production of new fronds from the mother plants' budding pouch was observed already in March (Figure 3a) and subsequently in cultivation.

The introduction of the species in Pieve Porto Morone seemingly occurred more than a year before its discovery: single-fronded individuals collected in early March (still winter season) were almost half the size (0.30–0.50 mm × 0.20–0.40 mm) of those examined in summer and autumn; they were certainly young fronds recently budded from overwintering turions, which had sunk to the bottom of the pond at the end of the previous vegetative season. Abundant turions were observed in January 2017 too, under the ice layer formed over the pond top surface.

Taking into account the size of the population and the establishment of the species, we consider *Wolffia columbiana* naturalized in Italy, in accordance with the definition provided by Celesti-Grapow et al. (2009).

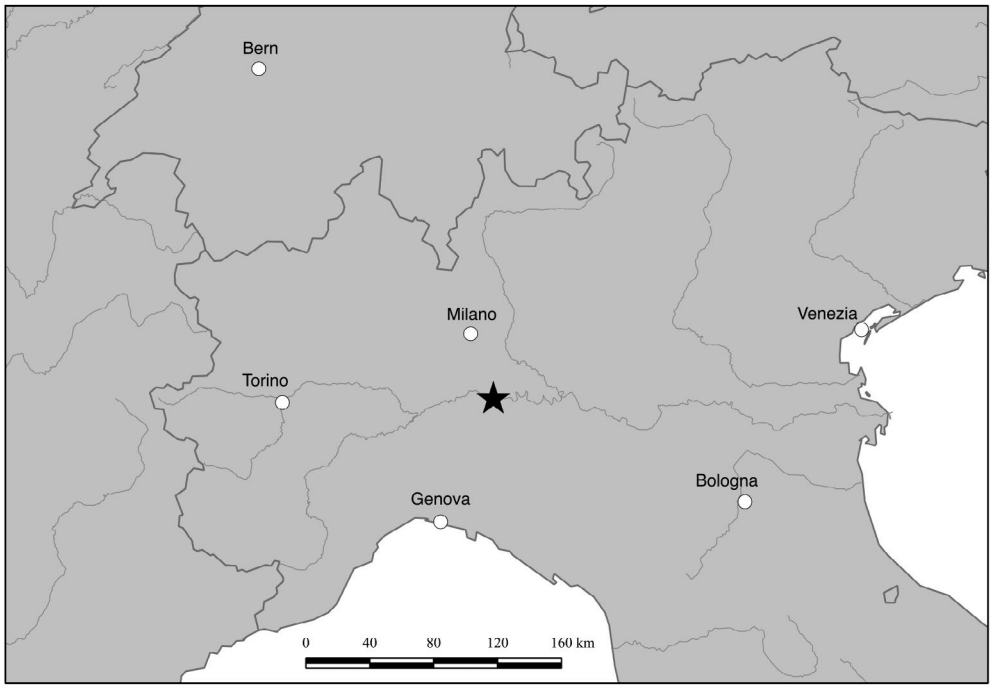

Figure 2. Location of the Italian population of *Wolffia columbiana*.

Figure 3. *Wolffia columbiana*: young fronds recently emerged from turions (a) (March 2016); probe covered with mature individuals (b) (September 2016). Photographs by N. M. G. Ardenghi (a) and R. Sconfietti (b).

Pathway of introduction and dispersal

The pathways of introduction of *Wolffia columbiana* from the Americas to the European continent are obscure. The arrival of the species was hypothesized by Schmitz, Köhler, and Hussner (2014), and Schmitz, Köhler, and Nesemann (2016) to be associated with the aquarium and aquatic ornamental plants trade; this supposition is shared by Armstrong and Thorne (1984), and Armstrong (1985, 1989) for different *Wolffia* species, and by Landolt (1986, 1998) for the subfamily Lemnoideae in general. These authors also take into consideration fish farming, a pathway highly compatible with the location of the Italian population: the river Po area is scattered with both natural (mainly "bodri" and oxbow lakes) and artificial ponds employed for recreational fishing (locally frequent in Pieve Porto Morone), where freshwater fishes, predominantly of non-native provenance, are released, not infrequently acting themselves as accidental vectors of further alien organisms (Savini et al. 2010).

Less likely is the introduction through rice seeds imported from the Americas (as has occurred historically, for instance, with *Heteranthera* sp. pl. or *Eleocharis obtusa* [Willd.] Schult. in the Italian rice fields, see Banfi and Galasso [2010]), as this pathway implies a prolonged and lethal desiccation of the fronds, while seeds, more drought-resistant in Lemnoideae, are rarely produced in *W. columbiana* (Landolt 1986, 1998).

On a local scale, *Wolffia* species, being small in size, not fixed on the ground, and capable of adhering in mass to different surfaces, are known to be easily dispersed by water currents, wind, clods of mud, debris, aquatic animals, and even man (Armstrong 1985; Armstrong and Thorne 1984; Jäger 1964; Landolt 1986, 1998; Lembi 2009; Schmitz, Köhler, and Hussner 2014; Schmitz, Köhler, and Nesemann 2016; Schulz 1962), as personally experienced by the authors on their instrumentation, clothing, and footwear during field surveys (Figure 3b).

Among the potential dispersal vectors, waterbirds are the most probable, according to the literature (e.g. Clausen et al. 2002; Figuerola and Green 2002; Green, Figuerola, and Sánchez 2002; Landolt 1986, 1998; Wołek 1981), and the specific environmental features of the growing site, where waterbirds, such as squacco herons (*Ardeola ralloides* [Scopoli, 1769]) and black-crowned night-herons (*Nycticorax nycticorax* [Linnaeus, 1758]), have been noticed among the visitors of the pond, and others, such as mallards (*Anas platyrhynchos* [Linnaeus, 1758]), grey herons (*Ardea cinerea* [Linnaeus, 1758]), and African sacred ibises (*Threskiornis aethiopicus* [Latham, 1790]), occurring in the surroundings. As remarked by Wołek (1981), duckweeds can be dispersed by birds mostly exozoically. In the case of *Wolffia columbiana*, the transportation may concern whole individuals or propagules (i.e. turions) and not fruits: flowering and fruiting are regarded as "occasional" in this species by Landolt (1986) and have not been observed by the authors either in nature or cultivation, despite plants bearing flowers being found in Germany and the Netherlands (Schmitz, Köhler, and Hussner 2014). As noticed by Landolt (1986), success in distributing live individuals is limited by the time in which fronds dry irreversibly. In consideration of this aspect, as already verified in congeneric *W. arrhiza* by Godziemba-Czyż (1970) and Wołek (1981), experimental investigation is needed (and is being undertaken by the authors) to assess the desiccation tolerance of *W. columbiana*, still unknown, especially with regards to transportation by waterbirds.

Impact and potential invasive behaviour

Taking into account the geographic origin and the invasion status of *Wolffia columbiana* discussed in the previous paragraphs, positive responses are given to questions A.1 and A.2 of the prioritization process scheme for evaluating the invasive behaviour of alien plants (EPPO/OEPP 2012), the species being a naturalized alien (neophyte) in the area under assessment.

The spread potential (question A.5) is "high": although no other individuals of *Wolffia columbiana* have been detected in surrounding ponds and waterbodies, its vegetative reproduction is rapid and vigorous in the study area, as revealed by our field observations (see previous paragraphs), which are in line with data reported in the literature for this species, whose capabilities of invasion and colonization are favoured by switching between a floating and dormant benthing stage (turions) (Witty 2009), and intense budding activity (White and Wise [1998] counted up to six daughter and granddaughter fronds, in various stages of development, attached to a single mother frond), pointing to a high potential dispersion capacity in the environment. Our choice can also partly rely on the remarkable number of growing sites discovered in the Netherlands after the first record of the species in 2013 (FLORON 2014; Schmitz, Köhler, and Hussner 2014): this is probably associated not only with a higher taxonomic awareness (which enabled identification of the overlooked *W. columbiana* from *W. arrhiza*), but also with a high spread capacity intrinsic to *W. columbiana*. Uncertainty rating is ranked as "low".

The potential negative impact on native species, habitats, and ecosystems in the area under assessment (question A.6) is considered "medium": *Wolffia columbiana* has colonized a natural environment, forming a dense and paucispecific population (Figure 1), yet the persistence of the recorded cover value needs to be monitored during the next years. As already observed for this species (Boyd 1975), other members of the genus *Wolffia* (Morris and Barker 1977), and Lemnoideae in general (Janes, Eaton, and Hardwick 1996; Landolt 1986; Pokorný and Rejmánková 1983), thick and even layers of *W. columbiana* can contribute to reducing the oxygen content of the water and its assimilation from phytoplankton, limiting light penetration, lowering pH, and increasing the water temperature and organic substances (especially tannins and lignins), with serious consequences on water quality and growth of submerged macrophytes. Effects may be amplified when the species is associated with more impacting duckweeds like *Spirodela polyrhiza* (Boyd 1975), similar to the pond in Pieve Porto Morone, where values of oxygen saturation have been recorded to be higher when the duckweed layer was absent in January 2017, in contrast to pH and the level of nitrogen compounds, which were higher during the winter sampling (see the "The new Italian population" paragraph). Furthermore, no risk of hybridization with native species (i.e. *W. arrhiza*) is evidenced, as no flowering has been recorded. The potential negative impact on agriculture, horticulture or forestry (question A.7) is ranked as "low", as the plant is not found in a crop; yet its future presence in local rice fields, where other alien duckweeds (such as *Lemna minuta* Kunth and *L. aequinoctialis* Welw.; see Banfi and Galasso 2010; Desfayes 1997) have been recorded, is not unlikely. The occurrence of additional impacts (question A.8) is "medium": dense mats, similar to that recorded from the study area, may affect recreational activities, such as sport fishing (Mudge, Gettys, and Haller 2007; Lembi 2009), and aesthetics in artificial waterbodies (Osgood 2012). Uncertainty ratings for questions A.5–A.8 are regarded as "low".

The combination of the spread potential ranking ("high") with the maximum rating obtained from the questions on the adverse impacts ("medium") suggests the inclusion of *Wolffia columbiana* in the "Observation list of invasive alien plants". On the basis of this result, the prioritization process for the identification of invasive aliens worthy of pest risk analysis (section B) is not required. Overall uncertainty is ranked as "medium", as the species, although present in the area under assessment, is a newcomer.

Specimens examined

ITALY: Lombardia, Pieve Porto Morone (Pavia), argine del Po tra Chiavica della Roggia Olonetta e Case Morea, bodri "Lanca degli Occhiali" [bank of the river Po between Chiavica della Roggia Olonetta and Case Morea, pond "Lanca degli Occhiali"], WGS84: 45.10153°N 09.41822°E, 51 m, bodri, con *Spirodela polyrhiza* e *Lemna minor* [pond, with *Spirodela polyrhiza* and *Lemna minor*], 4 March 2016, *N. Ardenghi s.n.* (Herb. N. Ardenghi); ibidem, 29 September 2016, *N. Ardenghi & D. Paganelli s.n.* (BR, FI, MSNM).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Nicola M. G. Ardenghi, PhD, is a research fellow at the University of Pavia, Italy. His main research fields are taxonomy, nomenclature, and distribution of vascular plants in Italy and southern Europe, with special regards to the critical genera *Festuca* (Poaceae) and *Vitis* (Vitaceae), and alien flora in general. He is author of numerous scientific papers in national and international journals. *Contribution*: finding and identification of the species, field work, preparation of the manuscript.

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Daniele Paganelli, PhD, is a Subject Expert in Ecology with specialization in Applied Ecology and Freshwater and Marine Ecology at the University of Pavia. He has a post-doc position at the Department of Earth and Environmental Sciences (University of Pavia, Italy) for the study of the freshwater ecosystems with special attention to alien macrozoobenthos species. *Contribution*: physico-chemical water analyses, field and laboratory work.

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