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A confirmed observation of *Oxalis dillenii* in Spain

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Abstract
A confirmed observation of *Oxalis dillenii* in Spain.— We confirm the presence of *Oxalis dillenii* Jacq. in the Catalonian province Lleida (Catalonia, Spain). Identification was confirmed morphologically and through DNA barcode sequencing. A map of the distribution of *O. dillenii* is supplied, as are notes on its identification.

Key words: DNA barcode; identification; non-native species.

Resumen
Observación confirmada de *Oxalis dillenii* en España.— Confirmamos la presencia de *Oxalis dillenii* Jacq. en la provincia de Lérida (Cataluña, España). La identidad de la especie fue confirmada tanto por sus características morfológicas como a través de la secuenciación del código de barras del ADN. Se proporciona un mapa de distribución de *O. dillenii*, así como notas sobre su identificación.

Palabras clave: código de barras del ADN; especie no nativa; identificación.

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INTRODUCTION

Oxalis is a remarkable genus for many reasons, but one of them is the large number of invasive species in this genus. These are not limited to one section of the genus, but spread within it and include *O. pes-caprae* L., *O. debilis* Kunth, *O. latifolia* Kunth and *O. incarnata* L. However, the section *Corniculatae* DC. seems well endowed with invasive members, the most infamous being *O. corniculata* L. that may be present in every country on the planet (Hassler, 2015). Other weedy species in this section are *O. dillenii* Jacq. and *O. stricta* L. from North America and *O. exilis* A. Cunn. and *O. perennans* Haw. from Australasia (Nesom, 2009; Gray, 2011).

Oxalis dillenii is native to North America, although its native distribution in that continent is obscured both by confusion with other species and its modern spread into new areas. It is one of the commonest *Oxalis* species in the eastern United States, where it usually grows in disturbed habitats (Nesom, 2009). Outside its native range, *O. dillenii* has been recorded in several countries, including France, Italy, Germany, Belgium, the Netherlands, Great Britain and Austria (Young, 1968; Moorsel *et al*., 2000; Hoste, 2012).

MATERIALS AND METHODS

Two small *Oxalis* plants were collected by the first author the 27 November 2013 from a site north of Solsona, Lleida, Catalonia, Spain (42° 00' 40.8" N, 1° 31' 01.5" E). The plants were growing on bare dry soil under a conifer hedge that surrounds a holiday camp.

Molecular protocols and sequence analyses

The modified CTAB protocol of Tel-Zur *et al*. (1999) was used for total genomic DNA isolation. Secondary metabolites were removed by washing ground dried leaf material with 1000 µL extraction buffer (100 mM Tris-HCl pH 8, 5 mM EDTA pH 8, 0.35 M sorbitol). After the addition of 800 µL CTAB lysis buffer, as described in Chase & Hills (1991) with addition of 1% PVP-40 and 0.3% 2-mercaptoethanol, the samples were incubated at 60°C for one hour. Chloroform-isoamylalcohol (24/1 v/v) extraction was done twice, followed by an isopropanol precipitation (0.8 volumes). After centrifugation, the pellet was washed in 70% ethanol, air-dried, and dissolved in 50 µL buffer (10 mM Tris-HCl pH 8, 1 mM EDTA pH 8).

Amplification reactions of ITS and *trnL-F* were carried out using a touchdown PCR protocol (25 µL). Reactions were initiated with a 3-min heating at 95°C followed by 35 cycles consisting of 95°C for 30 s, 50–56°C for 30 s, and 72°C for 30–90 s. Reactions ended with a 5-min incubation at 72°C. Amplification and sequencing of ITS and *trnL-F* was performed using the primers of White *et al*. (1990) and Taberlet *et al*. (1991), respectively. Enzymatically purified PCR products were sequenced by the Macrogen sequencing facilities (Macrogen Europe, Amsterdam, Netherlands).

Contiguous sequences were assembled using Geneious v7.0.6 (Biomatters, New Zealand). Automatic alignments were carried with MAFFT (Katoh *et al*., 2002) under an E-INS-i algorithm, a 100 PAM/*k* = 2 scoring matrix, a gap open penalty of 1.3 and an offset value of 0.123. Subsequent manual fine-tuning of the aligned dataset was done in Geneious v7.0.6. Sequence gaps were treated as missing data, whereas potentially informative base insertions and deletions were coded according to the "simple indel coding" method of Simmons & Ochoterena (2000).

The global distribution of *O. dillenii* was created from observations from the Global Biodiversity Information Facility on the 5 August 2015 (http://doi.org/10.15468/dl.9imeus).

Voucher specimens for *O. dillenii*, *O. stricta* and *O. corniculata* are deposited at the Botanic Garden Meise (BR) (Table 1).

RESULTS

Compared to *O. corniculata* the most consistent vegetative characteristics of *O. dillenii* are the narrow adnate stipules which are fused to the petioles and the antrorse appressed stem hairs, which are usually densest towards the top of the stem (Fig. 1).

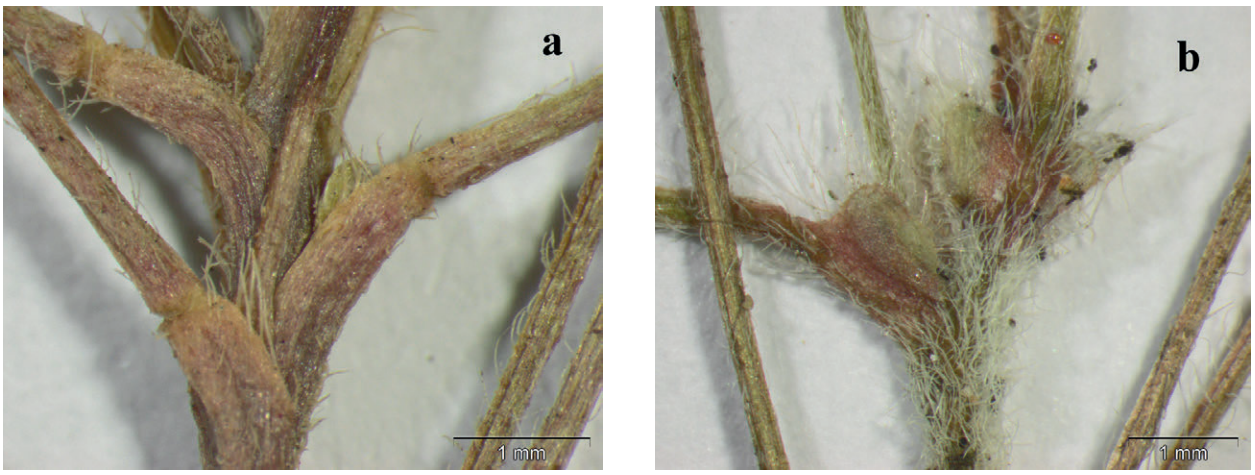

Figure 1. Detail of the stipules of *Oxalis dillenii* (A) and *Oxalis corniculata* (B). Both pictures are of dried herbarium material. The *Oxalis dillenii* photograph is from the Lleida material and the *Oxalis corniculata* is taken from plants collected in the Botanic Garden Meise, where it grows as a weed.

In *O. stricta* the stipules have no free margins (and are therefore seemingly lacking); in this species at least part of the stem hairs are long, patent and septate, a feature that separates *O. stricta* from both *O. dillenii* and *O. corniculata*. Since *O. dillenii* is easily confused with two closely-related species of the Iberian flora (Sánchez-Pedraja, 2015), we have provided a table that compares the distinguishing characteristics of *O. dillenii*, *O. corniculata*, *O. stricta* and *O. exilis* (Table 2). Very rarely *O. conorrhiza* Jacq. and *O. filiformis* Kunth have also been found in Spain, but are easily recognized by their single flowered inflorescences and somewhat larger flowers with long, very slender pedicels. They have been omitted from the table.

In the specimen from Lleida the narrow stipules and antrorse stem hairs are present as typical for *O. dillenii*. Other features of *O. dillenii* are more variable but present in these specimens. The young plants are erect, but with age stems can root at the nodes giving the impression of either a creeping stolon or, if covered by soil, a rhizome. However, the Lleida plants were too young for this to have occurred. Well grown plants in rich soil are usually bright green on both sides of the leaf, but the Lleida specimens have pink-purple leaves apparently due to the nutrient-poor dry soil they were growing in and possibly because they were collected in winter when they frequently experienced cold conditions. Another distinctive character of this species are the fasciculate leaves, whereas other closely related species (with the exception of *O. stricta*) have more evenly spaced alternate leaves.

The DNA barcode sequences of the Lleida specimens were compared to an *O. corniculata* specimen from Belgium, an *O. stricta* specimen from the United States and four specimens of *O. dillenii*, one from each of Belgium and Germany and two from the United States (Table 1). The chloroplast *trnL-F* sequence is almost identical for all species. However, for the nuclear ITS sequence the Lleida specimen shows 99.7–100% identity with the verified *O. dillenii* specimens; but only 93.2% and 95.8% identity with *O. corniculata* and *O. stricta*, respectively.

Oxalis dillenii is an annual or short lived perennial of disturbed ground. It can inhabit waste-places and become a weed of cultivation. Figure 2 shows the distribution of *O. dillenii* in the Northern Hemisphere. In both Asia and North America it grows at latitudes more southerly than Spain, so there is little reason to think that it is at the boundaries of its potential distribution in Europe.

DISCUSSION

The results demonstrate the presence of *O. dillenii* in Spain using both morphological and molecular approaches. Aymerich (2013) previously reported *O. dillenii* from Spain and an image of his specimen was obtained from the Vascular Plant Herbarium of the Real Jardín Botánico (MA specimen number 823538). This specimen is recognisable as *O. dillenii* and should be considered the first confirmed observation of *O. dillenii* in Spain. It is a larger specimen than the specimens from Lleida and is more typical of an older plant, as it has the typical fasciculate leaves. This specimen was collected at Fonollet, municipality of Casserres, Barcelona, at 610 m on the 3 November 2010 by P. Aymerich. The label describes the site as "*entorno*

Table 1. Details of the specimens used in this study. Their accession numbers at the Botanic Garden Meise Herbarium (BR); The GenBank accession numbers of the ITS and *trnL-F* barcode sequences and the geographic origins of the specimens.

Species	Accession number	ITS	*trnL-F*	Origin
Oxalis corniculata	BR0000013236847	KT737723	KT737730	Flanders, Belgium
Oxalis dillenii	BR0000009721166	KT737724	KT737731	Louisiana, USA
Oxalis dillenii	BR0000013234409	KT737725	KT737732	Lleida, Spain
Oxalis dillenii	BR0000013236830	KT737726	KT737733	Flanders, Belgium
Oxalis dillenii	BR0000013234225	KT737727	KT737734	Berlin, Germany
Oxalis dillenii	BR0000013498894	KT737728	KT737735	Texas, USA
Oxalis stricta	BR0000013234621	KT737722	KT737729	Pennsylvania, USA

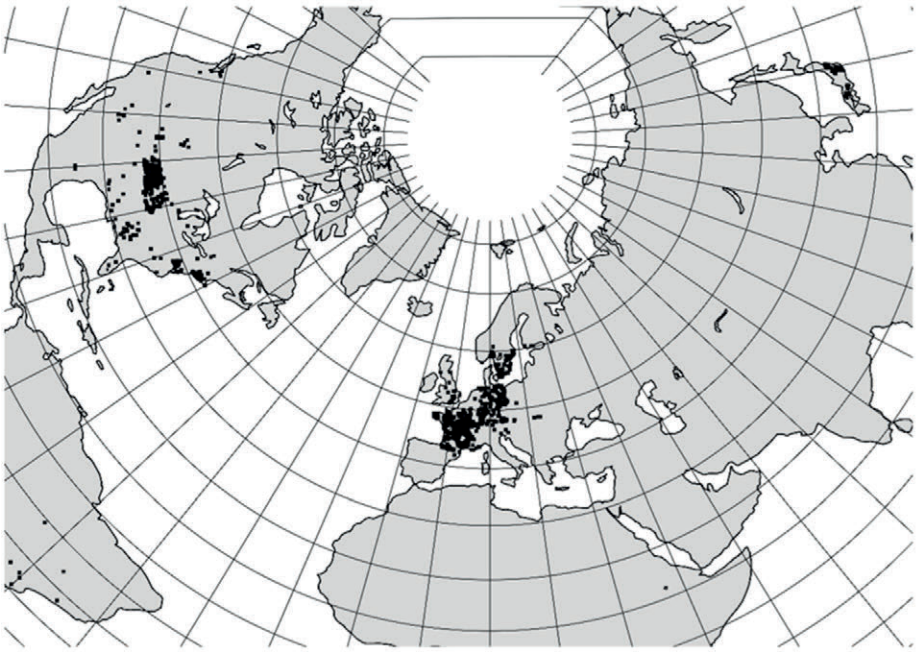

Figure 2. The approximate distribution of *Oxalis dillenii* in the Northern Hemisphere created from the observations in the Global Biodiversity Information Facility (http://doi.org/10.15468/dl.9imeus). The map uses an Albers equal-area conic projection.

de un camino rural, en taludes con algunos escombros y en matorrales de romero", which translates to "in the environment of a country road, on slopes with some debris and rosemary bushes". However, when he visited the site two years later the species was not refound.

It is unlikely that these are the only two sites of *O. dillenii* in Spain and we suspect that this species is likely to spread further. Indeed, there have been unsubstantiated records from elsewhere in Spain (Bolòs *et al*., 1990; Sánchez-Pedraja, 2015). It is an opportunistic invader and has probably not filled its climatic niche in Europe yet. Therefore its current distribution may reflect its introduction pathway rather than suitable habitat availability. Nevertheless, this species is a minor weed where it has become naturalised. It is easy to control with standard horticultural practices and has not yet invaded more natural habitats. We hope that publishing these observations will make botanists more aware of *O. dillenii* in Spain.

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Table 2. The *Oxalis corniculata* group. Underlined text summarizes the key features for identification of *Oxalis dillenii*.

	Oxalis stricta	***Oxalis dillenii***	***Oxalis corniculata***	***Oxalis exilis***
Habit (The habit of each of the four species shows a remarkable range of variation)	Stem usually single and erect, but older plants often toppling and decumbent; not rooting at the nodes; at the base often with green or reddish ground-level or white underground rhizomes	Stem usually single and erect when young. Older plants often bushy, with several erect stems arising at short distances from either prostrate aboveground or horizontal underground stems that often root	Stems few to numerous, radiating from a central taproot. Stems decumbent or, especially in var. *atropurpurea*, procumbent, sometimes erect in young plants; procumbent stems not, only sparsely or, especially in var. *atropurpurea*, freely rooting	Stems few to numerous, radiating from a central taproot. Stems procumbent and freely rooting at the nodes or, occasionally, decumbent and then more sparsely rooting at the nodes
Stem hairiness (In all four species the young upper part is usually more densely hairy)	Sparsely to, less often, densely hairy, sometimes almost glabrous; long, patent, septate hairs usually present (if not, then such hairs typically present on the pedicels); often mixed with some short, curved, whitish, unicellular hairs	Usually rather densely, but sometimes only sparsely covered with <u>antrorsely appressed unicellular hairs</u>; these may give the young distal part of the stem a whitish appearance	Usually moderately densely covered with whitish nonseptate hairs, some patent, some appressed, most of them curled	
Leaf colour and size	Green, sometimes with a tinge of purple, especially at the underside. The rarely cultivated var. *rufa* has dark purple leaves	Green, sometimes suffused with purple, especially at the underside of the leaves	Green or purple; if purple, leaflets often with a green midrib. Usually smaller in var. *atropurpurea* than in var. *corniculata*	Usually green; purple-leaved plants very rare among exotics introduced in Europe. Usually smaller than those of the other species
Stipules	Seemingly absent (no free membranous part visible)	<u>Present but reduced</u>, with a narrow membranous portion (Fig. 1A)	Present and well developed; at least some stipules widened into a triangular auricle in the apical part (Fig. 1B)	
Leaves	<u>Fasciculed or whorled</u>		Alternate and clearly distanced, each with an axillary peduncle	
Throat of the corolla	Usually without markings, but sometimes with fine red stripes (these mostly tiny and few)		With or without markings. If present: each petal with 3–4 thin red stripes (var. *corniculata*) or with a dim to clearly visible orange band (var. *atropurpurea*)	Never (?) with fine red stripes or an orange band

Table 2. (cont.) The *Oxalis corniculata* group. Underlined text summarizes the key features for identification of *Oxalis dillenii*.

	Oxalis stricta	***Oxalis dillenii***	***Oxalis corniculata***	***Oxalis exilis***
Stamens	Typically all stamens (five long and five short) with anthers, but quite often one or more of the short ones with somewhat less well-developed or completely aborted anthers			Typically the 5 long stamens with and the 5 short stamens without anthers; not uncommonly, however, 1 or more of the short stamens with a poorly developed anther
Inflorescences	Flowers in regular or irregular, often umbelliform cymes	Flowers most often in umbelliform cymes	Flowers in umbelliform or sometimes irregular cymes	1-flowered, very rarely 2-flowered
Pedicels	Erect	Erect during flowering, patent or reflexed during maturation of the capsule, eventually upright again when the capsule is ripe and ready for seed dispersal		
Capsules (In all four species the short hairs may be inconspicuous)	Usually sparsely hairy with long, patent, septate hairs, sometimes mixed with a few short hairs; less often densely hairy or glabrate. Typically most capsules at least 10 mm long	Densely hairy with short, whitish, retrorse hairs, usually mixed with at least some long, patent, septate, hyaline hairs. Typically most capsules >12 mm long (often >20 mm)	Usually densely hairy with short, whitish, retrorse hairs; long, patent, septate, hyaline hairs zero to numerous. Typically most capsules >10 mm long, sometimes shorter (especially in var. *atropurpurea*)	Usually densely hairy with short retrorse hairs, often mixed with a few to numerous patent, septate hairs. Capsules typically <10 mm long, with <4 seeds per cell
Seeds	Usually uniform brown; ridges rarely with ill-defined paler patches or stripes	Brown, with sharply defined white dots or lines on the ridges	Usually brown, but not infrequently with paler, vaguely defined dots or lines on the ridges	Usually uniform brown