

# Phylogenetic Relationships and Genetic Diversity among *Orobanche cumana* Wallr. and *O. cernua* L. (Orobanchaceae) Populations in the Iberian Peninsula

[Rocío Pineda-Martos](#)<sup>1</sup> / [Leonardo Velasco](#)<sup>1</sup> / [Antonio J. Pujadas-Salvà](#)<sup>2</sup> / [José M. Fernández-Martínez](#)<sup>1</sup> / [Begoña Pérez-Vich](#)<sup>1</sup>

<sup>1</sup>Department of Plant Breeding, Institute for Sustainable Agriculture (IAS-CSIC), Finca Alameda del Obispo, Avda. Menéndez-Pidal s/n, 14004 Córdoba, Spain

<sup>2</sup>Department of Agricultural and Forestry Sciences and Resources, University of Córdoba, Campus de Rabanales, Edificio Celestino Mutis, Carretera de Madrid Km 396, 14071 Córdoba, Spain

©2014 Pineda-Martos et al. published by De Gruyter. This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 3.0 License. ([CC BY-NC-ND 3.0](#))

**Citation Information:** *Helia*. Volume 37, Issue 61, Pages 161–171, ISSN (Online) 2197-0483, ISSN (Print) 1018-1806, DOI: [10.1515/helia-2014-0036](#), October 2014



## Publication History

Received:  
2014-09-25

Accepted:  
2014-10-10

Published Online:  
2014-10-29

## Abstract

*Orobanche cumana* is found in the Iberian Peninsula as an allochthonous species parasitizing exclusively sunflower, in contrast to the closely related species *Orobanche cernua*, which is an autochthonous species that only parasitizes wild Asteraceae hosts. Ten *O. cumana* populations were collected in the two traditional areas of sunflower broomrape occurrence, the Guadalquivir Valley, Southern Spain (six populations) and Cuenca province, Central Spain (four populations). Twelve *O. cernua* populations were collected on wild hosts across its natural distribution area in Southeastern Spain. Genetic relationships within and between both sets of populations were studied using a set of 50 robust and co-dominant SSR markers from *O. cumana*. The results supported the taxonomic separation of the two species and the existence of two distant genetic groups for *O. cumana*, one in Guadalquivir Valley and another one in Cuenca province. The inter- and intra-population variability was extremely low for *O. cumana*, whereas the overall genetic diversity was much higher for *O. cernua*. The genetic structure of *O. cumana* populations probably reflects a founder effect, with the two

genetically distant groups deriving from separate introduction events. The high degree of genetic differentiation observed in *O. cernua* is mainly explained on the basis of restricted gene flow due to ecological barriers together with the occurrence of a predominantly self-pollinating mating system. Complementary diversity studies on both species in its current distribution area are required for understanding global genetic variability and evolutionary characteristics of the parasitism.

**Keywords:** [genetic diversity](#); [Helianthus annuus](#); [microsatellite markers](#); [Orobanche cernua](#); [Orobanche cumana](#); [sunflower broomrape](#)

## Introduction

*Orobanche cumana* Wallr. (sunflower broomrape) is a holoparasitic plant that parasitizes sunflower roots. It is present in sunflower crops in many countries around the world, especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, China ([Škorić et al., 2010](#)), and more recently in new areas such as France ([Jouffret and Lecomte, 2010](#)). *O. cumana* was first described in the Iberian Peninsula parasitizing confectionary sunflower (*Helianthus annuus* L.) crops in 1958 in Toledo province ([Díaz-Celayeta, 1974](#)). The presence of *O. cumana* in oilseed sunflower fields was observed later in wide areas of Cuenca province in Castilla-La Mancha region (Central Spain) and the Guadalquivir Valley in Andalucía (Southern Spain) ([González-Torres et al., 1982](#)). Since then, *O. cumana* has spread over the whole sunflower cultivation regions, comprising new and traditional areas of Castilla-León (Northern Spain), Castilla-La Mancha, and Andalucía, causing severe yield losses in sunflower crops ([Alonso et al., 1996](#); [Fernández-Martínez et al., 2012](#)).

The closely related species *Orobanche cernua* L. was observed for the first time near Aranjuez (Central Spain) ([Loefling, 1758](#)). The species is mainly distributed in the North- and the South-East of the Iberian Peninsula and is only found in the wild, in arid areas of degraded, xerothermic scrub, parasitizing different species of the Asteraceae, being most frequently found on plants of the genus *Artemisia* ([Pujadas-Salvà and Velasco, 2000](#)). *O. cumana* and *O. cernua* have been traditionally considered as very closely related taxa ([Pujadas-Salvà and Velasco, 2000](#)). Several studies based on different molecular marker systems, such as RAPDs (Random Amplified Polymorphic DNAs) ([Katziret et al., 1996](#); [Paran et al., 1997](#); [Román et al., 2003](#)) or ISSRs (Inter Simple Sequence Repeats) ([Benharrat et al., 2002](#)), as well as those based on ecological, morphological, and biochemical data ([Pujadas-Salvà and Velasco, 2000](#)) or seed morphology analysis ([Plaza et al., 2004](#)), clearly support the taxonomic separation of *O. cumana* and *O. cernua* and the treatment of both taxa as different species.

Specific and joint studies on genetic diversity and phylogenetic relationships between both *O. cumana* and *O. cernua* species growing in the Iberian Peninsula based on a larger number of populations could be of interest to clarify the relationships between the two species. Coupled with this, alternative markers such as simple sequence repeat (SSR) markers, which are reproducible, neutrally evolving, multiallelic, and co-dominant, are needed to enable more powerful genetic analyses in the genus *Orobanche*. A recently developed collection of SSR markers is available for molecular research in *O. cumana*, which proved to be highly transferable to *O. cernua* ([Pineda-Martos et al., 2014](#)). Accordingly, the objective of this research was to study genetic diversity in a large set of *O. cumana* and *O. cernua* populations from the Iberian Peninsula using a subset of the newly SSR markers reported.

## Materials and methods

## Results and discussion

*O. cumana* and *O. cernua* have traditionally been considered closely related species. [Putt \(1978\)](#) suggested the possibility that *O. cumana* developed from a single population of *O. cernua* after the sunflower crop began to have economic importance in Russia in the nineteenth century. Although *O. cumana* has often been regarded as a variant of *O. cernua*, [Joel \(1987\)](#) and [Jacobsohn et al. \(1991\)](#) clearly differentiated the two species based on morphological differences and host. Subsequent molecular studies clearly supported the distinction between *O. cumana* and *O. cernua* ([Katzir et al., 1996](#); [Paran et al., 1997](#); [Benharrat et al., 2002](#); [Román et al., 2003](#)). The results reported by [Katzir et al. \(1996\)](#) revealed identical diagnostic markers in *O. cumana* samples, supporting the hypothesis that these populations were different from those of *O. cernua* collected from the wild. These results suggest that the two species are genetically different, which has been supported by [Pujadas-Salvà and Thalouran \(1998\)](#) and by studies of morphological, phenological, ecological, and biochemical characters performed in both species by [Pujadas-Salvà and Velasco \(2000\)](#).

SSRs are currently considered the markers of choice in many areas of molecular genetics, due to their co-dominance and high level of polymorphism, even between closely related species. A valuable set of 217 SSR markers has been isolated from *O. cumana* and characterized in diverse populations of this species and its closely relative *O. cernua* ([Pineda-Martos et al., 2014](#)). In the present research, a subset of 50 of these *O. cumana* SSR markers was used to evaluate the evolutionary relationships and genetic characteristics in the genetic makeup of these *Orobanche* species. SSR-cluster analysis resulted in a dendrogram with a high cophenetic value ( $r = 0.9952$ ,  $p < 0.001$ ) that separated the populations of both species into two main clusters, corresponding with the two species analyzed

(Figure 1). *O. cumana* populations clustered together at similarity values of 0.68 or higher, while *O. cernua* populations clustered together at similarity values of 0.32, with the exception of populations Boro-46 and Boro-47. *O. cumana* populations were grouped into two main groups: one group contained all the populations from Southern Spain (provinces of Córdoba and Sevilla), and a second one contained all the populations from Central Spain (province of Cuenca) (Figure 1). *O. cernua* cluster was separated into four different groups. These groups, listed in decreasing similarity order, comprised four populations from the south-west of Almería province (Boro-37, Boro-39, Boro-40, and Boro-42), three populations from the south of Jaén province (Boro-43, Boro-44, and Boro-45), one population from the south of Granada province (Boro-48), and two populations from the central area of Almería province (Boro-38 and Boro-41) (Figure 1). The two populations from the central area of Granada province (Boro-46 and Boro-47) (Figure 1), not included in the main *O. cernua* cluster, would require a new sampling determining their host and a re-evaluation for establishing a more accurate classification.

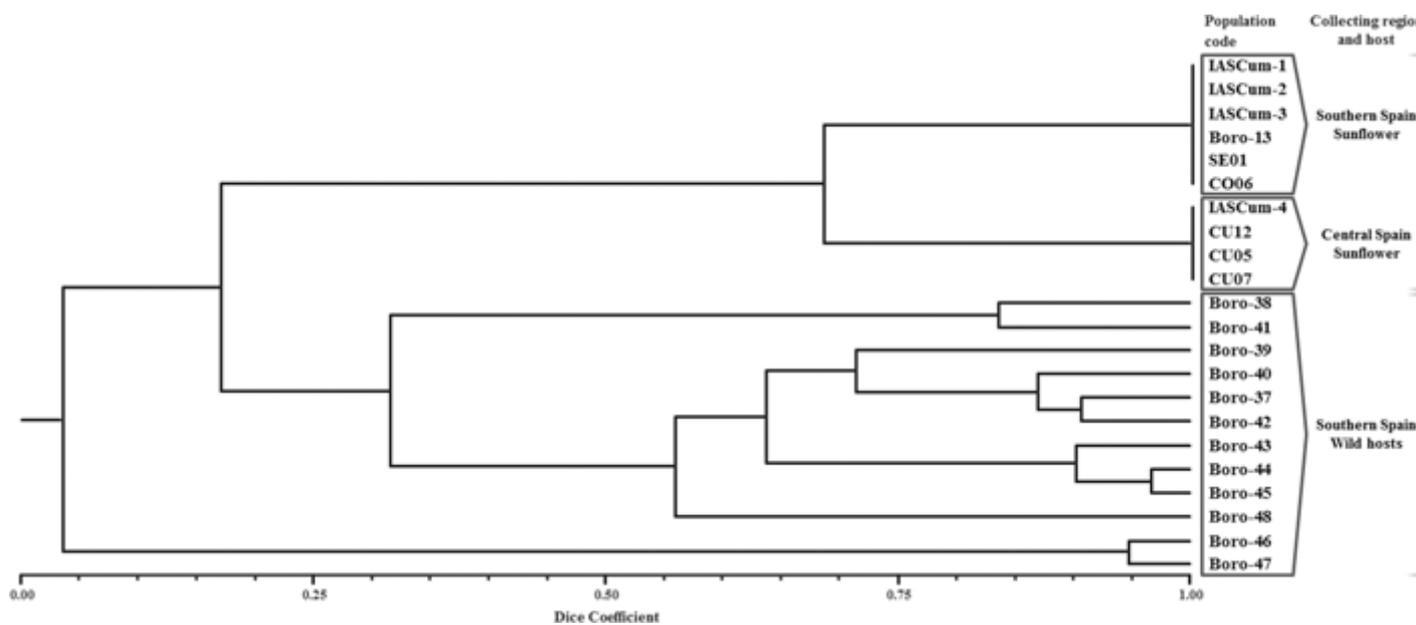


Figure 1:

UPGMA dendrogram based on Dice similarity matrix between 10 populations of *O. cumana* and 12 populations of *O. cernua* collected in the Iberian Peninsula obtained with 48 SSR polymorphic markers (see Table 1 for additional population details)

In the Iberian Peninsula, *O. cumana* is not found in the wild, but exclusively within sunflower fields (Pujadas-Salvà and Velasco, 2000). The great genetic separation between populations of Cuenca and the Guadalquivir Valley suggests that they may derive from seed introductions from different areas. It is also interesting to note that genetic diversity observed in *O. cumana* was considerably lower than in *O. cernua*, despite the geographically proximal populations used in the *O. cernua* set. Gagne et al.

[\(1998\)](#) concluded that *O. cumana* populations from different geographical origins were genetically very similar, pointing to a monophyletic origin. The high genetic differentiation observed among the groups of *O. cernua* populations suggested the presence of effective ecological barriers preventing gene flow between the populations together with the occurrence of a predominantly self-pollinating mating system.

## **Acknowledgments**

## **References**