



Disentangling the taxonomical uncertainties about the presence of *Cistus pouzolzii* (Cistaceae) in the Iberian Peninsula

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Abstract. *Cistus pouzolzii* is a species distributed in the western Mediterranean. Its populations are located in France and North Africa, being absent in the Iberian Peninsula. It was described by Delile, but its taxonomic and nomenclatural status has been discussed by several authors, mainly based on its particular combination of morphological characters. We have recently found several populations of a *Cistus* species whose morphological characters match those of *C. pouzolzii*. To confirm the taxonomic identity of these populations, a comparative study was performed using herbarium specimens of *C. pouzolzii* from France and North Africa. In addition, two chloroplast regions were sequenced to study the phylogenetic relationships between the newly discovered Iberian populations and the rest of the species of the genus *Cistus*, including the sequences belonging to *C. pouzolzii* from France and Morocco previously used. Both analyses (morphological and phylogenetic studies) confirmed that the specimens sampled in the Iberian populations were *C. pouzolzii*. Furthermore, both the nomenclature of this species and its conservation status are briefly discussed.

Keywords: *Cistus pouzolzii*, Iberian Peninsula, plastid sequences, western Mediterranean.

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Introduction

The family Cistaceae Juss. consists of annual or perennial herbs and shrubs that usually inhabit poor soils. It comprises approximately 220 species included in 8 genera (Stevens, 2001; POWO, 2023). It is distributed mainly in subtropical and temperate regions of the northern hemisphere, being the Mediterranean Basin's richest in number of species. *Cistus* L. is composed of approximately 49 taxa (including *Halimum* (Dunal) Spach, species and subspecies) mainly distributed in the Western Mediterranean basin and the Canary Islands, being both considered as a major centre of present-day differentiation. The taxonomy of the genus has been extensively studied based on the morphology of leaves and reproductive characteristics such as the sepal number, colour of petals and number of capsule valves (Dunal, 1824; Grosser, 1903; Demoly & Montserrat-Recoder, 1993; Nogueira *et al.*, 1993; Guzmán & Vargas, 2005, 2009; Civeyrel *et al.*, 2011).

The different phylogenies have pointed out that *Cistus* is a monophyletic genus, although some relationships among species are still unclear. Also,

they suggest that the species diversification might have occurred with the onset of the Mediterranean climate, which would have promoted the adaptation of species to summer drought and recurrent forest fires (Guzmán & Vargas, 2005; Guzmán *et al.*, 2009). Two lineages evolve independently, one consisting of pink flowers and another consisting of white flowers, except for *C. parviflorus* Lam. Moreover, populations in each of the above-mentioned centres of diversification (Mediterranean vs. Canary Islands populations) might have evolved at the same time. (Guzmán & Vargas, 2010).

Some of the species show a Mediterranean or Western-Mediterranean distribution, being scarce the endemics in the genus. However, the reproductive barriers between species seem to be very weak, due to the high number of known hybrids, although the influence of introgression in the rising of new taxa is negligible at least in the white-flowered species (Guzmán *et al.*, 2009; Guzmán & Vargas, 2009b; Fernández-Mazuecos & Vargas, 2010).

One of the species that follows a typical pattern of distribution in the Western Mediterranean is *Cistus pouzolzii* Delile. This species has been considered a

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hybrid between *C. monspeliensis* L. and *C. crispus* L. (Grosser, 1903). However, several authors argued against these taxonomic interpretations. Furthermore, in several phylogenetic studies (Guzman & Vargas, 2005; Guzman & Vargas, 2010) it is placed in the white and whitish pink *Cistus*, and no incongruences have been found regardless of the marker used, which support its specific status. Nevertheless, it shows several unique morphologic characters, such as the length style, that is equal to stamens, which led Rouy & Foucaud (1895) to define the section *Stephanocarpoidea* Rouy & Foucaud to include this taxon. Other authors suggested that *C. pouzolzii* should be synonymized with *C. varius* Pourr. (Dansereau, 1939; Warburg, 1968). *Cistus pouzolzii* is distributed in two disjunct areas; the South of France and Northern Africa (Morocco and Argelia) (Dobignard & Chatelain, 2011; Tison & de Foucault, 2014; FCBN, 2022), being absent in the Iberian Peninsula (Demoly & Montserrat, 1993). However, recently we have discovered new populations in the Southeast of Spain, which show morphological characters that are typical of *C. pouzolzii*.

So, the main objectives of this work are: to compare both nuclear and plastid sequences and morphological characters of the newly located populations with accessions of *C. pouzolzii* from the known populations and to shed light on the taxonomical issues that have been found about both the presence and the taxonomic status of this species in the Iberian Peninsula. Moreover, we add several comments about the biogeography and conservation status of the newly discovered populations of *C. pouzolzii*.

Material and Methods

Morphological analysis and nomenclatural study

The morphological analysis was based on collected material deposited in ABH, BC, BM, BR, COI, LY, MPU, MUB, MW and P herbaria. Acronyms of herbaria follow Index Herbariorum (<https://sweetgum.nybg.org/science/ih/>). Morphological characters of *C. pouzolzii* samples from Southeastern Spain were studied from direct examination of fresh material, a stereo microscope Optika Lab-20 was used to perform the morphological study. In addition, part of the material has been revised through digital images of preserved plants from some of the mentioned herbaria. Digital Annotate-on v.1.9.56 tool (RECOLNAT-ANR-11-INBS-0004) has been used to manage digital images of herbarium accessions belonging to *C. pouzolzii*. The main morphological characters used to differentiate *C. pouzolzii* from other species of *Cistus* mentioned in this study are listed in Table 1. We followed the International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) (Turland *et al.*, 2018) to perform the discussion based on the several interpretations of its nomenclature, indicating references, and synonyms.

QGIS 3.28 Geographic Information System software (<https://www.qgis.org>) was used to perform

a distribution map of *C. pouzolzii* using the confirmed locations shown in the labels of the reviewed herbarium accessions and the new collected populations. The ETRS89-LAEA (EPSG:3035) geographic coordinate system was used to georeference populations. In addition, comments relative to the habitat of locations of *C. pouzolzii* have been added. We compared the biogeography of the recently discovered populations with those published for the French and African populations.

Molecular analysis

For this work, we sequenced two plastid regions (trnS-trnG and trnL-trnF spacers) from 8 individuals of *Cistus pouzolzii*, which were complemented by two previously published sequences (downloaded from GenBank) belonging to *C. pouzolzii* from France and Morocco, and other 17 species of *Cistus*. We used *Tuberaria guttata* (L.) Fourr. as an outgroup taxon to root the tree. The taxa studied are listed in Table 2, with GenBank accession numbers and voucher details.

DNA extraction, PCR amplification and sequencing

Total DNA was extracted using the cetyltrimethyl ammonium bromide method (Doyle & Doyle, 1987) with slight modifications and DNA extracts were immediately diluted at 1:100 to perform PCR amplification. The rest of the DNA was stored frozen at -80°C to avoid degradation in case to be necessary. The two plastid regions were amplified with the following primer pairs: trnS and trnG (Hamilton, 1999) for the trnS-trnG intergenic spacer, and c plus f primers (Taberlet *et al.*, 1991) for the trnL(UUA)-trnF(GAA) region. The PCR reactions were carried out in an Eppendorf Mastercycler thermocycler using the following program: initial cycle of 94°C for 5 min, 35 cycles of 94°C for 30 s, 52°C for 45 s, 72°C for 1 min, and a final extension step of 72°C for 8 min to complete the PCR. The reactions were conducted in a final volume of 50 µl using approximately 40 ng of DNA, 0.4 µM of direct and reverse primers, 5 µl of polymerase buffer (provided by the supplier of the enzyme), 0.2 mM of each of dNTPs, 2 mM of MgCl₂ and 2 U of Taq polymerase (Biotools). Finally, 2 µl of each amplification product was visualized on 1.5% agarose gels, and successful amplifications were cleaned using the GenElute PCR Clean-Up kit (SIGMA). The same primers were used in the sequencing reactions with the Big Dye sequencing kit and the products were separated in an automatic ABI 3700 sequencer following standard protocols. For each DNA sample, both strands were sequenced.

Sequences were checked for inaccurate base identification using Chromas Lite v2.01 (Technelysium Pty Ltd.). A concatenated alignment of consensus sequences of the two plastid regions was obtained using MUSCLE alignment tool included in Geneious 7. BioEdit (Hall, 1999) was used to make minor alignment adjustments. Bayesian and Maximum Likelihood (ML)

Table 1. Morphological characters used to differentiate *Cistus pouzolzii* from the taxa involved in its different taxonomic interpretations.

Character	<i>C. pouzolzii</i>	<i>C. crispus</i>	<i>C. monspeliensis</i>	<i>C. salviifolius</i>	<i>C. ×florentinus</i>
Leaves shape	Sessile, semi-amplexicaul, oblongue-lanceolate	Sessile, connate ovate, ovate-lanceolate, lanceolate	Subsessile, linear-lanceolate	Petiolate, ovate, ovate-oblongue	Short-petiolate, ovate-lanceolate
Leaves size (mm)	15-46 × 4-13	12-35 × 4-13	15-45 × 2-7(13)	8-18(45) × 7-12(30)	10-40(50) × 3-8(14)
Leaves margin	Usually undulate	Undulate	Recurved, hirsute	Denticulate	Recurved, undulate, denticulate
Leaves indumentum	Simple and fasciculate hairs and glandular trichomes (upper part), stellate (lower part)	Mainly stellate hairs. Also simple and glandular trichomes	Simple hairs (upper part), stellate hairs (lower part)	Stellate hairs (upper part), sub-fasciculate (lower part)	Stellate hairs, scarce (upper part), stellate hairs (lower part)
Inflorescence	Unilateral cyme (1)2-5(8) flowers 5, deciduous in fruiting, 8-18 × 5-10 mm, cordate-ovate, acuminate	Cimose, short, covered by bracts	Scorpioid cyme, with 2-9 flowers	Cimose, 2-9 cm, with 1-2 flowers 5, deciduous in fruiting, 9-18 × 7-12 mm cordate, acuminate	Scorpioid cyme, with (1)2-5 flowers 5, 6-8 × 4-5(7) mm, ovate-acuminate, cordate
Sepals	White, yellow near base, (6)10-13 × 5-9 mm equal to sepals in anthesis. Very fugaceous	Purple, pink, 12-20 × 10-20 mm, undulate	White, 9-14 × 6-10 mm, truncate, obcordate	White, sometimes yellow near base, 14-20 × 12-16	White, yellow near base, 9-15 × 8-11 mm, truncate
Petals	Not equal	Subequals	Not equal	Not equal	Not equal, sometimes vestigial
Style	Filiform, equal to stamens 5-6 mm, globose, penicillate-pubescent, finally sub-sub-glabrous, except for the apex	Shorter than stamens, linear	Shorter than stamens	Absent, sessile stigma	Absent, sessile stigma
Capsule	c. 2.2 mm, angulous, rugulose	5-6 mm ovate, oblongue, sub-glabrous	4 mm, globose, puberulent	5-7 globose, pubescent	4-6 mm, ovate, puberulent
Seeds	c. 1.1 mm, smooth	c. 1.1 mm, smooth	c. 1.5 mm, reticulate-rugose	c. 1.5 mm, reticulate	usually sterile

analyses were performed. Both analyses were carried out under GTR + G + I model of evolution according to JModeltest 2 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012). MrBayes, v. 3.2 (Ronquist *et al.*, 2012) was used to perform Bayesian analyses. Two simultaneous runs were initiated by starting from random trees. To ensure that the two runs converged onto a stationary distribution, analyses were run until the average standard deviation of the split frequencies was 0.01. Convergence was evaluated using the potential scale reduction factor (PSRF), and 2,000,000 generations were run by sampling every 100th generation at the following settings: Nst = 6, rates = invgamma. Burnin (the number of starting generations ruled out from further analyses) was set at 200,000 generations after visually inspecting the likelihood values in Excel. A 50% majority rule consensus tree was constructed using the “sumt” command of MrBayes. ML phylogenetic trees were conducted with 10,000 bootstrap replicates by the rapid Bootstrap analysis in v.1.5b1 of RAxMLGUI (Silvestro & Michalak, 2012). The best-scoring ML trees were chosen as the final trees and bootstrap values were added to nodes. Trees were edited with Figtree, v.1.4 (Rambaut, 2012).

Results

Morphological results

Morphological examination and comparison between herbarium accessions and the newly collected material suggested that plants belonging to Southeastern Spain should be considered as *Cistus pouzolzii*. Specifically, the individuals from Southeastern Spain showed high similarities in the combination of characters used to differentiate *C. pouzolzii* from other *Cistus* species as the size and shape of leaves, the indumentum, inflorescences, size and shape of sepals and petals, and the equal length of stamens and style (Table 1).

Molecular results

The concatenated alignment of two plastid regions from 38 samples of *Cistus* plus *Tuberaria guttata* (outgroup species) was 1227 bp long (741 from trnS-trnG spacer, and 486 belonging to trnL-trnF spacer). Of these 930 sites were constant, 225 were variable but parsimony uninformative and 72 were parsimoniously informative.

Both the ML and Bayesian inference searches resulted in trees with a similar topology. Therefore, the Bayesian posterior probability (PP) and ML bootstrap values (Bs) are provided in the same tree for all the analyses (Figure 1). The phylogenetic tree is quite similar to those obtained in previous phylogenies (Guzmán & Vargas, 2005, 2010; Civeyrel *et al.*, 2011; Totta *et al.*, 2016). Species of *Cistus* are subdivided into two clades with high statistic support. The first one includes the purple-

flowered *Cistus* whereas the second clade includes the white-flowered *Cistus* species.

Regarding *C. pouzolzii*, the samples from the recently known populations showed almost identical sequences to those obtained from Genbank database, which belonged to individuals from France and Morocco (Table 2). Consequently, they are located in the same clade of the phylogenetic tree, being *C. monspeliensis* and *C. salviifolius* the sister species.

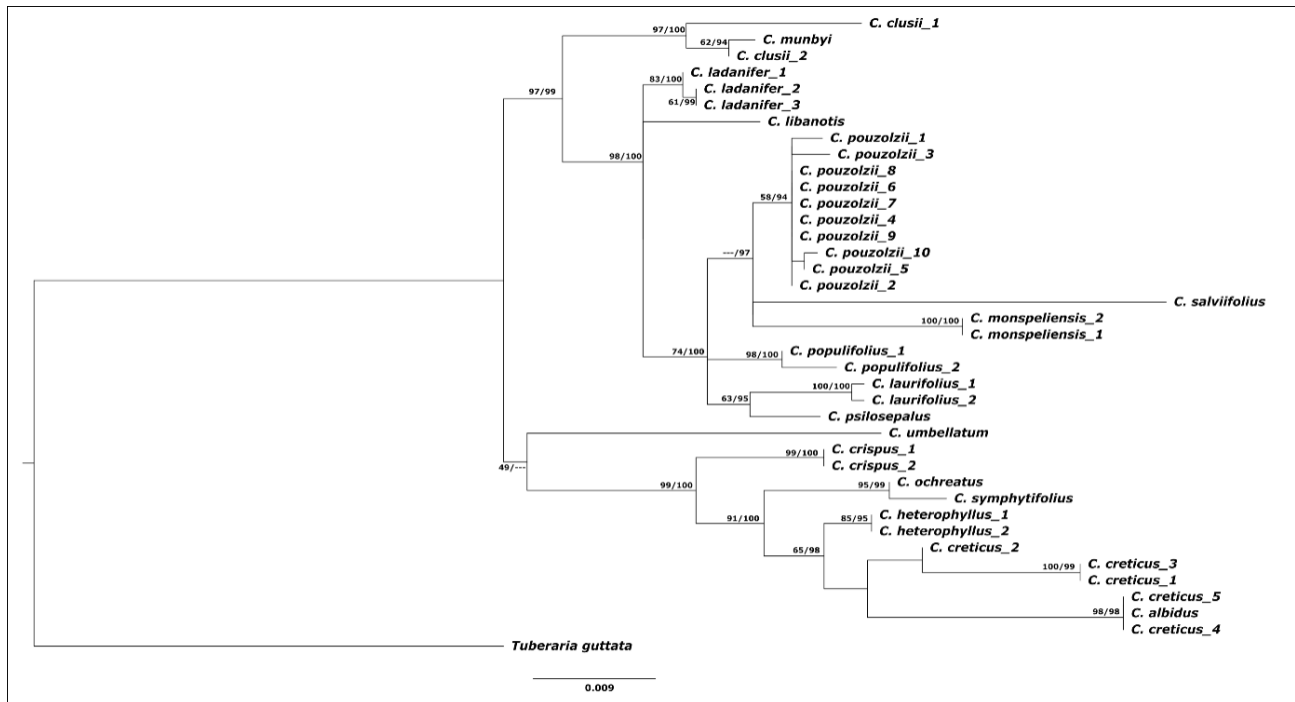


Figure 1. Maximum likelihood phylogenetic tree inferred for a sampling of select species of *Cistus*. Bootstrap values (Bs) followed by Bayesian posterior probabilities (PP) above 50% are given below the branches. The tree was rooted with *Tuberaria guttata* as outgroup.

Distribution and habitat of *Cistus pouzolzii*

According to the available data from studied herbarium accessions, as well as bibliographic references in which are indicated locations of *Cistus pouzolzii*, its distribution area would cover the Western Mediterranean Region. It is present in Southeastern France, North Africa (Morocco and Algeria) (Munby, 1847; Coste & Soulié, 1911; Mouline, 1996; Guzmán *et al.*, 2009; Poumailloux, 2014; Raab-Straube, 2018), and in the Southeast of the Iberian Peninsula, according to Guzman *et al.* 2009 (Figure 2). It inhabits sub-heliophyll shrubs on siliceous soils, in both the mesomediterranean and supramediterranean bioclimatic belts, with subhumid and humid ombrotypes (Guzmán *et al.*, 2009).

In France it is widely distributed in the Aveyron, Gard, Lozère and Archède Departments (Southern Cévennes). It inhabits in mesomediterranean and supramediterranean belt, between 150–650 m asl, on schistose soils as part of scrubland and thickets of *Lavanduletalia stoechadis* Br.-Bl. (1931) 1940 em. Rivas Martínez, 1968, in pine woods of *Pinus nigra* J.F. Arnold subsp. *salzmannii* (Dunal) Franco and *Pinus pinaster* Aiton, and it also inhabits, to a lesser extent, chestnut groves of *Castanea sativa* Mill. (Aubin, 1986;

Revol, 1992; Mouline, 1996; Bensettiti *et al.*, 2001). In Morocco, its main populations are located in the Tangier area, West and Central Rif Mountains, Targuist area y Tazekka National Park. In these locations inhabits in mesomediterranean and supramediterranean soils, between 800–1800 m asl, where it also occurs on crystalline schists and in decalcified soils as part of shrubs of *Cistus sp.* of *Lavanduletalia stoechadis* Br.-Bl (Mateos & Valdés, 2009), in *Quercus sp. pl.* or even *Cedrus atlantica* (End.) Carrière forests. Its distribution is more diffuse in Algeria, where it is considered a rare species. It is at least located in the Saharian Atlas, near Zaccar, and Atlas Mountain Blida (Quèzel & Santa, 1962), on decalcified soils in more or less humid mountains.

In the Iberian Peninsula (in this paper) it is distributed along the Baetic mountains in the Subbaetic ranges Segura (Jaén province) and foothills of Calar del Mundo (Albacete province). The newly discovered populations are listed below. For each population, we show the UTM coordinates, date of collection and voucher number (Appendix 1). All the accessions have been deposited in the MUB herbarium (Appendix 1). It inhabits on siliceous sands of the Utrillas facies, forming part of *Cistion laurifolii* Rivas Goday in Rivas Goday *et al.*, 1956 em.

Table 2. Origin of the material included in the molecular analysis and GenBank accession numbers. The accessions used to generate plastid sequence data in the present study are listed in *Italics*. First appeared publications with GenBank accessions are indicated with asterisks: *Guzmán & Vargas (2005); **Guzmán & Vargas (2009b); *** (Guzmán *et al.*, 2009); **** Guzmán & Vargas (2010); ***** (Fernández-Mazuecos & Vargas, 2010).

Species/accessions	Origin of sequence/voucher	Genbank accession number	
		TrnS-trnG spacer	trnL-trnF spacer
<i>C. albidus</i>	25PV03	FJ228730**	DQ093021*
<i>C. clusii_1</i>	MA618671	GQ281693***	GQ281667***
<i>C. clusii_2</i>	8bBGA04	FJ228739**	DQ093056*
<i>C. creticus_1</i>	86PV05	GQ927055****	-
<i>C. creticus_2</i>	68PV05	GQ927056****	-
<i>C. creticus_3</i>	131PV03	GQ927061****	-
<i>C. creticus_4</i>	209PV04	-	DQ093025*
<i>C. creticus_5</i>	392PV02	-	DQ093026*
<i>C. crispus_1</i>	32PV03	-	DQ093061*
<i>C. crispus_2</i>	58BGA04	GQ281694***	DQ093060*
<i>C. heterophyllus</i>	7BGA04	-	DQ093035*
<i>C. heterophyllus</i>	99BGA04	GQ281690***	DQ933036*
<i>C. ladanifer_1</i>	28PV03	FJ189395**	DQ093047*
<i>C. ladanifer_2</i>	109BGA04	FJ189400**	DQ093048*
<i>C. ladanifer_3</i>	179PV04	FJ189411**	DQ093044*
<i>C. laurifolius_1</i>	13BGA03	FJ228731**	DQ093052*
<i>C. laurifolius_2</i>	12PV03	GU288890*****	DQ093051*
<i>C. libanotis</i>	149BGA04	FJ228732**	DQ093040*
<i>C. monspeliensis_1</i>	30PV03	GU288910*****	DQ093058*
<i>C. monspeliensis_2</i>	35BGA04	GU288916*****	DQ093059*
<i>C. munbyi</i>	4BGA04	FJ228738**	DQ093053*
<i>C. ochreatus</i>	8BGA04	GQ281687***	DQ093032*
<i>C. populifolius_1</i>	5PV03	FJ228733**	DQ093050*
<i>C. populifolius_2</i>	20BGA04	GQ281692***	DQ093049*
<i>C. pouzolzii_1</i>	698247MA	GQ927105****	DQ093055*
<i>C. pouzolzii_2</i>	8tBGA04	FJ228734**	DQ093054*
<i>C. pouzolzii_3</i>	Sierra de Segura, Jaén	OR237430	OR237438
<i>C. pouzolzii_4</i>	Sierra de Segura, Jaén	OR237437	OR237445
<i>C. pouzolzii_5</i>	Cortijo de las Acebeas, Jaén	OR237432	OR237440
<i>C. pouzolzii_6</i>	Cortijo de las Acebeas, Jaén	OR237436	OR237444
<i>C. pouzolzii_7</i>	Campamento de San Juan, Albacete	OR237434	OR237442
<i>C. pouzolzii_8</i>	Campamento de San Juan, Albacete	OR237435	OR237443
<i>C. pouzolzii_9</i>	Prox. Padroncillo, Albacete	OR237433	OR237441
<i>C. pouzolzii_10</i>	Prox. Padroncillo, Albacete	OR237431	OR237439
<i>C. psilosepalus</i>	7PV03	FJ228737**	DQ093041*
<i>C. salviifolius</i>	119PV04	FJ228735**	DQ093038*
<i>C. symphytifolius</i>	143BGA04	GQ281686***	DQ093030*
<i>Cistus umbellatus</i>	71BGA04	GQ281676***	DQ093019*
<i>Tuberaria guttata</i>	44BGA04	GQ281674***	DQ093018*

Rivas Martínez, 1979, between 1045–1400 m asl, within the upper mesomediterranean-supramediterranean belts with subhumid-humid ombrotypes. Although they currently live in the pine forests of *Pinus pinaster* and

P. nigra subsp. *salzmannii*, in both populations there are remains of old Pyrenean oaks (*Quercus pyrenaica* Willd.). It flowers from April to June and fruit formation takes place from July to August.



Figure 2. Distribution map of *Cistus pouzolzii*. Each square represents a 10×10 km UTM coordinate.

Discussion

In this study, we demonstrated that the individuals from the newly located populations shared the typical morphological characters used to discriminate *Cistus pouzolzii* (Table 1). Also, the habitat of the species is quite similar in each of the locations where it appears. Moreover, the plastid sequences used in the phylogenetic study are almost identical to those obtained from Genbank database, belonging to individuals from France and Morocco (Figure 1).

Cistus pouzolzii was first collected by Pouzolz at Gard department (France), being described by Delile (1840) as a new species. A complete description of *C. pouzolzii* was made by Grenier & Godron (1847) and Pouzolz (1857), and Willkomm (1857–1863) provided a detailed iconography. The former authors confirmed that it was not a hybrid, despite its morphological characters, which are similar to several *Cistus* species. Gard's studies pointed in that direction. He carried out several studies on comparative anatomy of pollen, seed, capsule, leaves and indumentum in the genus *Cistus*, and ruled out that *C. pouzolzii* was a hybrid. Furthermore, the species produces seeds without difficulty by forming populations in places where the supposed parents do not coexist, and in artificial crossing experiments by M. Bornet the hybrid *C. crispus* × *C. monspeliensis* (Gard, 1907, 1910, 1912) could not be obtained; Warburg & Warburg (1930) also argue against the hybrid origin and consider it to be a

valid species. The hybrid *C. crispus* × *C. monspeliensis* has also not been found in nature.

This species has undergone numerous nomenclatural changes, most probably due to its particular combination of morphological characters. Clos (1858) and Timbal-Lagrave (1875) identified this species as *C. varius*, a name adopted by some authors (Willkomm, 1878; Battandier & Trabut, 1888; Warburg & Warburg, 1930; Dansereau, 1939; Warburg, 1968; Greuter *et al.*, 1984). Clarifying the identity of *C. varius* is complicated (Rouy & Foucaud, 1895), as only the brief diagnosis of Pourret (1788) is available. Grosser (1903) also synonymized it to *C. varius*, but interpreted it as the hybrid *C. crispus* × *C. monspeliensis*. However, neither the locality of the protologue nor the description of Pourret (1788) matched the characteristics of *C. pouzolzii* (Rouy & Foucaud, 1895; Aubin, 1985, 1986, 1990; Demoly, 1996).

As commented before, the presence of *Cistus pouzolzii* in France is restricted to Aveyron, Gard, Lozère, and Archède Departments (Southern Cevennes). The localities cited for *C. pouzolzii* in Montpellier and Narbonne by Grenier & Godron, would correspond to a hybrid between *C. albidus* and *C. monspeliensis* called *C. ×ambiguus* by Rouy & Foucaud (1895). Subsequently, they were also discarded by Bubani (1901) and Breistroffer (1974).

As for its presence in the Iberian Peninsula, Costa (1877) reported *C. pouzolzii* from the vicinity of Barcelona, which was taken up by Willkomm (1878) and by Cadevall (1913) as a synonym of *C. varius*. Pau



Figure 3. Images of *Cistus pouzolzii* from Southeastern Spain. a, Branches showing the flowers before flowering; b, individual in flowering; c, Population from Villaverde de Guadalimar (Albacete), in which there are individuals growing in a burnt down pine forest.

(1921) studied vouchers from Costa's herbarium, which were very incomplete, and concluded that they belong to the hybrid *C. ×florentinus* Lam. In the COI-Willkomm herbarium there is a specimen (COI00061278!) with a handwritten label by Costa, in which it can be read "Montes cerca de Barna" (mountains near Barcelona), which is indeed *C. ×florentinus*. Later Sennen (1936, p. 70) suggested that *C. pouzolzii* was a different taxon from *C. varius* and also ruled out its presence in

Barcelona. Demoly (1996) also studied these specimens, concluding that both the Catalonia and Narbonne records corresponded to *C. ×florentinus*.

Martín-Bolaños & Guinea (1949) indicated that *C. pouzolzii* (sub *C. varius*) was a hybrid between *C. crispus* and *C. monspeliensis*, although with some doubts. They also included an iconography of a Carlos Pau voucher (MA-01-00079844!) in which no locality appears on the label. López Vélez (1996), based on these authors, indicated the

presence of *C. ×varius* (interpreted as the hybrid between *C. salviifolius* L. and *C. monspeliensis*) as present among the parents, at Arroyo del Pradoncillo (Albacete); however, *C. monspeliensis* is not present at that locality. She also indicated an erroneous UTM coordinate (30SWH4749). Subsequently, Sánchez-Gómez *et al.* (2016), based on this coordinate, where both parents were present, suggested that this was a plausible citation, together with another reference from a phytosociological inventory of a nearby point (López Vélez, 1996). However, after reviewing the only herbarium specimen recently provided by the author from Arroyo del Pradoncillo, where the correct location was indicated (30SWH4759), we found out that this specimen was *C. pouzolzii*. However, the presence of *C. ×florentinus* (*C. salviifolius* × *C. monspeliensis*) in nearby localities of the Sierra de Segura Mountains (Natural Park of Calares del Mundo y de la Sima), where the parents coexist, cannot be ruled out. At the same time, all adjacent areas with similar edaphoclimatic characteristics were sampled, as well as other nearby localities in the Sierra de Segura (Jaén), where the presence of *C. pouzolzii* was probable. The discovery of new localities in the province of Jaén from phytosociological inventories where we suspected that the references to *C. crispus* corresponded to *C. pouzolzii* (Valle *et al.*, 1988) has been particularly relevant. Finally, Bellard & Hervás (2021) cited *C. ×pouzolzii* as a hybrid between *C. crispus* and *C. monspeliensis* from Jaén, without voucher specimen, but with a photograph of the habit of the plant in vegetative state and a detail of the leaves. Based on its morphology this sample does not look like *C. pouzolzii*, but it should be studied in case it corresponds to an undescribed hybrid.

Conservation

Cistus pouzolzii is considered a western Mediterranean species that cannot be considered a threatened species, however, at the regional level, it is likely to present a higher degree of threat. It has been included in the “next threatened” (NT) category of the red list of Rhone-Alpes and the red list of the vascular flora of Midi-Pyrénées. It has been also protected at national level (“Arrêté du 20 janvier 1982 fixant la liste des espèces végétales protégées sur l’ensemble du territoire”). However, it is included in the category “low concern” (LC) (Olivier *et al.*, 1995; Poumailloux, 2014; Guillaume *et al.*, 2015; INPN, 2022). The threats to African populations are poorly known. *C. pouzolzii* is considered a rare species, being scarcer in Algeria than in Morocco (Munby, 1847; Quèzel & Santa, 1962; Mateos & Valdés, 2009; Dobignard & Chatelain, 2011). A large number of the populations are found in protected areas and National Parks, but it is known that in these two countries, livestock pressure, deforestation, and the transformation of the territory in recent decades are risks that should be taken into account. According to IUCN (2012) criteria, the Iberian populations should be considered Data Deficient (DD). However, the fact that it has gone unnoticed by botanists leads us to infer that it is a rare species. The available data show its presence in 12 UTM squares of 1x1 km in Jaén and 9 squares in Albacete

(total area of occupancy of 21 km²), with approximately 2500 individuals counted between the two populations. The main threats to the Iberian populations would be forest clearance, forest exploitation of pine forests, and forest fires. However, according to our observations in the population from Villaverde de Guadalimar, it seems a pyrophyte with good response to fire (Figure 3). As a precautionary measure, its legal protection is recommended in the autonomous communities where it occurs (Andalusia and Castilla-La Mancha), even though all the populations are protected as regional protected natural areas and within the Natura 2000 network at the European level.

Concerning the Iberian populations, sampling is needed in nearby areas of the Subbaetic Mountains where the edaphic and climatic conditions are similar to assess with certainty the degree of threat to the species and to establish appropriate management measures at the regional and national level. It cannot be ruled out that the species might be found in other localities with similar edaphoclimatic conditions in other Baetic Mountains and the Eastern of the Iberian Peninsula.

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Authorship contribution

PSG: Design, supervision, field sampling, taxonomic and morphological analysis, and writing (first draft and review); JLC: field sampling and laboratory analyses; AL: taxonomic and morphological analysis, field sampling and writing (first draft and review); AEC: writing (review) and field sampling; JFJ: Supervision, molecular analyses, writing (first draft, review and editing).

Conflict of interest

None.

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Appendix 1. Specimens of *C. pouzolzii* from Spain used in the morphological analysis.

SPAIN. Albacete: Riópar, Arroyo del Padroncillo, 30SWH4759, 1100 m asl, 16/VI/1981, G. López-Vélez, MUB 31117 [sub *Cistus x varius* Pourret]; Riópar, Campamento de San Juan, 30SWH4759, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1090 m asl, 07/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114739, MUB 114740, MUB 114741; Riópar, Campamento de San Juan, 30SWH4759, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1055 m asl, 07/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114742, MUB 114743; Riópar, Campamento de San Juan, 30SWH4759, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1080 m asl, 07/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114744; Riópar, proximidades Campamento de San Juan, 30SWH4760, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1070 m asl, 07/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114745; Riópar, proximidades del Padroncillo, 30SWH4659, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1160 m asl, 16/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114746; Riópar, proximidades del Padroncillo, 30SWH4659, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1175 m asl, 16/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114747; Riópar, entre el Puerto del Arenal y Cerro del Padroncillo, 30SWH4758, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1175 m asl, 16/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114748; Riópar, Campamento de San Juan, 30SWH4759, rockrose communities on siliceous sand soils of Facies Utrillas under *Pinus pinaster* formations, 1090 m asl, 14/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114750, MUB 114751, MUB 114752, MUB 114753; Riópar, proximidades Hueco de Los Chorros, 30SWH4857, shrublands in open *Pinus* woodland on siliceous substrata, 1050 m asl, 02/VI/2023, J.L. Cánovas, M. González & P. Sánchez Gómez, MUB 115390; Villaverde de Guadalimar, proximidades Fuente de las Raigadas, 30SWH4653, open shrubland in postfire areas on siliceous substrata, 1225 m asl, 05/VI/2023, P. Sánchez Gómez, J.L. Cánovas & F. Medina, MUB 115391. Jaén: Siles, proximidades Cortijo de las Acebeas, 30SWH3640, rockrose communities under *Pinus nigra* and broad-leaved oak formations, 1350 m asl, 16/VI/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114749; Benatae, Sierra de Segura, 30SWH3739, rockrose communities under *Pinus nigra* and *Quercus pyrenaica* formations, 1310 m asl, 20/VII/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114754; Siles, Sierra de Segura, 30SWH3839, rockrose communities under *Pinus nigra* and *Quercus pyrenaica* formations, 1390 m asl, 20/VII/2022, P. Sánchez Gómez & J.L. Cánovas, MUB 114755, MUB 114756, MUB 114757. Benatae, Sierra de Segura, 30SWH3839, under *Pinus nigra* and *Quercus pyrenaica* formations, 1365 m asl, 28/VI/2023, P. Sánchez Gómez, J.L. Cánovas & A Lahora, MUB 115540.