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# *Cistus ladanifer* (Cistaceae): a natural resource in Mediterranean-type ecosystems

David F. Frazão<sup>1</sup> · Joana R. Raimundo<sup>1</sup> · Joana L. Domingues<sup>1</sup> · Celestino Quintela-Sabaris<sup>2</sup> · José C. Gonçalves<sup>1,3,4</sup> · Fernanda Delgado<sup>1,3,4</sup> 

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## Abstract

**Main conclusion** *Cistus ladanifer* has a well-defined taxonomic identity. 2,2,6-trimethylcyclohexanone may be an authenticity and taxonomic marker. Its traits and applications make it a possible economic resource fitted for Mediterranean areas.

*Cistus ladanifer* is a dominant shrub species endemic to the western Mediterranean region. Due to its dominant nature and its potential ecological, aromatic or pharmacological applications, *C. ladanifer* has been the object of numerous studies. In this review current knowledge on different aspects of this species is summarized, from its taxonomy to its chemical characterisation or its competitive traits. There are no doubts about the taxonomic entity of *C. ladanifer*, although the recognition of infraspecific taxa deserves more attention. Given that the fragrant exudate of *C. ladanifer* holds a very specific composition, one species specific carotenoid, 2,2,6-trimethylcyclohexanone, derivative is proposed as an authenticity marker for uses of *C. ladanifer* in pharmacological or aromatic industries. Evidence is also gathered on the extreme adaptation of *C. ladanifer* to stressful conditions in the Mediterranean region, such as the ability to survive in low hydric and high solar exposition conditions, persistence in poor and contaminated soils, and growth inhibition of several other plants through the release of allelochemicals. Thus, the finding of potential applications for this plant may contribute to enhance the economic dimension of derelict lands, such as mine tailings or poor agricultural Mediterranean areas.

**Keywords** Rockrose · Labdanum · 2,2,6-Trimethylcyclohexanone · Taxonomy · Competitive traits

## Introduction

*Cistus ladanifer* L. (Cistaceae), also known as rockrose, and called “esteva” (Portuguese) or “jara” (Spanish), is a resinous and fragrant evergreen shrub, very abundant among other wild species in the forest and uncultivated areas of the western Mediterranean region (Portugal, Spain, south of France, north of Morocco). This plant is frequently associated to negative impacts, such as agroecosystems occupation and competition, as well as fire promotion. Knowledge about the shrub’s survival mechanisms may constitute a way either to fight and control it or to exploit it. Cistaceae plants have gained high biological interest, both at the level of rapid colonization of burnt and poor agricultural areas, as well as revegetation of metal-polluted sites, and at the level of phytotoxic and symbiotic interactions with the ecosystem, which consequently highlights its relevant aromatic and pharmacological potential applications (Dias et al. 2005; Martín-Pinto

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et al. 2006; Papaefthimiou et al. 2014; Santos et al. 2016). This shrub produces a resin, called labdanum, rich in valuable compounds, which have been extracted in countries such as France, Spain and Portugal, mainly to be used in the perfume industry due to its fixative and aromatic properties (Moyler and Clery 1997). In Spain, *C. ladanifer* labdanum has been exported to some Arabic countries, where it is used as incense (Papaefthimiou et al. 2014). Moreover, some labdanum compounds constitute an important alternative to ambergris, which is a rare ingredient obtained from a protected animal source (sperm whale), and provides starting materials for the manufacturing of synthetic ambers (Groom 1997). Essential oils are valuable products obtained from plants. Although in rockrose it has a low yield extraction of around 0.1%, essential oil has a potential use in medical, cosmetic (Papaefthimiou et al. 2014) and food industry (Barrajón-Catalán et al. 2016).

Most of the current labdanum exploitation is based on wild *C. ladanifer* populations, although possibilities are emerging of cultivation this species on derelict land, such as low-productive agricultural fields or mine tailings (Santos et al. 2017). However, in order to develop *C. ladanifer* cultivation, knowledge on the ecology of the species, its reproductive biology, or inherent variability in secondary compounds is needed.

To fulfil this task, this study compiled and reviewed the existing scientific literature on *C. ladanifer*, focusing on its taxonomy, phylogeny, and phylogeography, its morphology and distribution, its reproductive biology and its chemical characterisation. The natural advantages of this species are also evidenced as competitive traits versus others species and/or as environmental responses.

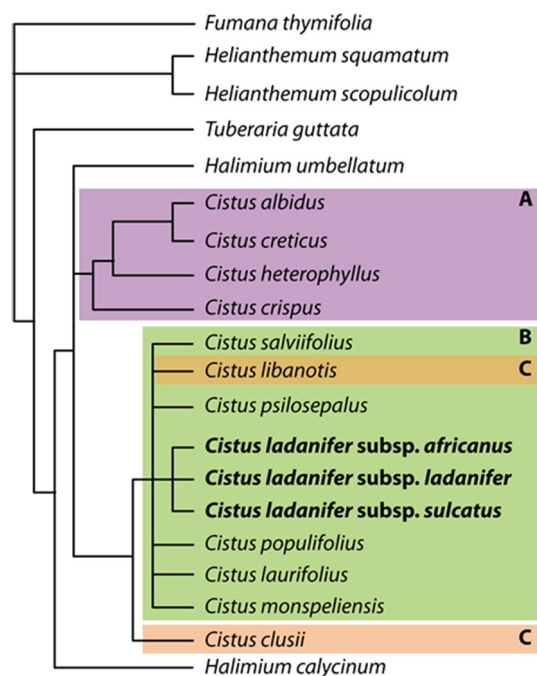
## Taxonomy, phylogeny and phylogeography

Cistaceae family comprises eight genera, five of which (*Cistus*, *Fumana*, *Halimium*, *Helianthemum* and *Xolantha*) are present in the Mediterranean area, and about 180 species. After several taxonomic re-evaluations, about twenty one species of *Cistus* are now recognized, spreading within white and pink-flowered lineages (Guzmán and Vargas 2005). These taxonomic changes have been mainly based on morphological characters. However, the type of pollen, specific genetic characteristics and chemical characterization of plants belonging to the genus *Cistus* have also been taken into account for taxonomy and phylogenetic divisions (Barrajón-Catalán et al. 2011). Ellul et al. (2002) reported significant differences in DNA content between pink-flowered species (subgenus *Cistus*) and white-flowered ones (subgenera *Leucocistus* and *Halimioides*).

Currently, twelve *Cistus* species are recognised in the *Flora Iberica* (Demoly and Montserrat 1993) distributed

along three subgenera (Fig. 1). However, frequent hybridisations have been registered in the genus (Demoly and Montserrat 1993). Within *C. ladanifer* three subspecies are recognized (Fig. 1).

Morphological differences of *Cistus* genus have been clarified by the application of DNA sequencing and molecular markers, allowing a more complete taxonomic classification. In these studies, many authors used universal primers for non-coded regions of the chloroplast DNA since these are structurally stable, haploid and heritability is maternal (Fernández-Mazuecos and Vargas 2010; Guzmán and Vargas 2005, 2010; Hamilton 1999; Johnson and Soltis 1994; Taberlet et al. 1991). Sequencing of the Internal Transcribed Spacer region (Sun et al. 1994; White et al. 1990) from the nuclear genome has also been used at the level of *Cistus* genus, but showed to be less useful for species identification than plastid sequences. Apart from sequence analyses, nuclear markers such as RAPDs (Williams et al. 1990), AFLPs (Vos et al. 1995) and ISSRs (Zietkiewicz et al. 1994) and sets of primers for plastid microsatellites (Sebastiani et al. 2004; Weising and Gardner 1999) have been applied to the study of phylogeography and population genetics in *C. ladanifer* (Quintela-Sabaris et al. 2005, 2011, 2012) or in *C. ladanifer* ssp. *sulcatus*, *Halimium halimifolium* and several other *Cistus* species from south of Portugal (Carlier et al. 2008).



**Fig. 1** Phylogenetic tree of *Cistus* species in Flora Iberica, where A—subgenus *Cistus*, B—subgenus *Leucocistus*, C—subgenus *Halimioides* (adapted from Guzmán and Vargas 2005). *Cistus ladanifer* and its subspecies are marked in bold type

The taxonomic identity of *C. ladanifer* has been confirmed by Guzmán and Vargas (2005) on the basis of nuclear and plastid genetic sequence data. A later study done by Guzmán and Vargas (2009), using also nuclear and plastid DNA sequences, dated the origin of *C. ladanifer* and the divergence of its different subspecies in the Upper Pleistocene, well after the onset of the Mediterranean climate and the flooding of the Strait of Gibraltar. Therefore, the current species range in northern Africa and southwest Europe is the result of recent natural long distance dispersal events across that sea barrier (Guzmán and Vargas 2009). These data were further confirmed by Quintela-Sabarís et al. (2011) who, using chloroplast microsatellites (cpSSRs), identified three population lineages that resulted from isolation by geographic barriers (such as the Betic ranges or the Strait of Gibraltar) during glacial periods. Thus, the current distribution area of *C. ladanifer* is actually divided in three main genetically homogenous groups in (1) north of Africa, (2) Betic area (south and southeast of Iberian Peninsula) and (3) an extensive area from the southwest Iberian Peninsula to the south of France. Later analysis, using nuclear AFLP markers, confirmed the existence of those population lineages (Quintela-Sabarís et al. 2012).

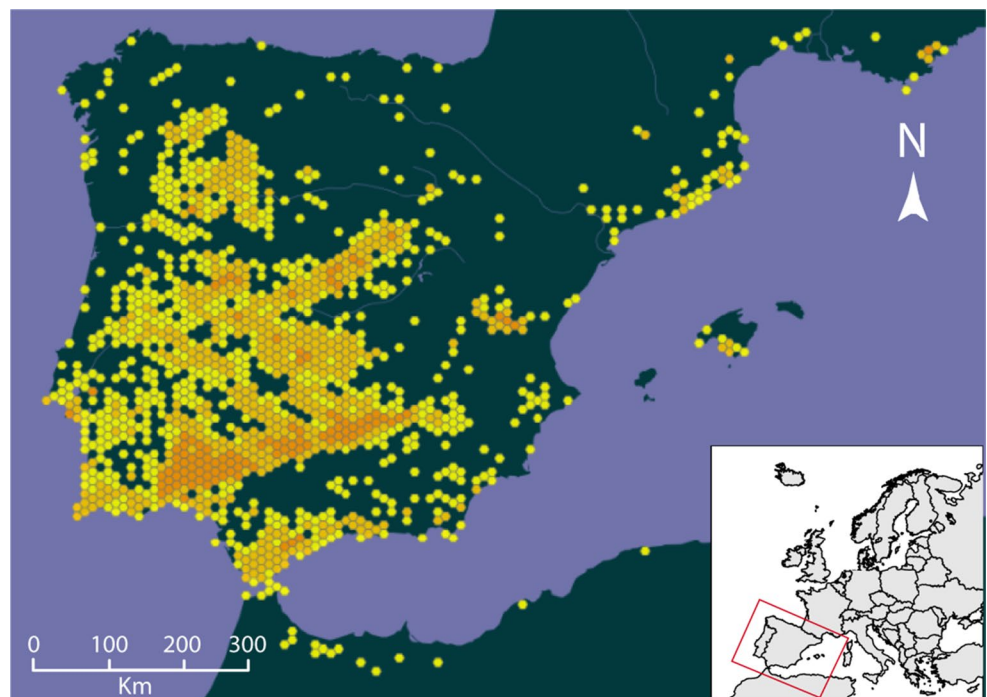
Different genetic analyses suggest the more ancient origin of the subspecies *africanus*, and a subsequent differentiation for subspecies *ladanifer* and *sulcatus* (Guzmán and Vargas 2009; Quintela-Sabarís et al. 2011, 2012). Moreover, a study on *C. ladanifer* populations from the Algarve region (south of Portugal) with AFLPs and ISSRs uncovered significant genetic differentiation between subspecies *ladanifer* and

*sulcatus* (Carrier et al. 2008). However, more recent analyses using cpSSRs and AFLPs (Quintela-Sabarís et al. 2011, 2012) showed no differentiation between subspecies *ladanifer* and *sulcatus*, and also indicated that Betic populations of *C. ladanifer* (identified as subspecies *ladanifer* on the basis of leaf morphology) formed an unusual cluster both at the chloroplast and nuclear level. Therefore, contrasting results between different studies, as well as the incongruence between morphology and genetic data pointed out the need for a taxonomic revision at the intraspecific level in these species.

## Morphology and distribution

*Cistus ladanifer* is a perennial and woody shrub from the western Mediterranean area, from southern France to the north of Morocco and Algeria (Fig. 2). It appears over extensive xerophilic shrub lands, mainly in the southwestern region of the Iberian Peninsula, since sea level goes up to 1500 m (Demoly and Montserrat 1993). It may form very dense populations that mainly integrate poor siliceous and acidic soils with shale, granite or sandstone origin (Ribeiro and Delgado 2016). *C. ladanifer* is the major component of shrub lands in oligotrophic acid soils in the western half of the Iberian Peninsula (Rivas-Martínez 1979). However, distribution models support the idea of its tolerance to calcareous soils (Gaston and Fuller 2009) and subsp. *sulcatus* is even restricted to limestone-derived soils on coasts from southwestern Portugal (Demoly and Montserrat 1993). This

**Fig. 2** Distribution of *C. ladanifer* in its native range, using georeferenced data from Global Biodiversity Information Facility—*Cistus ladanifer* L. in GBIF Secretariat (2017). GBIF Backbone Taxonomy. Checklist Dataset <https://doi.org/10.15468/39omei> accessed via GBIF.org on 2017-11-06. Inset presents the geographic context of *C. ladanifer* distribution area in southwestern Europe. Red square in the inset marks the area zoomed in the detail map



species behaves as colonizer on highly degraded areas (Alías et al. 2006; Pérez-García 1997; Valbuena et al. 1992) and has an increasing distribution as a result of human disturbance (Trabaud and Oustric 1989).

The subspecies *ladanifer* is primarily distributed in the Iberian Peninsula, northern Africa, southern France and isolated patches in the Canary Islands (being considered an introduced species in the two latter places); subspecies *sulcatus* is endemic to southwestern Portugal (Algarve region); and subspecies *africanus* is present in southern Spain (Cádiz, Málaga), but more commonly found in northern Africa (Demoly and Montserrat 1993).

An adult *C. ladanifer* plant may reach 2 m in height, with dense root and shoot systems (Bolaños and López 1949). It is a very viscous and erect shrub that has lanceolate green leaves with a glabrous upper surface and a back surface covered with a white tomentum. Leaves are presented in a decussate arrangement and welded at their base.

The morphology of leaves and capsule is used for the identification of its three recognized subspecies: *africanus*, *ladanifer* and *sulcatus* (Demoly and Montserrat 1993). Subspecies *africanus* has petiolate leaves, whereas those of subspecies *ladanifer* and *sulcatus* are sessile. Moreover, the subspecies *africanus* usually has lanceolate leaves with little apparent nerves and the capsule can be divided into 6–10 compartments. In subspecies *ladanifer*, the leaves have visible nerves and the capsule can be divided into 9–10 compartments. In subspecies *sulcatus* (originally described as *Cistus palhinhae*) the leaves, usually elliptic, are split by very visible nerves and the capsule can be divided into 8–9 compartments (Demoly and Montserrat 1993).

Overall *C. ladanifer* presents short peduncles (5–16 cm) covered by yellowish peltate hairs. Stigma is sessile and surrounded by a great number (> 100) of unequal stamens and the fruit is a loculicide capsule with 5–12 locules. The number of valves showed to be variable at the inter-populations, inter-individual, intra-individual and inter-annual levels (Narbona et al. 2010).

The exemplars of *C. ladanifer* present the largest and showiest flowers (5–10 cm in diameter) of genus *Cistus* and overall Spanish flora, constituted by three oval sepals with yellowish-green hairs and five petals. In these large and solitary flowers of subspecies *ladanifer* the five petals may be totally white (var. *albiflorus*) or have a red to maroon blotch at the base of each petal (var. *maculatus*) (Rouy and Foucaud 1895; Talavera et al. 1993). These varieties can occur separated in monomorphic populations or in mixed populations with both varieties present (Guzmán et al. 2011).

Apart from its big flowers and the high number of locules, another anatomic distinguishing trait of *C. ladanifer* is the production and exudation of a fragrant, sticky oleoresin, labdanum, comprising more than 10% (w/w) of its dry weight (Demoly and Montserrat 1993; Dias et al. 2005; Gulz

1984; Mariotti et al. 1997; Vogt et al. 1987) or between 2 and 3.5% (w/w) of its fresh weight (Lawrence 1999), from secretory trichomes found in leaves and photosynthetic stems (Chaves et al. 1997a; Masa et al. 2016a). According to Gulz et al. (1996), two kinds of trichomes are found in *C. ladanifer* leaves, the secretory ones, called glands, multicellular structures with the format of 20–30 µm short stalked small heads containing and secreting the labdanum, and the non-secretory ones, called hairs, unicellular stellate hairs with 6–20 branches. Hairs are only found, in a very dense arrangement, on the lower leaf surface (Gulz et al. 1996). Labdanum accumulates under the cuticle of the head cells of glands, which finally breaks due to pressure (Ascensão and Pais 1987; Borges 1987; Dias et al. 2005), resulting in total covering of leaf surface and glands completely soaked in it (Dias et al. 2005; Gulz et al. 1996). These secretory glands are also present in photosynthetic stems (Borges 1987, 1989), but it is not yet clear if they coexist with hairs at the lower leaves surface (Dias et al. 2005). Apart from the clear seasonal variation through the year, young leaves secrete greater amounts of labdanum than photosynthetic stems and these, in turn, secrete more than mature leaves (Masa et al. 2016a). Annually, labdanum production increases when minimum temperature increases (Dias et al. 2005) which means usually in the summer. According to Trabaud and Oustric (1989), labdanum production has a daily cycle too, in which it increases at noon. The role of labdanum is thought to be autoregulation and protection against herbivores and environmental stresses, such as low water and excessive UV light (Dias et al. 2005).

Like all other *Cistus* species, *C. ladanifer* is diploid ( $2n = 18$ ) (Demoly and Montserrat 1993) and its nuclear DNA content ( $2C$ ) is  $4.45 \pm 0.17$  pg (Ellul et al. 2002).

## Reproductive biology

During spring, *C. ladanifer* produces a large number of ephemeral flowers (1–3 days). The flowering ends with the falling of petals and the refolding of sepals around the ovary tomentose. A flower that has not been fertilized will fall a few days after the end of the anthesis, its pedicel remaining on the plant (Guzmán et al. 2011).

Pollination in *C. ladanifer* is entomophyllous (mainly by individuals Diptera, Hymenoptera and Coleoptera). Flowers last for a few days and attract insects by nectar and high pollen production (more than 700,000 pollen grains per flower) (Talavera et al. 1993). Pollinators have been shown to exert a selection effect towards large flowers (Barrio and Teixido 2015). A gametophytic mechanism of incompatibility makes *C. ladanifer* an obligate outcrosser (Guzmán et al. 2015; Talavera et al. 1993). This feature, together with the short distances of pollen dispersal, results in reduced reproductive

output in isolated plants, e.g. pollination success in isolated *C. ladanifer* plants dropped to zero when the distance to the nearest neighbour was around 33 m (Metcalf and Kunin 2006).

Morphological diversity of white flowers (*albiflorus* or *maculatus*) showed no effect on *C. ladanifer* reproductive success, measured as flower production, fruit set or seed set (Guzmán et al. 2011). Moreover, weak reproductive barriers were found between floral morphs (Guzmán et al. 2015).

A single *C. ladanifer* capsule may contain around 1000 (from 318 to 1185) small (1 mm) polyhedral seeds and the total seed production by a single adult *C. ladanifer* plant may be up to 158,000 seeds each year (Bastida and Talavera 2002). Narbona et al. (2010) reported a positive relationship between number of valves and number of seeds per valve.

Seed release starts in the middle of summer and continues for 8–10 months. This dispersion is mainly barochorous, and more than 80% of seeds fall beneath the mother-plant canopy (Bastida and Talavera 2002). However, different granivorous ants of genus *Goniomma* and the red-deer (*Cervus elaphus*), which feed on seeds and fruits, respectively, may play an important role in seed dispersal over longer distances (Bastida and Talavera 2002; Malo and Suárez 1998). Cistaceae family is characterized to have “hardseededness” which is a specific type of physical dormancy (Thanos et al. 1992; Valbuena et al. 1992). *C. ladanifer* has small seeds with size between 0.5–1 mm and weight around 0.27 mg, which facilitates their accumulation and penetration in soils and leads to the formation of soil seed banks. These seed banks allow the escape from unfavorable conditions and guarantees propagation, for example, after a fire (Bastida and Talavera 2002; Talavera et al. 1993; Thanos et al. 1992). Hard seeds have a crusty and impermeable coat and, usually, germinate by thermal shock, smoke and nitrogenous salts treatments (Pérez-Fernández and Rodríguez-Echeverría 2003; Pérez-García 1997). *Cistus* species are not exclusively dependent on fire however they have a slow germination without it (Delgado et al. 2001; Valbuena et al. 1992). The dry-heat pretreatment is one of the most effective methods increasing germination in the seeds of *C. ladanifer* (Valbuena et al. 1992). Interestingly, a small soft-coat fraction of seeds is also produced, which can soak water and, eventually, germinate (Alías et al. 2006; Pérez-García 1997; Thanos et al. 1992).

*Cistus ladanifer* seeds have considerable longevity as a result of the protection given by the impermeable coats (Thanos et al. 1992). Furthermore, the ability to produce seeds with different degrees of dormancy is, most probably, a mechanism by which *C. ladanifer* adapts to new environmental scenarios (Gutterman 1994; Kigel 1995). This peculiar two ways adaptation also contributes for seed characteristics variation in *C. ladanifer* (Delgado et al. 2001). Most germination happens in first year after fire, usually

in autumn (first rains) but, in low rainfall years, hard seeds may germinate in the second or third year after production (Moreno et al. 2011; Quintana et al. 2004).

## Chemical characterization

Apart from the classification based on morphological, anatomical and genetic characteristics, secondary metabolites can contribute to the taxonomic classification of plants (Demetzos and Dimas 2001). Although biotic and abiotic stimuli change the production level of some secondary metabolites (Alías et al. 2012; Papaefthimiou et al. 2014; Sangswan et al. 2011), the chemical profile or fingerprint depends on plants genotype.

Most studies about the chemical profile characterization of *C. ladanifer*, have mainly focused on its essential oil (cistus oil), restricting analysis to mono- and sesquiterpenes, the main constituents (Gomes et al. 2005; Greche et al. 2009; Mariotti et al. 1997; Robles et al. 2003). Other *C. ladanifer* extracts, usually called bioextracts, have also been studied, but to a lesser extent.

Labdanum, the exuded aromatic resin from *C. ladanifer*, is economically important for countries like Spain and France (Papaefthimiou et al. 2014) and constitutes a specific kind of bioextract from intact plant material. It is traditionally obtained by boiling the plant material in water (Dias et al. 2005). However, other solvents have been applied either to plant material or resin, for analytical purposes of labdanum constituents: chloroform (Alías et al. 2012; Chaves et al. 1997a, 1998; Vogt et al. 1987), ethanol (Chaves et al. 1993), acetone (Wollenweber and Mann 1984), hexane (Pascual et al. 1982) and with water followed by organic solvents such as dichloromethane (Ramalho et al. 1999) and ethanol (Greche et al. 2009). Extraction with water has the advantage (over organic solvents) of being more ecologic and environmentally friendly (Barrajón-Catalán et al. 2011). Labdanum is characterized by its high content in diterpenes and flavonoids (Alías et al. 2012; Masa et al. 2016a), but it also contains mono- and sesquiterpenes. These later compounds are also markedly found in the oil obtained from distillation of labdanum (Oller-López et al. 2005; Weyerstahl et al. 1998).

Other bioextracts from *C. ladanifer* found in literature comprise extracts of milled plant samples with organic solvents (Barros et al. 2013; Guimarães et al. 2009 Santos et al. 2017) and water (Barrajón-Catalán et al. 2011; Fernández-Arroyo et al. 2010), supercritical CO<sub>2</sub> extract (Rincón et al. 2000) and simultaneous distillation-solvent extract (Teixeira et al. 2007). Different types of extracts and different solvents result in different target chemical compounds extracted and analysed, thus it is often difficult to compare results between different studies.

On the one hand, cistus and labdanum oils, as well as in the supercritical extract, have many identified mono- and sesquiterpenes and also co-distilled or co-extracted alkanes derived from waxes. On the other hand, many other non- or less volatile compounds have been identified in the exudate, such as diterpenes, flavonoids and phenolic compounds, apart from the mono- and sesquiterpenes (Fig. 3). Additionally, labdanum oil was found to have a significant constitution on diterpenes (Weyerstahl et al. 1998).

### Odoriferous compounds

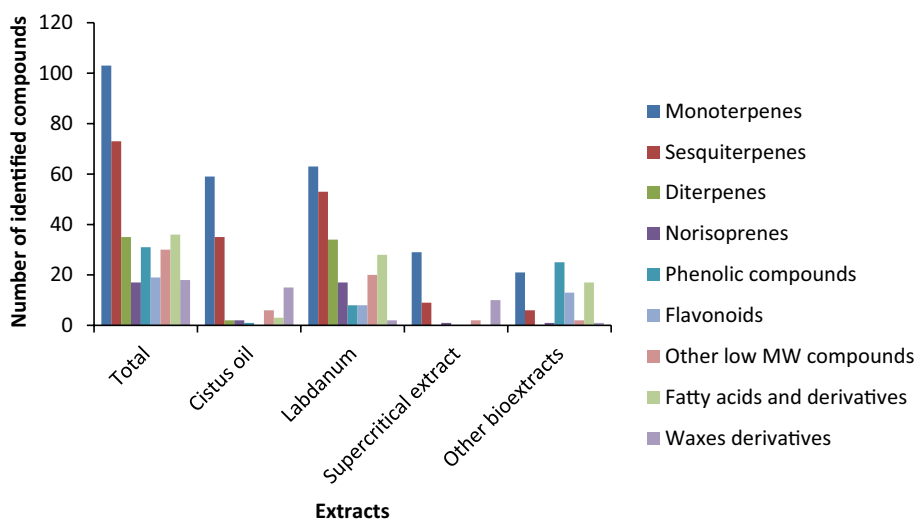
The most relevant volatile terpenes, according to the frequency of reports in the literature and their relative quantities in extracts, mainly labdanum and cistus oils, are listed in Table 1. *C. ladanifer* shows a different terpene profile from other *Cistus* species, with a close taxonomic relationship (Gulz 1984; Oller-López et al. 2005). Regarding mono- and sesquiterpenes, the most common reported major compounds are the monoterpenes  $\alpha$ -pinene (Gomes et al. 2005; Gulz 1984; Mariotti et al. 1997; Rincón et al. 2000; Robles et al. 2003; Teixeira et al. 2007; Vieira et al. 2017), camphene (Zidane et al. 2013) and pinocarveol (Oller-López et al. 2005; Verdeguer et al. 2012) and the sesquiterpene viridiflorol (Gomes et al. 2005; Greche et al. 2009; Weyerstahl et al. 1998). In their literature review, Gomes et al. (2005) also observed that  $\alpha$ -pinene was the most commonly reported major compound. According to Robles et al. (2003), *C. ladanifer* var. *maculatus* produces more  $\alpha$ -pinene but less viridiflorol than var. *albiflorus*. However, Mariotti

**Table 1** Most relevant low molecular weight terpenes found in *C. ladanifer* L.

	Non-oxygenated	Oxygenated
Monoterpenes	$\alpha$ -Pinene	Pinocarveol
	$\beta$ -Pinene	Pinocarvone
	Camphene	Myrtenol
	Limonene	Myrtenal
	p-Cymene	Verbenone
		Borneol
		Bornyl acetate
		Terpinen-4-ol
Sesquiterpenes	$\delta$ -Cadinene	Viridiflorol
	Aromadendrene	Ledol
Norisoprenes	–	2,2,6-Trimethylcyclohexanone

et al. (1997) and Santos et al. (2017) observed a quantitative variation of a similar chemical profile inside *C. ladanifer* populations not reported to be multivarietal.

Another important compound found in high proportions in *C. ladanifer* extracts is the carotenoid derivative norisoprene called 2,2,6-trimethylcyclohexanone (Gomes et al. 2005; Greche et al. 2009; Mariotti et al. 1997; Oller-López et al. 2005; Ramalho et al. 1999; Robles et al. 2003; Santos et al. 2017; Teixeira et al. 2007; Verdeguer et al. 2012; Weyerstahl et al. 1998; Zidane et al. 2013), which is one



**Fig. 3** Number of identified compounds in *C. ladanifer* extracts reported in literature: Alías et al. (2006, 2012); Barrajón-Catalán et al. (2011); Barros et al. (2013); Chaves et al. (1997a, b, 1998, 2001); Fernández-Arroyo et al. (2010); Guimarães et al. (2009); Gomes et al. (2005); Greche et al. (2009); Gulz (1984); Mariotti et al.

(1997); Oller-López et al. (2005); Pascual et al. (1982); Proksch and Gulz (1980a, b); Ramalho et al. (1999); Rincón et al. (2000); Robles et al. (2003); Santos et al. (2017); Sosa et al. (2004); Teixeira et al. (2007); Verdeguer et al. (2012); Vieira et al. (2017); Vogt et al. (1987); Weyerstahl et al. (1998); Wollenweber and Mann (1984)

of the main compound responsible for *C. ladanifer* odour (Ramalho et al. 1999). This compound is not reported in other *Cistus* species, namely *C. monspeliensis* (Oller-López et al. 2005), *C. laurifolius* (Öğütveren and Tetik 2004), *C. salvifolius* (Demetzos et al. 2002), *C. creticus* (Paolini et al. 2009) and *C. libanotis* (Zidane et al. 2013). Thus, it may be a good chemotaxonomic marker at species level and even a *C. ladanifer* authenticity marker.

## Diterpenes

Diterpenes are rarely reported in cistus and labdanum oil (Gomes et al. 2005; Mariotti et al. 1997; Oller-López et al. 2005; Weyerstahl et al. 1998) due to their low volatility. Rincón et al. (2000) did not report, also, diterpenes presence in their CO<sub>2</sub> supercritical extracts. In contrast, labdanum is very rich in diterpenes mainly labdane-type ones (Alías et al. 2006, 2012; Greche et al. 2009; Pascual et al. 1982; Weyerstahl et al. 1998). According to Alías et al. (2006), the main diterpenes present in labdanum are 6-acetoxy-7-oxo-8-labden-15-oic acid, 7-oxo-8-labden-15-oic acid and oxocativic acid. Besides these, Pascual et al. (1982) also identified 6,8(17)-labdadien-15-oic acid, labdanolic acid, 7-hydroxy-8(17)-labden-15-oic acid, 8-methoxy-labda-15-oic acid and 8-hydroxy-13-labden-15-oic acid. Greche et al. (2009) obtained sclareol and 16-kaurene as the major diterpenes in their labdanum extract. Nor-diterpenes and a few diterpenes such as 16-kaurene, manoyl oxide and labdenols were reported to be present in labdanum oil (Oller-López et al. 2005; Weyerstahl et al. 1998). Results from Gomes et al. (2005) demonstrate a quantitative variation of 15-nor-labdan-8-ol between essential oils of two different *C. ladanifer* populations from Alentejo, Portugal. Apart from this, to our knowledge, there is no study comparing the diterpene profiles of *C. ladanifer* subspecies and their varieties, as it had been done for the *C. creticus* subspecies (Anastasaki et al. 1999). Regarding environment effects on diterpenes production, Alías et al. (2012) observed that 6-acetoxy-7-oxo-8-labden-15-oic acid and 7-oxo-8-labden-15-oic acid production is influenced by low temperature and low hydric stress and oxoativic acid diterpene production is only affected by temperature in the same way.

## Polyphenolic compounds and flavonoids

Phenolic and other aromatic compounds accumulate as end-products from shikimate and acetate pathways and can range from relatively simple molecules (e.g. phenolic acids, flavonoids and benzenoids) to highly polymerised compounds (lignins, melanins and tannins) (Guimarães et al. 2010). Ellagitannins are reported to be the most

significant compounds found in *C. ladanifer* leaves and stems (Barrajón-Catalán et al. 2010, 2011; Barros et al. 2013). However, according to Barrajón-Catalán et al. (2010) and Dentinho et al. (2007), they also exhibit high amounts of condensed tannins. Analysis of the chemical profile regarding polyphenolic compounds, such as hydrolysable tannins, shows a clear separation between the three *Cistus* subgenus: *Cistus*, *Leucocistus* and *Halimioides*. There is a clear separation between subgenus *Cistus* and subgenera *Leucocistus* and *Halimioides* and also between the latter, confirming previous evolutionary and phylogenetic studies (Barrajón-Catalán et al. 2011). Furthermore, the same authors also confirm the close taxonomic relationship between *C. ladanifer* and *C. salvifolius* within the subgenus *Leucocistus* due to their high concentration in ellagitannins.

Flavonoids aglycones are another major group of compounds present in the oleoresin exuded by the plant (Alías et al. 2012), represented by apigenin, kaempferol and their methylated derivatives, mainly 3-methyl-kaempferol and 3,7-dimethyl-kaempferol between others: 3,4'-dimethyl-kaempferol 3,7,4'-trimethyl-kaempferol, 4'-*O*-methyl-apigenin, 7-*O*-methyl-apigenin, 7,4'-di-*O*-methyl-apigenin (Chaves et al. 1997a, b, 1998; Sosa et al. 2004; Vogt et al. 1987; Wollenweber and Mann 1984). Secreted flavonoids aglycones (epicuticular) chemical patterns are usually complex and species specific. Thus, the application of lipophilic flavonoids patterns to systematic studies of *Cistus* seems more promising than that of uniform flavonoid glycosides (Dias et al. 2005; Vogt et al. 1987). *C. ladanifer* seems to exhibit differences in biosynthesis regulation between flavonoid glycosides and secreted flavonoid aglycones, since differences were found between flavonoid glycosides in vacuoles (quercetin, myricetin and kaempferol) and secreted flavonoids (kaempferol and apigenin) (Gulz 1984). Only kaempferol derivatives, apigenin and apigenin derivatives appear in *C. ladanifer* as exuded flavonoids aglycones, while quercetin, myricetin, luteolin and other flavonoid aglycones are present in other *Cistus* species (Vogt et al. 1987). Chaves et al. (1997b) observed different patterns of dimethylated kaempferols (3,4'- or 3,7-dimethyl-kaempferol) secretion, but not of apigenins, coexisting in plants of a single population, which suggests the existence of different chemotypes, the 4'-methyltransferase activity being enhanced in the one and the 7-methyltransferase activity being enhanced in the other one. Exposure to high solar irradiance (UV) and hydric stress (drought), therefore in summer, are the most recognized phytochemical stresses, which increase flavonoid aglycones, specially methylated ones, as well as labdanum production and secretion (Chaves et al. 1993, 1997a; Vogt et al. 1987).



## Competitive traits

In the Mediterranean region, *C. ladanifer* plays a fundamental role in the occupation of habitats and interactions with the ecosystem (Águeda et al. 2008; Guzmán et al. 2011; Hernández-Rodríguez et al. 2013), establishing symbiotic relationships with some microorganisms (Comandini et al. 2006; Fogel and Trappe 1978; Martín-Pinto et al. 2006; Mediavilla et al. 2016).

*Cistus ladanifer* populations also constitute early succession stages adapted to disturbances operating in Mediterranean ecosystems, especially fire, as they are extremely resistant to changes in the physical, chemical and biochemical properties of soils, as water and light stress. It develops dense shallow root systems that favour water uptake in transient wet periods (Bolaños and López 1949). In addition, it is semi-deciduous, retaining small-sized potentially active leaves through the summer drought (Nunez-Olivera et al. 1996). Their ability to develop in nutrient-poor areas may have been favoured by the establishment of symbioses with microorganisms in roots. Several bacteria strains have been isolated from *C. ladanifer* roots, some with a capacity of phosphate solubilisation or siderophore production (one of them identified as Plant Growth Promoting Rhizobacteria—PGPR) (Carro et al. 2017; Díez-Méndez et al. 2017; Solano et al. 2007).

Some authors suggest that in young leaves lacking epidermis, trichomes and their exudates may serve as a functional analogue of the epidermis in mature leaves (Masa et al. 2016a, b). They play a protective role against phytopathogenic fungi, herbivores and UV radiation (Sosa et al. 2004; Masa et al. 2016b). Due to its content in flavonoids and other phenolic compounds, the secretion of labdanum has several beneficial effects for *C. ladanifer* plants, such as the photoprotective role on leaves, especially in summer when exudation increases (Chaves et al. 1993) and the defence against herbivores eating *C. ladanifer* leaves through impairment of mouth skeletal muscle relaxation (Sosa et al. 2004).

Phenols, terpenes, alkaloids and other secondary metabolites showing phytotoxic activity can reduce the growing of other plants, a mechanism known as allelopathy (Agra-Coelho et al. 1980; Chaves et al. 2001; Dias 2001; Dias et al. 2005; Herranz et al. 2006; Malato-Beliz et al. 1992; Verdeguer et al. 2012). It is thought that this effect is caused by competition for nutrients, moisture, chemical interference, foliage accumulation, and light penetration (Aerts et al. 1991; Chaves and Escudero 1997; Friedman 1995; Kuuluvainen 1994; Muller and Muller 1956). In parallel, auto-toxicity was demonstrated in *C. ladanifer* and it may be involved in the control of the species itself (Alfás et al. 2006; Herranz et al. 2006; Manzano et al. 2005).

Studies about synthesis of secondary metabolites demonstrated that the intra-population variability may be conditioned by environmental and/or individual factors. Spatial arrangement of *C. ladanifer* populations may occur and this may be a strategy to save energy, while preserving the response capacity and protection against external factors. This will be a clear advantage over other species, providing good possibilities to adapt to variable environmental effects and increasing survival (Masa et al. 2016b). This trait may explain the quantitative variation of the chemical profile found by many authors within a *C. ladanifer* population as stated before.

Regarding its relation to soils, *C. ladanifer* is a pseudo-metallophyte, meaning that it has populations on both normal and metalliferous (metal rich) substrates (Pollard et al. 2002). More precisely, subspecies *ladanifer* and *africanus* have successfully colonised and established populations on serpentine areas and on mine tailings, where in some cases they have become the dominant species (Alvarenga et al. 2004; Ater et al. 2000; Lázaro et al. 2006; Quintela-Sabarís 2012). The colonisation of metalliferous substrates by plants is accompanied by micro evolutionary processes leading in most cases to the formation of metal-tolerant ecotypes (Linhart and Grant 1996). Tolerant plants are thus able to develop on metal-rich soils, toxic for most other plants (Antonovics 1975; Macnair 1993) by the development of mechanisms for efficient control of metal fluxes inside and through the plant (Hall 2002). It has been proposed that tolerance to metals may be a widespread trait in *C. ladanifer*, on the basis of two evidences: (1) the multiple origin of metallicolous populations of *C. ladanifer*, with no genetic imprints (such as genetic bottlenecks or founder effects) related to soil type on chloroplast or nuclear markers (Quintela-Sabarís et al. 2010, 2012), (2) no differences between metallicolous and non-metallicolous populations in growth response to treatments with Co, Ni and Zn under hydroponic conditions (Quintela-Sabarís 2012). However, further studies are needed to determine if this apparent tolerance is based on plant mechanisms or whether it is mediated by metal-tolerant ectomycorrhizal fungi as advocated by Colpaert et al. (2011).

## Conclusion

Apart from clear morphological differences, molecular data support the entity of *C. ladanifer* as a true species, although the unresolved incongruence between phylogeographic data and the morphological subspecies deserves the development of new research on this topic. *C. ladanifer* also has a specific composition of its fragrant exudate, carotenoid derivative norisoprene 2,2,6-trimethylcyclohexanone having been proposed as an authenticity marker in case of using this species

for pharmacological or aromatic industries. Furthermore, in what regards chemical composition, *C. ladanifer* seems to show some variation at subspecies, varieties and even populations and at the individual level, which shows the need for research and selection of better subspecies, varieties and ecotypes in accordance to specific applications. Finally, evidence was given for the extreme adaptation of *C. ladanifer* to stressful conditions in Mediterranean region, such as the ability to survive in low hydric and high solar exposition conditions, presistence in poor and contaminated soils and growth inhibition of several other plants through the release of allelochemicals. Thus, finding new potential applications for this plant may contribute to enhance the economic dimension of derelict lands, such as mine tailings or poor agricultural Mediterranean areas.

**Author contribution statement** JRR, CQS and FD worked on taxonomy, phylogeny and phylogeography section. DFF, JRR, CQS and FD worked on morphology and distribution section. JLD, CQS and FD worked on reproductive biology section. DFF worked on chemical characterization section. DFF, JRR, JLD and CQS worked on competitive traits section. DFF, JRR, JLD, CQS and FD gathered the information and wrote and edited the manuscript. JCG promoted, revised and approved the work.

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