

Molecular systematics, character evolution, and pollen morphology of *Cistus* and *Halimium* (Cistaceae)

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Abstract Pollen analysis and parsimony-based phylogenetic analyses of the genera *Cistus* and *Halimium*, two Mediterranean shrubs typical of Mediterranean vegetation, were undertaken, on the basis of cpDNA sequence data from the *trnL-trnF*, and *trnS-trnG* regions, to evaluate limits between the genera. Neither of the two genera examined formed a monophyletic group. Several monophyletic clades were recognized for the ingroup. (1) The “white and whitish pink *Cistus*”, where most of the *Cistus* sections were present, with very diverse pollen ornamentations ranging from striato-reticulate to largely reticulate, sometimes with supratectal elements; (2) The “purple pink *Cistus*” clade grouping all the species with purple pink flowers belonging to the *Macrostyliia* and *Cistus* sections, with rugulate or microreticulate pollen. Within this clade, the pink-flowered endemic Canarian species formed a monophyletic group, but with weak support. (3) Three *Halimium* clades were recovered, each with 100% bootstrap support; all *Halimium* species had striato-reticulate

pollen. Two *Halimium* clades were characterized by yellow flowers, and the other by white flowers.

Keywords *TrnL-F* · *TrnS-G* · Pollen · Exine · Cistaceae · *Cistus* · *Halimium*

Introduction

Specialists on the Cistaceae usually acknowledge eight genera for this family (Arrington and Kubitzki 2003; Dansereau 1939; Guzmán and Vargas 2009; Janchen 1925): *Cistus*, *Crocanthemum*, *Fumana*, *Halimium*, *Helianthemum*, *Hudsonia*, *Lechea* and *Tuberaria* (*Xolantha*). Two of these, *Lechea* and *Hudsonia*, occur in North America, and *Crocanthemum* is present in both North America and South America. The other genera are found in the northern part of the Old World. The two shrubby genera of the family, *Cistus* (22 species) and *Halimium* (nine to 14 species), are restricted to the Mediterranean basin and are mainly found in open vegetation (matorral) (Dansereau 1939). They are both sun lovers and are large to small shrubs, reaching up to 3 m in height (*Cistus ladanifer* L.).

Cistus and *Halimium* are recognized as distinct by most botanists, although they share some characters, for example chromosome number ($2n = 18$), which is different from all the other genera of the family (*Helianthemum* $2n = 20-24$; *Tuberaria* $2n = 14$; *Fumana* $2n = 32$; *Xolantha* $2n = 36$; *Crocanthemum* $2n = 20$ in Demoly and Montserrat 1993). They hybridize in the wild and in cultivation, making genus delimitation more tedious. Intergeneric hybrids have been described: $\times$ *Halimiocistus revolii* (Coste) Dans., with two nothosubspecies, $\times$ *H. sahucii* (Coste and Soulié) Janch., and $\times$ *H. humilis* Demoly, and a fourth has been reported (*Cistus* $\times$ *heterogenus* Bornet, n.n.) (Demoly 1998). Some

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intrageneric hybrids of *Cistus* are more difficult to obtain artificially than the intergeneric hybrids (Demoly 1996).

Classification of these two genera has mainly been based on morphological characters. *Cistus* has pink or white flowers with five locules in each ovary (except for *Cistus ladanifer* which has 6–12). *Halimium* has yellow or white flowers with three locules in each ovary. Nevertheless, white flowers and four locules in each ovary are found in both genera, as shown by Demoly (1998). Moreover, the number of sepals can be variable in both genera. Pink-flowered *Cistus* species, which were considered by Dansereau (1939) as “basal”, generally have five large sepals (with the exception of Canarian species). Other species in *Cistus* or *Halimium* have either three large sepals or 3 + 2 sepals (three large and two small). All these distinctive characters sometimes overlap and transitions between the two genera are almost continuous. Guzmán and Vargas (2005) proposed a phylogenetic hypothesis for 20 species of Cistaceae based on plastid and nuclear DNA sequence data. More recently, they conducted a total evidence analysis combining nuclear and plastid DNA sequences to assess the adaptive radiation of Mediterranean *Cistus* (Guzmán et al. 2009). In a third paper, Guzmán and Vargas (2009) reconstructed the first phylogeny comprising a representative sample of all known Cistaceae genera using sequences of plastid DNA. Guzmán and Vargas (2009), Nandi (1998), and Arrington and Kubitzki (2003) followed Ukrantseva’s Cistaceae pollen classification (1993), in which *Tuberaria*, *Halimium*, and *Cistus* share the “same pollen type”. Palynological studies of Cistaceae published by Ukrantseva in 1993 are mainly based on light microscopy from her previous work in Russian (Ukrantseva 1991), and on previous work by Kultina (in Russian; cited in Ukrantseva 1993). Because it involves work dealing with the whole family, her simplification, grouping *Tuberaria*, *Halimium*, and *Cistus* under the same pollen type, is understandable. One of her objectives was to provide tools for paleoecological or paleofloristic reconstructions of the past by paleoflorists (Ukrantseva 1993), but her classification is oversimplistic when dealing with Cistaceae species.

Pollen of the Cistaceae attracted the attention of botanists and paleoclimatologists long before Ukrantseva, as they are clearly typical of Mediterranean vegetation. Several palynologists have studied the pollen morphology of *Cistus* or of *Halimium* (Heydacker 1963; Jean and Pons 1962, 1963; Jiménez-Albarrán 1984; Marquez et al. 1996; Palacios-Chavez et al. 1999; Reille 1990; Saens de Rivas 1979; Ukrantseva 1993), but the number of species studied varied depending on the authors, from two (Heydacker 1963) to 17 (Saens de Rivas 1979). Strong contradictions have been encountered in the literature for pollen size and exine thickness (mainly between Saens de Rivas 1979; Jean and Pons 1963 and Ukrantseva 1991, 1993) and there

is no complete study of these two genera. In our study, we examined all the available species of *Cistus* and *Halimium* in order to evaluate palynological characters (Table 1).

Non-coding chloroplast regions are another source of information for evaluating relationships between closely-related taxa. They display higher levels of variation than coding regions and have been used extensively for lower taxonomic studies (Borsch and Quandt 2009; Clegg and Zurawski 1992; Downie and Palmer 1992; Mort et al. 2007; Shaw et al. 2005; 2007). Mapping characters on to a molecular phylogeny provides insights into patterns of character evolution independently of the characters themselves.

The *trnL-trnF* region (hereafter *trnL-F*) is one of the most frequently used molecular markers in phylogenetic reconstruction (Borsch and Quandt 2009; Quandt et al. 2004; Shaw et al. 2005; Taberlet et al. 1991). It consists of two transfer RNA genes, *trnL*_{UAA} *trnF*_{GAA}, separated by non-coding regions: the spacer *trnL-trnF*, and the *trnL* intron, the latter being the only group I intron in the plastid genome of land plants (Borsch and Quandt 2009). The *trnL* intron is generally more conserved than the *trnL-F* spacer and, for that reason, it is assumed to structure the topology, whereas the relatively high variation of the *trnL-F* spacer is assumed to resolve inter-specific relationships (Borsch and Quandt 2009; Lahaye et al. 2007).

We also used a second region to increase the robustness of the molecular phylogeny by including the *trnS*_{GCU}-*trnG*_{UCC} intergenic region (hereafter *trnS-G*), for which universal primers have been designed. This region is rapidly evolving and has been used to assess genetic variation within populations of *Corythophora* (Hamilton 1999). Gaskin and Schaal (2003) showed that in *Tamarix* the *trnS-G* spacer can be five times more variable than the *trnL-trnF* spacer and thus may provide more variable characters than even ITS. Unfortunately, this high level of variation can render some sequences unalignable between distant genera within the same family (Olson 2002; Shaw et al. 2005) and they are sometimes difficult to amplify (Shaw et al. 2005).

The objectives of this study were to assess phylogenetics relationships for *Cistus* and *Halimium*. Morphological and palynological characters were used to establish limits between the two genera and to provide new information on the evolutionary history of these genera in the Mediterranean region, and especially on Mediterranean islands where speciation of this group has occurred.

Materials and methods

Material

Samples for DNA, morphological, or pollen character studies were collected in the field or taken from herbarium

Table 1 Plant vouchers for DNA and pollen studies

Taxon	Origin	Pollen voucher	Pollen no.	DNA voucher	<i>trnS-G</i>	<i>trnL</i>	<i>trnL-F</i>
<i>Cistus albidus</i> L.	France	Civeyrel no. 1192	332	Civeyrel no. 1191	This study	This study	This study
<i>Cistus albidus</i> L. f. <i>subalbus</i> (Dun.) Dans.	Cultivated	Demoly no. 69	363				
<i>Cistus asper</i> Demoly & Mesa	Canary Is H	Demoly no. 1931	1931	Demoly no. 1934	This study	This study	This study
<i>Cistus chinamadensis</i> ssp. <i>chinamadensis</i> Bañares & Romero	Canary Is T	Demoly no. 4 18/05/1995 cult CBN Brest	391	Demoly no. 1211	This study	This study	This study
<i>Cistus chinamadensis</i> ssp. <i>gomeræ</i> Bañares & Romero	Canary Is G	Demoly no. 6 23/05/1995 cult CBN Porq.	390	Demoly no. 907	This study	This study	This study
<i>Cistus chinamadensis</i> ssp. <i>ombriosus</i> Demoly & Marrero	Canary Is H	Demoly no. 1924	1924	Demoly no. 1922	This study	This study	This study
<i>Cistus clusii</i> Dunal	Morocco	Faure sn 30/05/1931 (K)	330	Civeyrel no. 1447	This study	This study	This study
<i>Cistus clusii</i> ssp. <i>multiflorus</i> Demoly	Spain	Charpin sn 9/06/1971 (G)	365				
<i>Cistus clusii</i> ssp. <i>multiflorus</i> Demoly	Balearic Is Mall	D'en Rabassa no. 10 (G)	366				
<i>Cistus creticus</i> L.	Greece			Demoly no. 2584	This study	This study	This study
<i>Cistus creticus</i> L.	Morocco	Demoly no. 10	359				
<i>Cistus creticus</i> L. (= <i>C. villosus</i> L.)	Yugoslavia	Kew no. 51843 (K)	288				
<i>Cistus creticus</i> L. grp <i>corsicus</i>	France Corsica	Collet sn April 1899 (K)	289				
<i>Cistus creticus</i> L. grp <i>tauricus</i> (= <i>C. villosus</i> L.)	Greece	Mattfeld sn 2/07/1926 (K)	280				
<i>Cistus creticus</i> L. grp <i>tauricus</i> (= <i>C. villosus</i> L.)	Crimea	Davis no. 33102 (K)	287				
<i>Cistus crispus</i> L.	France	Civeyrel sn 16/5/1993	336	Civeyrel no. 336	This study	This study	This study
<i>Cistus crispus</i> L.	France	Gauthier sn 20/05/1894 (MPU)	326				
<i>Cistus crispus</i> L.	Spain	Sennen no. 235 (MPU)	327				
<i>Cistus heterophyllus</i> Desf.	Morocco			Civeyrel no. 1460	This study	This study	This study
<i>Cistus heterophyllus</i> Desf.	Algeria	Bove sn April/1839 (K)	314				
<i>Cistus heterophyllus</i> Desf. ssp. <i>carthaginensis</i> (Pau) Crespo & Mateo	Spain	Demoly no. 8 14/5/1990	281				
<i>Cistus horrens</i> Demoly	Canary Is G C	Demoly no. 2218	2218	Demoly no. 2218	This study	This study	This study
<i>Cistus horrens</i> Demoly	Canary Is G C	Lowe no. 38 bis (K)	301				
<i>Cistus inflatus</i> Pourr. ex Demoly	Spain		323	Civeyrel no. 1456	This study	This study	This study
<i>Cistus inflatus</i> Pourr. ex Demoly	France	G. Hibon no. 435 (P)	273				
<i>Cistus inflatus</i> Pourr. ex Demoly	Spain	Bourgeau sn 07/6/1863 (K)	320				
<i>Cistus inflatus</i> Pourr. ex Demoly	Spain	Durieu no. 398 (K)	321				
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	Spain			Demoly no. 2582	This study	This study	This study
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i>	France	Demoly no. 125	333				
<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i> f. <i>albiflorus</i> (Dun.) Dans.	France	Demoly no. 126	334				

Table 1 continued

Taxon	Origin	Pollen voucher	Pollen no.	DNA voucher	<i>trnS-G</i>	<i>trnL</i>	<i>trnL-F</i>
<i>Cistus ladanifer</i> L. ssp. <i>mauritanus</i> Pau & Sennen (= <i>Cistus ladanifer</i> L. var. <i>petiolatus</i> Maire)	Morocco			Civeyrel no. 1462 Demoly no. 2583	This study	This study	This study
<i>Cistus ladanifer</i> L. ssp. <i>mauritanus</i> Pau & Sennen (= <i>Cistus ladanifer</i> L. var. <i>petiolatus</i> Maire)	Spain	Demoly no. 128	282				
<i>Cistus ladanifer</i> L. ssp. <i>mauritanus</i> Pau & Sennen (= <i>Cistus ladanifer</i> L. var. <i>petiolatus</i> Maire)	Algeria	Faure sn 16/04/32 (K)	307				
<i>Cistus ladanifer</i> L. var. <i>sulcatus</i> Demoly (= <i>C. palhinhae</i> Ingram)	Portugal			Demoly no. 1788	This study	This study	This study
<i>Cistus ladanifer</i> L. var. <i>sulcatus</i> Demoly (= <i>C. palhinhae</i> Ingram)	Cultivated	Ingram sn 02/06/1949 (K)	305				
<i>Cistus laurifolius</i> L.	France	Demoly no. 112	361	Demoly no. 638	This study	This study	This study
<i>Cistus laurifolius</i> L.	Turkey	Civeyrel no. 1182	313				
<i>Cistus laurifolius</i> L. ssp. <i>atlanticus</i> (Pitard) Sennen & Mauricio	Morocco	Crookhank no. 38 (K)	308	Demoly no. 124	This study	This study	This study
<i>Cistus libanotis</i> (auct. non L.) var. <i>sedjera</i> (Pomel) Dans.	Algeria	Letourneux sn 30 3 1862 (P)	274				
<i>Cistus libanotis</i> L.	Spain	Bourgeau sn 01/04/1850 (K)	315	Civeyrel no. 1464	This study	This study	This study
<i>Cistus libanotis</i> L. f. <i>major</i> n.n.	Cultivated	Demoly no. 136	357				
<i>Cistus monspeliensis</i> L.	France	Demoly no. 89	331	Civeyrel no. 331	This study	This study	This study
<i>Cistus monspeliensis</i> L.	Canary Is P	Sprague & Hutchinson no. 196 (K)	303				
<i>Cistus monspeliensis</i> L.	France Oléron Is	Demoly sn 07/05/1994	375				
<i>Cistus munbyi</i> aff.	Morocco			Demoly no. 2073	This study	This study	This study
<i>Cistus munbyi</i> Pom.	Algeria	Faure sn 10/05/1931 (K)	309				
<i>Cistus ochreateus</i> Chr. Sm.	Canary Is G C	Demoly no. 2248	2248	Demoly no. 1709			
<i>Cistus ochreateus</i> Chr. Sm.	Canary Is G C			Demoly no. 1717	This study		
<i>Cistus ochreateus</i> Chr. Sm.	Canary Is G C	Austin no. M3 25/7/1960 (K)	311			This study	This study
<i>Cistus osbeckiifolius</i> ssp. <i>tomentosus</i> Bañares & Demoly	Canary Is T			Demoly no. 1957		This study	This study
<i>Cistus osbeckiifolius</i> ssp. <i>tomentosus</i> Bañares & Demoly	Canary Is T	Demoly no. 2494	2494	Demoly no. 2480	This study		
<i>Cistus osbeckiifolius</i> Webb. ex Christ.	Canary Is T	Demoly sn 28/04/1993	341	Demoly no. 1210	This study	This study	This study
<i>Cistus osbeckiifolius</i> Webb. ex Christ.	Canary Is C	Bramwell no. 2101 (K)	310				
<i>Cistus palmensis</i> Bañares & Demoly	Canary Is P	Demoly no. 1989	1989	Demoly no. 1908	This study	This study	This study
<i>Cistus parviflorus</i> Lam.	Crete			Civeyrel no. 1446	This study	This study	This study
<i>Cistus parviflorus</i> Lam.	Greece	Atchley sn 1932 (K)	316				

Table 1 continued

Taxon	Origin	Pollen voucher	Pollen no.	DNA voucher	<i>trnS</i> -G	<i>trnL</i>	<i>trnL</i> -F
<i>Cistus parviflorus</i> Lam.	Greece	Atchley no. 677 (K)	317				
<i>Cistus parviflorus</i> Lam.	Greece	Gandoger no. 7696 (K)	322				
<i>Cistus parviflorus</i> Lam.	Turkey	Balansa no. 87 (MPU)	360				
<i>Cistus parviflorus</i> Lam. (= <i>C. asperrimus</i>)	Cyprius	Tracey no. 36 (K)	312				
<i>Cistus populifolius</i> L. ssp. <i>major</i> (Dun.) Heywood	Spain			Civeyrel no. 1454	This study	This study	This study
<i>Cistus populifolius</i> L. ssp. <i>major</i> (Dun.) Heywood	Morocco	Trettewy no. 448 (K)	306				
<i>Cistus populifolius</i> L. ssp. <i>major</i> (Dun.) Heywood	Spain	Ellman & Hubbard no. 611 (K)	318				
<i>Cistus populifolius</i> L. ssp. <i>populifolius</i>	Cultivated	Demoly no. 111	338				
<i>Cistus populifolius</i> L. ssp. <i>populifolius</i>	France			Civeyrel no. 1459	This study	This study	This study
<i>Cistus populifolius</i> L. ssp. <i>populifolius</i> (= <i>C. narbonensis</i> Rouy & Foucaud)	Portugal	Bourgeau no. 1778 (P)	272				
<i>Cistus populifolius</i> L. ssp. <i>populifolius</i> (= <i>C. narbonensis</i> Rouy & Foucaud)	France	Schultz no. 19265 1884 (P)	276				
<i>Cistus pouzolzii</i> Delile	France	Delille sn June 1837 (MPU)	350	Civeyrel no. 1461	This study	This study	This study
<i>Cistus pouzolzii</i> Delile	Morocco	Maire sn (MPU)	351				
<i>Cistus salviifolius</i> L.	France	Civeyrel sn 16/05/93	335	Civeyrel no. 335	This study	This study	This study
<i>Cistus salviifolius</i> L.	France Corsica	Demoly sn 3/05/1994	383				
<i>Cistus sintenisii</i> de Lit.	Albania	Demoly no. 84	356	Demoly no. 84	This study	This study	This study
<i>Cistus sintenisii</i> de Lit.	Albania	Civeyrel no. 1463	325				
<i>Cistus symphytifolius</i> Lam.	Canary Is T			Civeyrel no. 1453	This study	This study	This study
<i>Cistus symphytifolius</i> Lam.	Canary Is T	De La Perraudière sn 27/05/1855 (K)	329				
<i>Cistus symphytifolius</i> Lam. (Pico de Cabras)	Canary Is T	Demoly no. 2462	2462	Demoly no. 2463	This study	This study	This study
<i>Cistus symphytifolius</i> Lam. (Punta Gorda)	Canary Is P			Demoly no. 1832	This study		
<i>Cistus symphytifolius</i> Lam. (Punta Gorda)	Canary Is P	Demoly no. 1851	1851	Demoly no. 1845		This study	This study
<i>Cistus symphytifolius</i> Lam. var. <i>canus</i> Demoly	Canary Is P			Demoly no. 882	This study		
<i>Cistus symphytifolius</i> Lam. var. <i>canus</i> Demoly	Canary Is P			Demoly no. 2444		This study	This study
<i>Cistus symphytifolius</i> Lam. var. <i>canus</i> Demoly	Canary Is P	Demoly no. 2023	2023				
<i>Cistus symphytifolius</i> Lam. var. <i>villosus</i> Demoly	Canary Is T			Demoly no. 1952	This study		
<i>Cistus symphytifolius</i> Lam. var. <i>villosus</i> Demoly	Canary Is T	Demoly no. 2319	2319	Demoly no. 1954		This study	This study

Table 1 continued

Taxon	Origin	Pollen voucher	Pollen no.	DNA voucher	<i>trnS-G</i>	<i>trnL</i>	<i>trnL-F</i>
<i>Fumana ericoides</i> Pau subsp. <i>montana</i> (Pomel) Güemes & Muñoz	France			Civeyrel sn 27/06/2005	This study	This study	This study
<i>Halimium antiatlanticum</i> Maire & Wilczek	Morocco	Maire sn (MPU)	271				
<i>Halimium antiatlanticum</i> Maire & Wilczek	Morocco	Maire no. 173 (MPU)	342				
<i>Halimium antiatlanticum</i> Maire & Wilczek	Morocco	Maire sn 08/04/1935 (MPU)	343				
<i>Halimium atlanticum</i> Humb. & Maire	Morocco			Civeyrel no. 1587	This study	This study	This study
<i>Halimium atlanticum</i> Humb. & Maire	Morocco	De Wilde & al. no. 2951 (P)	275	Civeyrel no. 1595	This study	This study	This study
<i>Halimium atriplicifolium</i> (Lam.) Spach	Spain	Brummitt et al. no. 5943 (K)	290	Civeyrel no. 1450	This study	This study	This study
<i>Halimium calycinum</i> (L.) K.Koch (= <i>H. commutatum</i> Pau)	Portugal			Civeyrel no. 1451	This study	This study	This study
<i>Halimium calycinum</i> (L.) K.Koch (= <i>H. commutatum</i> Pau)	Spain	Belmonte et al. sn 16/04/1982 (K)	299				
<i>Halimium halimifolium</i> (L.) Willk.	Portugal	Sauvage no. 157 (MPU)	349				
<i>Halimium halimifolium</i> f. <i>coronatum</i> Sennen	Morocco			Civeyrel no. 1457	This study	This study	This study
<i>Halimium halimifolium</i> f. <i>lasiocalycinum</i> (Boiss. & Reut.) Gross.	Morocco	Maire sn 12/06/1928 (MPU)	346				
<i>Halimium halimifolium</i> f. <i>lasiocalycinum</i> (Boiss. & Reut.) Gross.	Morocco	Maire sn 25/04/1924 (MPU)	347				
<i>Halimium halimifolium</i> ssp. <i>halimifolium</i> (L.) Willk	Morocco	Maire sn 06/04/1921 (MPU)	344				
<i>Halimium halimifolium</i> ssp. <i>multiflorum</i> auct. non Salzm.	Morocco	Demoly no. 1045	1045	Demoly no. 1045	This study	This study	This study
<i>Halimium halimifolium</i> ssp. <i>multiflorum</i> auct. non Salzm.	Morocco	Maire sn 21/06/1930 (MPU)	345				
<i>Halimium lasianthum</i> (Lam.) Spach var. <i>formosum</i> (Curt.) Gross.	Portugal			Civeyrel no. 1465	This study	This study	This study
<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Greuter & Burdet (= <i>H. alyssoides</i> (Lam.) Koch)	France			Demoly no. 2080	This study	This study	This study
<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Greuter & Burdet	Portugal	Vertcourt no. 4396 (2) (K)	291				
<i>Halimium lasianthum</i> ssp. <i>lasianthum</i> (Lam.) Spach	Spain	Breckle no. 1008 (K)	295				
<i>Halimium lasianthum</i> ssp. <i>lasianthum</i> (Lam.) Spach	Spain	Lindberg sn 10/04/1926 (K)	296				
<i>Halimium lasianthum</i> ssp. <i>lasianthum</i> (Lam.) Spach	Spain	Demoly sn 25/05/1995	394				
<i>Halimium lasiocalycinum</i> ssp. <i>rhypaeum</i> (Pau & Font Quer) Maire	Morocco	Dr. Font Quer no. 406 (G)	388				

Table 1 continued

Taxon	Origin	Pollen voucher	Pollen no.	DNA voucher	<i>trnS-G</i>	<i>trnL</i>	<i>trnL-F</i>
<i>Halimium lasiocalycinum</i> ssp. <i>rhypaeum</i> var. <i>elatum</i> Pau & Font Quer	Morocco			Civeyrel no. 1583	This study	This study	This study
<i>Halimium lasiocalycinum</i> ssp. <i>rhypaeum</i> var. <i>elatum</i> Pau & Font Quer	Morocco	Maire sn 24/06/1926 (MPU)	348				
<i>Halimium ocymoides</i> (Lam.) Willk.	Spain			Civeyrel no. 1445	This study	This study	This study
<i>Halimium ocymoides</i> (Lam.) Willk. f. <i>elongatum</i> (Vahl.) Gross.	Spain	Bourgeau no. 240 (K)	292				
<i>Halimium ocymoides</i> (Lam.) Willk. f. <i>sampsucifolium</i> (Cav.) Gross.	Portugal	Atchley no. 127 (K)	293				
<i>Halimium umbellatum</i> (L.) Spach	France			Demoly no. 641	This study	This study	This study
<i>Halimium umbellatum</i> (L.) Spach	France	Urgel no. 51906 (K)	298				
<i>Halimium umbellatum</i> (L.) Spach (= <i>H. verticillatum</i> (Brot.) Sennen)	Portugal			Demoly no. 1356	This study	This study	This study
<i>Halimium umbellatum</i> (L.) Spach f. <i>syriacum</i> (Boiss.) Willk.	Lebanon	Gombault no. 4274 (P)	278				
<i>Halimium umbellatum</i> (L.) Spach ssp. <i>viscosum</i> (Willk.) Bolos & Vigo	Algeria	Bourgeau sn (P)	277				
<i>Halimium umbellatum</i> (L.) Spach ssp. <i>viscosum</i> (Willk.) Bolos & Vigo (= <i>H. viscosum</i> (Willk.) P. Silva)	France			Civeyrel no. 1449	This study	This study	This study
<i>Halimium umbellatum</i> (L.) Spach ssp. <i>viscosum</i> (Willk.) Bolos & Vigo (= <i>H. viscosum</i> (Willk.) P. Silva)	France	Demoly no. 164	392				
<i>Halimium voldii</i> Kit Tan, Perdetzoglou & Raus	Greece	Demoly no. 2571	2571	Demoly no. 2571	This study	This study	This study
<i>Helianthemum caput-felis</i> Boiss.	Morocco			Civeyrel no. 1455	This study	This study	This study
<i>Helianthemum oelandicum</i> (L.) DC. subsp. <i>incanum</i> (Willk.) López (= <i>H. canum</i> (L.) Hornem.)	France			Civeyrel no. 1192	This study	This study	This study
<i>Helianthemum syriacum</i> (Jacq.) Dum.-Cours.	Spain			Civeyrel no. 1466	This study	This study	This study
<i>Muntingia calabura</i> L.	Porto-Rico			Chase 346 (K)	This study	This study	This study
<i>Tuberaria guttata</i> (L.) Raf.	France			Civeyrel no. 1194	This study	This study	This study

Abbreviations for the Canary Islands: G, La Gomera; G C, Gran Canaria; H, El Hierro; P, La Palma; T, Tenerife

specimens (from G, K, MPU and P herbaria). When possible, we sampled recognized sub-species or varieties, or strongly isolated populations. The list of samples is given in Table 1 with the reference voucher and geographical origin.

Samples used for pollen or DNA studies, voucher specimens, and sources are listed in Table 1. All the 106 sequences of Cistaceae were produced for this study. For *trnL-F*, all available species or subspecies of *Cistus* and *Halimium* and representatives of the other European genera

of Cistaceae were included and constituted the ingroup, with *Muntingia calabura* L. as the outgroup. The genus *Muntingia* appeared as one of the sister taxa of Cistaceae (Alverson et al. 1998, fig. 3, p. 880). It was also included in the outgroups in Guzmán and Vargas (2009). It shares some characters with the Cistaceae, for example crumpled petals in buds (Bayer et al. 1998). *Muntingia* was unalignable for *trnS*-G, so we used our *trnL*-F analyses and the results of Guzmán and Vargas (2009) to select *Fumana ericoides* Pau subsp. *montana* (Pomel) Güemes and Muñoz as the outgroup to root the trees for the combined analysis.

Methods

Palynology

Pollen grains were removed from anthers and acetolysed according to Erdtman's (1960) standard acetolysis method. For electron microscopy, pollen grains were air dried on SEM stubs from 100% ethanol and coated with platinum by use of an SPD 050 Balzers sputter-coater. They were examined with an Hitachi S-2400 scanning electron microscope (SEM) at the Royal Botanic Gardens, Kew. Exine ornamentation and structure were described from SEM images at the same magnification (15,000 $\times$) on the same region between two apertures on the equator. For light microscopy, pollen grains were mounted in glycerol jelly sealed with paraffin and observed with an optical microscope. The type and number of apertures were examined by light and electron microscopy. We considered three palynological characters for this study: pollen shape,

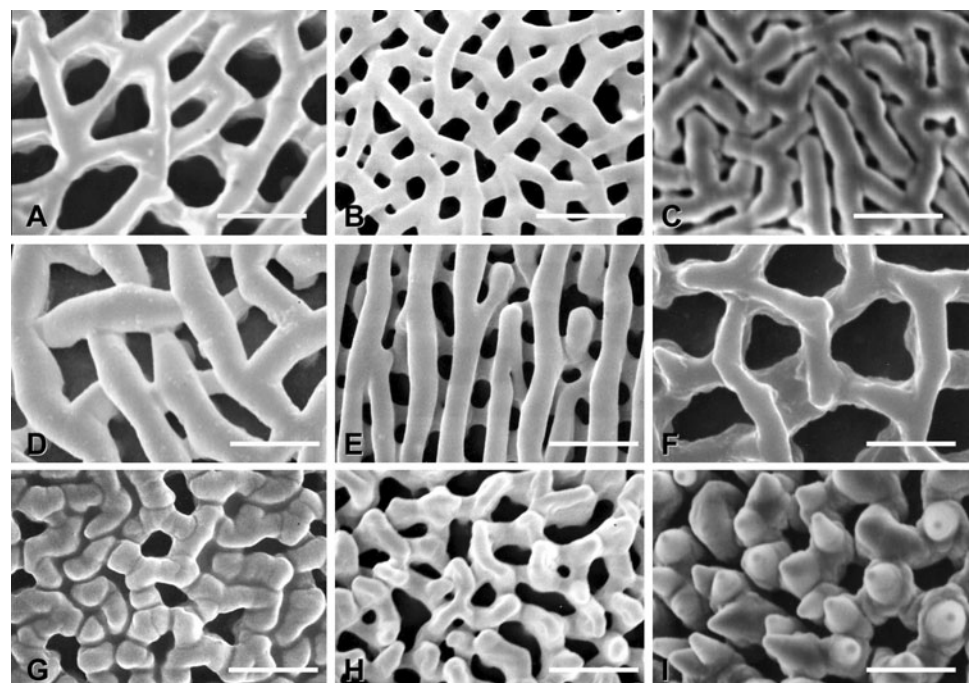
exine thickness, and exine sculpture. Character states were defined according to reference works in pollen terminology (Punt et al. 1994, 2007; Hesse et al. 2009; <http://www.palдат.org/> 2010) and completed by personal observations.

Pollen shape terminology was defined by Erdtman (1943, 1952), and shape classes were based on the relationship *P/E* between the polar axis (*P*) and the equatorial diameter (*E*); they were determined on 25 pollen grains for each sample (when possible).

Exine thickness was also measured on 25 pollen grains for each sample. Thickness states are quantitative characters and it was sometimes difficult to define limits in a continuous dataset. We considered the presence of consequent gaps within the numerical order of data and reduced it to four states <2.5 μm , between 2.5 and 2.8 μm , between 2.8 and 3.15 μm and over 3.15 μm . Exine thickness was somehow related more to exine ornamentation than to pollen size.

In the literature, interpretation of the exine sculpture of Cistaceae varies from one author to another, maybe because of misinterpretation, so for this study we redefined the terms we used. Exine is *reticulate* when muri form a network-like pattern with lumina wider than 1 μm (Fig. 1a), and is *microreticulate* if the lumina and the muri are less than 1 μm , and equivalent in size (Fig. 1b) (Punt et al. 2007; Hesse et al. 2009). There has been some confusion in the literature of Cistaceae between microreticulate and rugulate ornamentation. For example *Cistus albidus* (Fig. 1c) is described as rugulate by Jean and Pons (1963), and microreticulate by Reille (1990). *Rugulate* exine, defined by Iversen and Troels Smith (1950),

Fig. 1 SEM micrographs of exine surface ornamentation types, all at identical magnification (originally $\times 15,000$). **a** *Cistus laurifolius* 313, reticulate exine. **b** *C. heterophyllus* subsp. *carthaginensis* 281, microreticulate exine. **c** *C. albidus* 332, rugulate exine. **d** *Halimium lasianthum* ssp. *lasianthum* 296, striato-reticulate exine. **e** *H. calycinum* 299, striato-reticulate exine. **f** *C. populifolius* var. *populifolius* 306, largely reticulate exine. **g** *C. monspeliensis* 303, micro-reticulate with smooth supratectal ornamentation. **h** *C. inflatus* 273, micro-reticulate with smooth supratectal ornamentation. **i** *C. salvifolius* 335, microechinate ornamentation. All scale bars = 2 μm



describes a type of “ornamentation of elongated muri more than 1 μm long intermediate between reticulate and striate”. We would add here, to complete that definition, that the lumina should be much smaller on average than the muri (Fig. 1c). *Striate* ornamentation of exine has been described for some of our taxa (Saens de Rivas 1979), but again with some confusion. For example, pollen of *Halimium umbellatum* (Table 3; Fig. 5i) has been described as striato-reticulate by Márquez et al. (1996), reticulate by Jiménez-Albarrán (1984) and striate by Saens de Rivas (1979). The definition of *striate* ornamentation given by Iversen and Troels-Smith (1950) in Punt et al. (2007) describes an exine with “*elongated and generally parallel elements separated by grooves*”. This is close to the definition given for the term *rugulate*, and to distinguish between the two ornamentations we complete this definition by: “elements (lumina and muri) should be more than 1 μm wide on average”. We have already given a definition of *reticulate*, and things would have remained simpler without the term *striato-reticulate*, which somewhat confuses any interpretation. Erdtman (1952) describes a *striato-reticulate* exine as “a pattern in which parallel or subparallel muri are cross-linked to form a reticulum in the grooves”. In the case of *Halimium umbellatum*, the exine is obviously striato-reticulate as interpreted by Márquez et al. (1996) (Fig. 5i).

Nevertheless, within the reticulate type of exine, muri and lumina can be approximately of the same size, but when the lumina is much wider than the muri (1.5 $\times$ wider), we used the term *largely reticulate* (Fig. 1a, f) as suggested by Reille (1990).

For some *Cistus* species, the exine has been described as retipilate (Saens de Rivas 1979). This word is used to describe a pattern of exine ornamentation when the reticulum is formed by a row of pila instead of muri (Erdtman 1952). Hesse et al. (2009) pointed out that there is no example of a reticulum formed by rows of pila instead of muri. Earlier observations were based on light microscopy (Hesse et al. 2009). Moreover, the four species examined in our study and described as being retipilate were basically reticulate, so we described the type of reticulation according to the size of the muri and lumina completed by the type of supratectal ornamentation. This gave us two more states, micro-reticulate with smooth supratectal ornamentation (Fig. 1g, h), or microechinate ornamentation (Fig. 1i).

Molecular techniques

DNA was extracted using fresh leaf tissues or dried material conserved in silica gel using a DNeasy Plant Mini Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. Double-stranded products of

CpDNA were amplified from total DNA using the universal primers designed by Hamilton (1999, *trnS-G*) or those of Taberlet et al. (*trnL-F*, 1991), and the PCR procedures given by the two authors (Hamilton 1999; Taberlet et al. 1991). PCR was performed in a 50 μL reaction mixture which contained 0.4 μM of each forward and reverse primer and 0.025 U μL^{-1} Taq-polymerase in 10 mM Tris-HCl pH 9, 50 mM KCl, 2.5 mM MgCl_2 , 0.1% Triton X100, and 0.2 mg mL^{-1} BSA. PCR amplifications were also carried out using 1–4 μL total DNA and the Master mix TAQ PCR Qiagen (Qiagen). PCR reactions were sent for purification and sequencing to Genome Express (Meylan, France). Sequences were edited, corrected and aligned using Sequencher 4.2.2 software (Gene Code Corporation, Ann Arbor, Michigan, USA). Consensus sequences were manually aligned in a matrix under Paup 4.0b10 software for Macintosh (Swofford 2002).

Analyses

For *trnL-F*, 54 sequences were analysed separately, and 52 for *trnS-G*. All but an average of the first 36 bases at the 5' end and 52 at the 3' end of *trnL-F*, and the first 40 bases at the 5' end and 54 at the 3' end of the of *trnS-G* were sequenced. Alignment was straightforward for *trnL-F*; the length of the individual sequences varied from 618 bp (incomplete sequence for *Cistus symphytifolius* PicoDC) to 736 bp for *trnL-F*, and from 250 bp (incomplete sequence for *Halimium umbellatum*) to 866 bp for *trnS-G*. The total length of the aligned matrix is given in Table 2.

Indels were found for *trnL-F* and *trnS-G*, and were quite variable in length (Table 2), often consisting of a repetition of short sequences of base pairs next to the indel itself (SSR: simple sequence repeat), or a short tandem repeat (STRs) or microsatellites (Borsch and Quandt 2009). Most of these indels can be phylogenetically informative. None of the indels were coded in the analysis, but inserted regions were retained and coded as missing. The total, matrix length, and number of informative characters are given in Table 2.

Maximum parsimony analyses of the sub-matrix were conducted separately for *trnL-F* and *trnS-G*. Two combined analyses were then performed. All analyses were implemented with PAUP 4.0b10 (Swofford 2002) with the options: unit weight, heuristic search, 1,000 replicates of random taxon-additions, and TBR swapping with ACC-TRAN optimization to save the shortest trees. Then, keeping all the shortest trees in memory, and with Mul-Trees on, an extensive search was conducted to find all the most parsimonious trees with a number limit of 20,000–50,000 trees. Successive approximation weighting (hereafter SW; Farris 1969), with characters reweighted according to their rescaled consistency index (RC) based

Table 2 Summary statistics for plastid DNA data analyses of phylogenetic relationships in *Cistus* and *Halimium*

Maximum parsimony analyses	<i>trnL-trnL-F</i>	<i>trnS-G</i>	<i>trnL-trnL-F</i> + <i>trnS-G</i> Combined molecular
Number of taxa x alignment length	54 × 833	52 × 1141	52 × 1922
Number of indels	28	36	54 (18 + 36)
Indels length	1–26	1–202	1–202
Phylogenetically informative characters (%)	102 (12.25%)	140 (12.27%)	226 (11.76%)
UW Number of shortest trees	>20,000	>20,000	>50,000
UW Tree length	331	491	725
UW Consistency index ^a	0.855 (0.7377)	0.8676 (0.7336)	0.8524 (0.7284)
UW Retention index	0.8797	0.8862	0.8863
UW Rescaled consistency ind.	0.7521	0.7689	0.7555
SW Number of shortest trees	>20,000	>20,000	>50,000
SW Tree length	256,697	390,418	567,752
SW Consistency index ^a	0.9544 (0.8923)	0.9665 (0.9087)	0.9602 (0.9046)
SW Retention index	0.9609	0.9684	0.9668
SW Rescaled consistency index	0.9171	0.9359	0.9283

UW, unit weight analyses; SW, successive weighting analysis

^a Without uninformative characters

on the best fit of characters on any of the trees, was conducted on the two combined analyses. The reasons for using SW were explained in a previous paper (Civeyrel et al. 1998). Re-weighting rounds were repeated until the tree length did not change in two consecutive iterations. The base weight of 1,000 applied in SW was removed for tree presentation. Confidence in specific clades of the resulting topology was estimated by bootstrap analysis. The settings used were: 1,000 replicates, keeping bootstrap frequencies from 50 to 100%, random addition of taxa, sampling characters with equal probability but applying weights (from SW), and TBR swapping (Tree Bisection and Reconnection) with 5 replications, but only keeping the optimum tree from each replicate, even if not optimum over all replicates. All illustrated trees used ACCTRAN optimization. We categorized bootstrap supports according to Kress et al. (2002) and considered strong for support >85%, moderate 70–85%, and weak 50–70%.

Results

Molecular results

DNA sequence summary

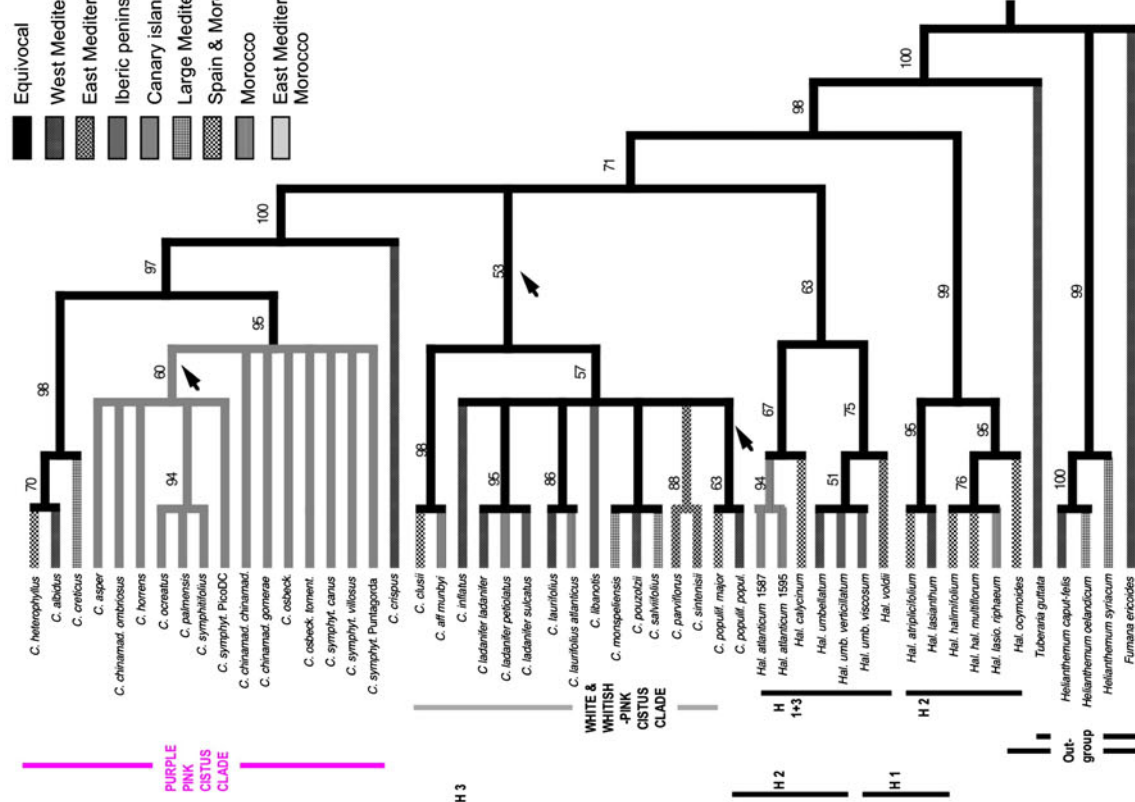
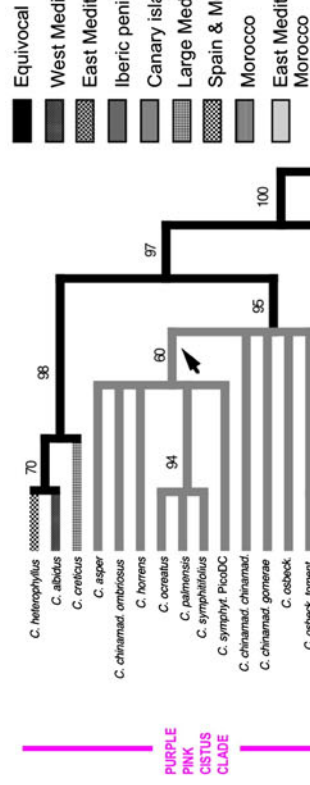
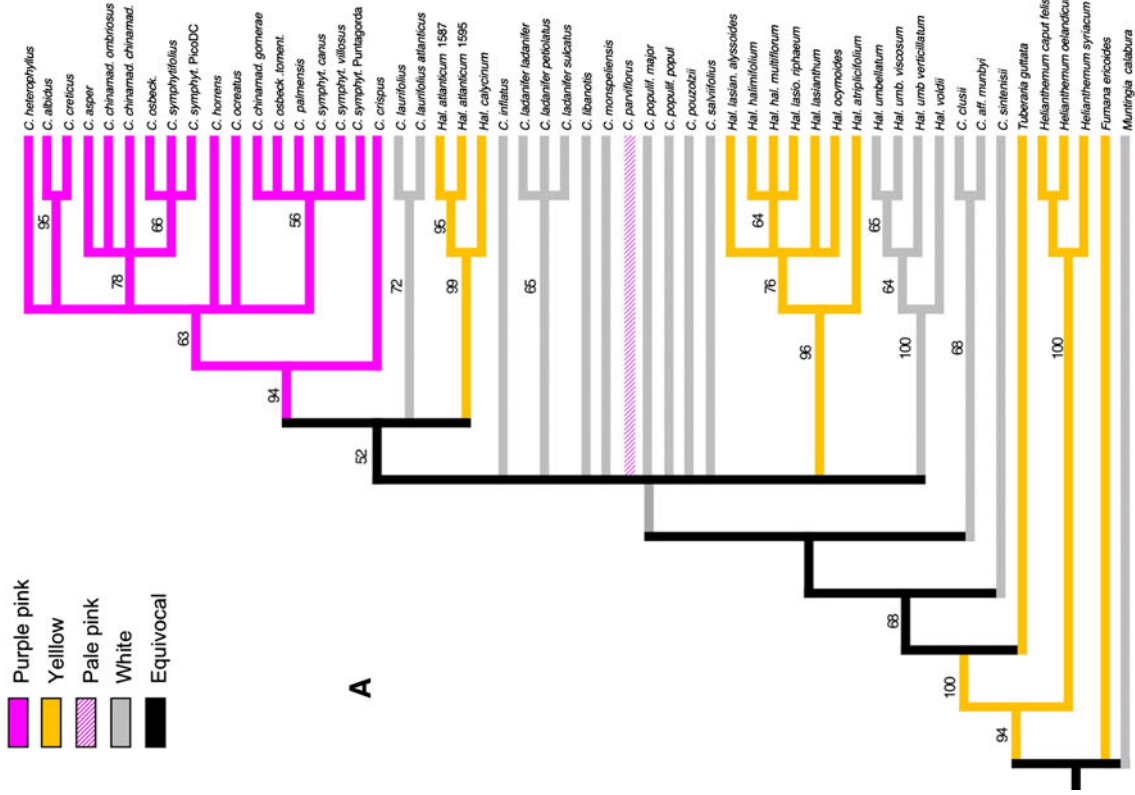
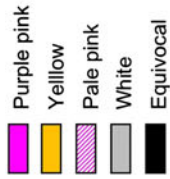
Table 2 provides a summary of statistics for the two molecular datasets. The aligned *trnS-G* data matrix contained more parsimony-informative characters (12.86% for

1141 bp) than the *trnL-F* region (12.25% for 833 bp) (Table 2). A parsimony analysis of the *trnL-F* dataset was conducted for 54 taxa. The removal of *Muntingia calabura* L. for the combined molecular analysis reduced the numbers of indels from 28 to 18, and the length of the aligned matrix from 833 to 780 bp. We removed a second taxon, *Halimium lasianthum* ssp. *alyssoides* (Lam.) Greuter & Burdet, that was too poor for *trnS-G*.

Parsimony analysis of the *trnL-F* matrix (54 taxa)

The parsimony analysis of the *trnL-F* matrix produced >20,000 trees with *Muntingia calabura* L. as the outgroup. The topology for the unit-weighted analysis (UW) was not different from the successive weighted analysis (SW), but the bootstrap supports were stronger. Only the former will be discussed. Flower colour defines groups quite well so we mapped this character on the strict consensus tree to help visualise groups to be discussed later (Fig. 2a). From the base to the top of the strict consensus tree, *Fumana ericoides* Pau subsp. *montana* (Pomel) Güemes and Muñoz

Fig. 2 a Complete *trnL-trnL-F* dataset, strict consensus tree of the 20,000 equally parsimonious trees from the successive weighting analysis. Petal colours are mapped on the tree to help visualise colour clades. **b** *trnS-G* dataset, strict consensus tree of the 20,000 equally parsimonious trees from the successive weighting analysis. Geographical origin is mapped on the branches. Numbers above branches are successive weighted bootstrap values. Branches not present in the strict consensus tree from the unit weight analysis are indicated with an arrow



formed a polytomy with *Muntingia*, followed by the three species of *Helianthemum* and *Tuberaria guttata* (L.) Raf. as the sister group of the *Cistus* and *Halimium* clades. The *Helianthemum* clade and the clade formed by *Tuberaria* with *Cistus* and *Halimium* were strongly supported by 94% bootstrap support (hereafter BS) and each of them by 100% BS. *Cistus sintenisii* de Lit. was sister to the rest of the ingroup, followed by a clade formed by *Cistus* aff. *munbyi* and *Cistus clusii* Dunal, supported by 100% BS. From the above we found a large polytomy including 2 separate clades grouping some of the *Halimium* species, H 1, with white flowers and H 2, with yellow flowers, all supported by very high BS. The following were an unresolved group of white or whitish pink (*Cistus parviflorus*) species and infraspecific taxa of *Cistus*, in which only subspecies of *C. ladanifer* L. were grouped together (Fig. 2a). At the top of the tree we found a polytomy grouping a third clade of *Halimium*, H 3 with yellow flowers, the two subspecies of *C. laurifolius* L. with white flowers, and the remaining *Cistus*, all with purple pink flowers. There was strong support for the “purple pink *Cistus* clade” with a BS of 94%. *Cistus crispus* L. was the sister taxon to a large *Cistus* polytomy with only one clade comprising *Cistus albidus* L. and *Cistus creticus* L., which was strongly supported, and three quite weakly supported clades mixing Canarian taxa and *Cistus heterophyllus*.

Parsimony analysis of the *trnS-G* matrix (52 taxa)

The parsimony analysis of the *trnS-G* matrix produced 20,000 trees (tree limit). We used the result of *trnL-F* to

select *Fumana ericoides* subsp. *montana* as the outgroup. The distribution of taxa was mapped on the tree according to the hot spot or floristic entities given in the distribution map (Fig. 3). The topology for the SW strict consensus tree was more resolved than that of UW, with three branches not present in the UW analysis and higher BS. Only the strict consensus tree from the SW is discussed (Fig. 2b). From the base to the top of the strict consensus tree we found the strongly supported clade of the three species of *Helianthemum* in polytomy with *Fumana*. *Tuberaria guttata* (L.) Raf. as the sister group of the *Cistus* and *Halimium* clades. For the ingroup, we only summarized the main differences between the *trnL-F* and *trnS-G* analyses. We found none of the *Cistus* present at the base of the tree from the *trnL-F* analyses, but instead we found the *Halimium* clades H 1 and H 3 and H 2, followed by a large group of *Cistus* with white or whitish pink flowers. This clade was also present in the UW analysis, but with the small clade grouping *Cistus* aff. *munbyi* and *Cistus clusii* in polytomy with the rest of the *Cistus*, and not apart as in the SW tree. Subspecies of *C. ladanifer* L., subspecies of *C. laurifolius* and a clade comprising *Cistus sintenisii* de Lit. and *Cistus parviflorus* Lam. had high BS. Two clades, the two subspecies of *C. populifolius* and a three-species clade comprising *Cistus monspeliensis* L., *Cistus salviifolius* L., and *Cistus pouzolzii* Delile, which were not present in the *trnL-F* analysis, had weak or no support at all (Fig. 2b). The purple pink *Cistus* clade was even better supported with a BS of 100%. *Cistus crispus* L. was again found at the base and sister to two strongly supported

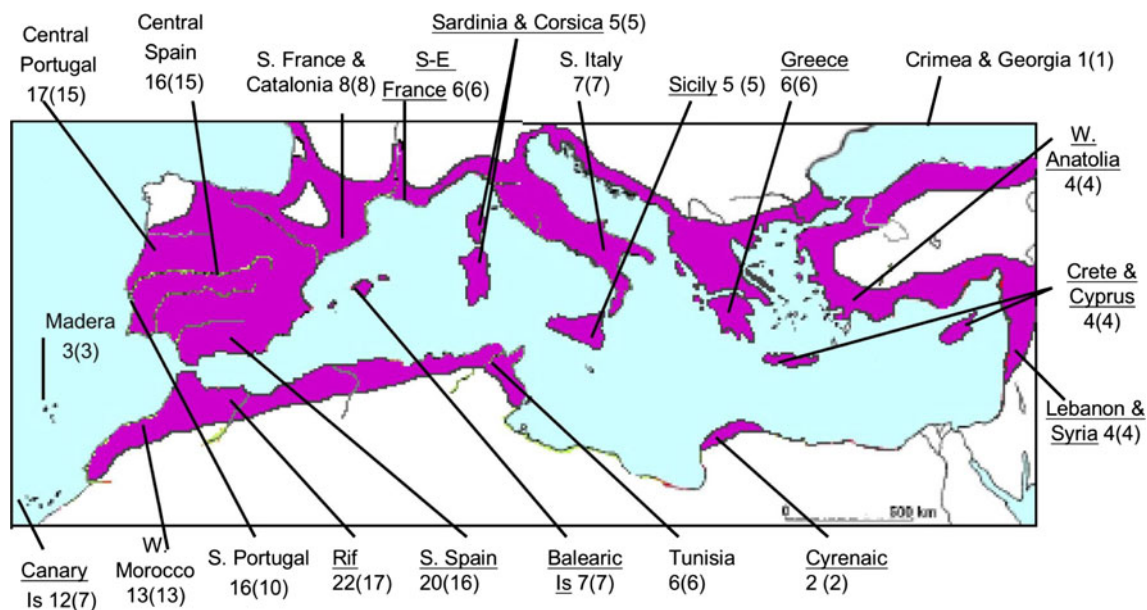


Fig. 3 Distribution map of the genera *Cistus* and *Halimium*. For each floristic entity the number of species, subspecies and varieties is given, followed by the number of species in brackets. Mediterranean

hot spots are underlined (from Médail and Quezel 1997). The distribution of species encompasses more or less the limits of the Mediterranean climate but goes further north

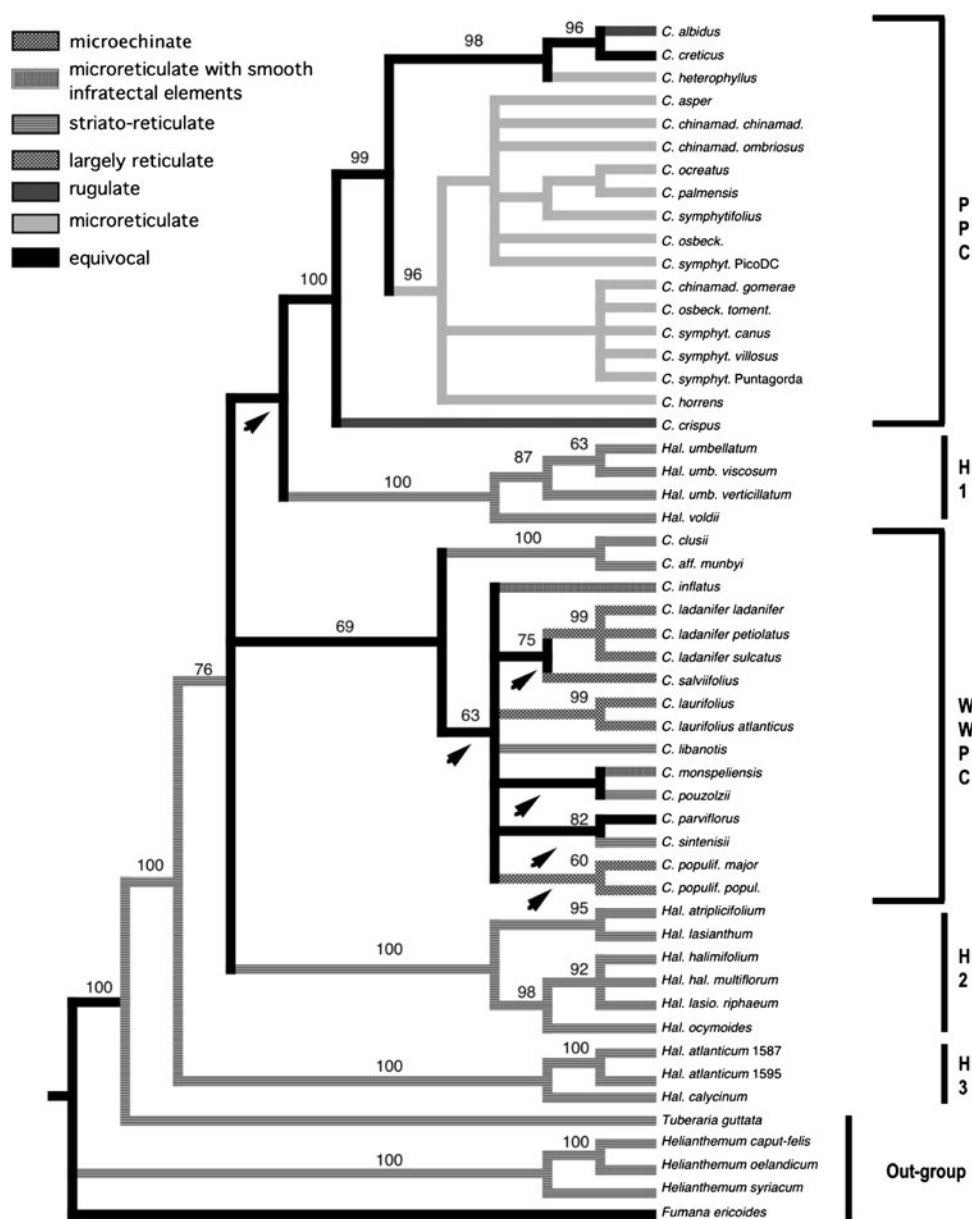
groups, *Cistus albidus* L., *Cistus creticus* L., and *Cistus heterophyllus* Desf. on one side, and a complex polytomy, with 14 Canarian taxa, on the other side (Fig. 2b).

Combined parsimony analysis of the *trnS*-G and *trnL*-F matrix (52 taxa)

The combined parsimony analysis of the *trnS*-G and *trnL*-F matrix produced more than 50,000 trees. *Fumana ericoides* subsp. *montana* was again used as the outgroup and to root the tree. The topology for the SW strict consensus tree was more resolved than that of the UW, with 6 branches not present in the UW analysis and higher BS, but in the UW analysis the H 2 clade was not in polytomy with the “white

and whitish pink *Cistus*”. However, because there was no BS for this branch in the UW, this did not really change the interpretation of the analysis much. Only the strict consensus tree from the SW is discussed (Fig. 4). At the base of the tree, the three species of *Helianthemum* were in polytomy with *Fumana*, and *Tuberaria guttata* (L.) Raf. was again the sister group of the *Cistus* and *Halimium* clades. The topology of the tree was a mixture of the two separate analyses but with stronger support on average. Each marker contributed to better resolution of the phylogeny. The three *Halimium* clades, H 1, H 2 and H 3, all had 100% BS, followed by the same large group of white and whitish pink *Cistus*. This former clade was very close to what was found in the *trnS*-G, but with the difference

Fig. 4 Combined parsimony analysis of the *trnS*-G and *trnL*-F matrix. Strict consensus tree of the 50,000 equally parsimonious trees from the successive weighting analysis. Exine ornamentation is mapped on the tree to help visualise pollen clades. Numbers above branches are successive weighted bootstrap values. Branches not present in the strict consensus tree from the unit weight analysis are indicated with *arrows*



that *Cistus salviifolius* was now sister to the *C. ladanifer* clade instead of being with *Cistus monspeliensis* L. and *Cistus pouzolzii*. This former clade was without any support. The “purple pink *Cistus* clade” was supported with a BS of 100%, again with *Cistus crispus* L. at the base and sister to two strongly supported groups found already in the *trnS*-G analysis. The 14 Canarian taxa clade found previously was better resolved, but still with some taxa in polytomy, and the only weakly supported clade was the group formed by *Cistus ochreatus* Chr. Sm., *Cistus palmensis* Bañares & Demoly and *Cistus symphytifolius* Lam. (Fig. 4). We mapped exine ornamentation on this strict consensus tree according to our observations (Fig. 4).

Palynological results

Pollen grains in *Cistus* and *Halimium* are stenopalynous single grains, tricolporate comprising three long ectoapertural colpi and three equatorial endoapertural pori. Their forms vary from oblate spheroidal to prolate, but 90% of the samples examined were spheroidal to sub-prolate. The exine was simplicolumellate with most samples with a microreticulate and striato-reticulate exine. Exine thickness varied from 2 to 5 µm (Table 3).

Six groups of taxa were distinguished for the combined analysis, hereafter the outgroup, the three *Halimium* clades H 1, H 2 and H 3, the “white and whitish pink *Cistus* clade” (hereafter WWPC) and the “purple pink *Cistus* clade” (hereafter PPC), in which the Canarian and the non-Canarian taxa could be distinguished. We discuss:

- 1 the pollen data taxa for each of these groups to establish whether there is a common trend in their palynological morphology; and
- 2 the discrepancies between our observations and the literature or in the literature itself, when applicable.

Pollen morphology of outgroups

We used the palynological characters given by the literature, with the exception of the surface of the exine, because of the discrepancies already found for the ingroup. We reinterpreted the exine surface from the original SEM images given by the authors when available. All species were straightforward, except *Fumana ericoides*, where two very different types of exine have been proposed. For Jean and Pons (1963) and Heydacker (1963), the exine is reticulate but it is retipilate for Saenz de Rivas (1979). In the absence of more data, the exine was coded for this species as equivocal. For other taxa, there is some confusion in the descriptions given by Jean and Pons (1963), the exine being described as

striate, but often authors talk about a deeper reticulum. For Saenz de Rivas (1979), the state striato-reticulate does not exist for Cistaceae; he describes either reticulate or striate exine with nothing in between. However, his images show that subparallel muri are crossed-linked to form a reticulum in the grooves. For all outgroups except *Fumana* we coded the surface of the exine as striato-reticulate.

Pollen morphology of *Halimium*

The authors who have examined the exine ornamentation of *Halimium* have described it as striate (Saenz de Rivas 1979), reticulate (Jean and Pons 1962, 1963; Jiménez-Albarrán 1984; Marquez et al. 1996), or striato-reticulate (Heydacker 1963; Jean and Pons 1962, 1963), but are usually coherent within the group. The exine thickness and grain shapes were quite variable, sometimes within the same species.

The first *Halimium* clade H 3 (Fig. 4) was at the base of the tree. It comprised two species of *Halimium* with a striato-reticulate exine (Figs. 1e, 5a–c). There was a difference in the thickness of the exine and the shape of the pollen grains. *Halimium atlanticum* pollen grains were thicker and with a more spheroidal shape than those of *H. calycinum*, but their exine ornamentation was very much the same.

The second *Halimium* clade H 2 (Fig. 4) comprised six taxa of *Halimium* in the combined analysis and seven in the *trnL*-F analysis (*H. lasianthum* ssp. *alyssoides* belonged to this group for *trnL*-F). All had a striato-reticulate exine with the exception of *H. lasiocalycinum* ssp. *rhaphaeum*, whose exine tended to be intermediate between rugulate and striato-reticulate (Fig. 5h), but with wider muri than the typical rugulate found in PPC (Fig. 1c).

The third *Halimium* clade H 1 comprised four taxa of *Halimium* in the combined analysis (Fig. 4), three belonging to the same species, *H. umbellatum* (Fig. 5i), and the fourth, *H. voldii* (Fig. 5k–m), which has been described recently from Greece (Greuter and Raus 2000), could be regarded as a subspecies of *H. umbellatum* to which it shows similarities (<http://www.cistuspage.org.uk/Halimium%20voldii.htm> 2010). All these four *Halimium* taxa have white flowers.

We also present a plate showing the pollen of *Halimium antiatlanticum* (Fig. 5n–p), but we did not find the plant in Morocco, and did not have a sequence to discuss for that taxon. However, on the basis of the samples examined in the Paris (P) and Montpellier (MPU) herbaria, we considered this taxon as a synonym of *Halimium halimifolium* f. *lasiocalycinum* (Boiss. & Reut.) Gross. The exine was striato-reticulate (Fig. 5n, p) but with a tendency to be rugulate (Fig. 5p).

Table 3 Selected palynological characters for Cistaceae taxa studied with combined SEM and LO observations

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
	<i>Cistus albidus</i> L.	Jean and Pons 1963	48–55	40–46	1.20	Sub prolate	2 µm	Rugulate
	<i>Cistus albidus</i> L.	Reille 1990					2–3 µm	Microreticulate
	<i>Cistus albidus</i> L.	Saenz de Rivas 1979	53	31	1.71	Prolate	1.4 µm	Rugulate
332	<i>Cistus albidus</i> L.	This study	47.26 µm ± 2.09	44.09 µm ± 2.15	1.07 ± 0.05	Prolate spheroidal	2.2 µm ± 0.36	Rugulate
1931	<i>Cistus asper</i> Demoly & Mesa	This study	59.71 µm ± 3.90	58.83 µm ± 2.90	1.01 ± 0.03	Prolate spheroidal	2.19 µm ± 0.13	Microreticulate
391	<i>Cistus chinamadensis</i> ssp. <i>chinamadensis</i> Bañares & Romero	This study	63.6 µm ± 2	61.5 µm ± 2	1.03 ± 0.03	Prolate spheroidal	2.16 µm ± 0.10	Microreticulate
390	<i>Cistus chinamadensis</i> ssp. <i>gomeræ</i> Bañares & Romero	This study	63.60 µm ± 0.76	60 µm ± 1.12	1.06 ± 0.03	Prolate spheroidal	2.19 µm ± 0.11	Microreticulate
1924	<i>Cistus chinamadensis</i> ssp. <i>ombriosus</i> Demoly & Marrero	This study	58.95 µm ± 3.38	58.61 µm ± 2.83	1.01 ± 0.04	Prolate spheroidal	2.17 µm ± 0.17	Microreticulate
	<i>Cistus clusii</i> Dunal	Saenz de Rivas 1979	53	45	1.18	Sub prolate	2.8 µm	Striate
330	<i>Cistus clusii</i> Dunal	This study					2–2.5 µm	Striato-reticulate
365	<i>Cistus clusii</i> ssp. <i>multiflorus</i> Demoly	This study	44.50 µm ± 3.17	35.54 µm ± 3.06	1.26 ± 0.10	Sub prolate	2.43 µm ± 0.41	Striato-reticulate
366	<i>Cistus clusii</i> ssp. <i>multiflorus</i> Demoly	This study					2–2.5 µm	Striato-reticulate
359	<i>Cistus creticus</i> L.	This study	36.06 µm ± 1.53	32.54 µm ± 1.30	1.11 ± 0.05	Prolate spheroidal	2.02 µm ± 0.27	Rugulate
	<i>Cistus creticus</i> L. (= <i>C. incanus</i> L.)	Jean and Pons 1963	39–47	32–40	1.19	Sub prolate	3 µm	Reticulate
288	<i>Cistus creticus</i> L. (= <i>C. villosus</i> L.)	This study					2–2.25 µm	Rugulate
289	<i>Cistus creticus</i> L. <i>grp corsicus</i>	This study	57.33 µm ± 4.22	45.80 µm ± 3.63	1.03 ± 0.07	Prolate spheroidal	2.26 µm ± 0.15	Microreticulate
287	<i>Cistus creticus</i> L. <i>grp tauricus</i> (= <i>C. villosus</i> L.)	This study					2 µm	Microreticulate
280	<i>Cistus creticus</i> L. <i>grp tauricus</i> (= <i>C. villosus</i> L.)	This study						Microreticulate
	<i>Cistus crispus</i> L.	Jean and Pons 1963	40–41	30–32	1.31	Sub prolate	2 µm	Rugulate
	<i>Cistus crispus</i> L.	Saenz de Rivas 1979	42	37	1.14	Prolate spheroidal	1.4 µm	Rugulate
336	<i>Cistus crispus</i> L.	This study	40.86 µm ± 1.73	39.54 µm ± 1.12	1.03 ± 0.04	Prolate spheroidal	2.41 µm ± 0.32	Rugulate
326	<i>Cistus crispus</i> L.	This study						Rugulate
327	<i>Cistus crispus</i> L.	This study						Rugulate
	<i>Cistus heterophyllus</i> Desf.	Saenz de Rivas 1979	50	46	1.09	Prolate spheroidal	1.4 µm	Rugulate to microreticulate

Table 3 continued

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
314	<i>Cistus heterophyllus</i> Desf.	This study						
281	<i>Cistus heterophyllus</i> Desf. ssp. <i>carthaginensis</i> (Pau) Crespo & Mateo	This study	76.27 $\mu\text{m} \pm 9.16$	63.07 $\mu\text{m} \pm 6.72$	1.21 ± 0.07	Sub prolate	2.20 μm	Microreticulate
2218	<i>Cistus horrens</i> Demoly	This study	64.15 $\mu\text{m} \pm 2.96$	57.38 $\mu\text{m} \pm 2.53$	1.12 ± 0.03	Prolate spheroidal	2.33 $\mu\text{m} \pm 0.14$	Microreticulate
301	<i>Cistus horrens</i> Demoly	This study	56.36 $\mu\text{m} \pm 4.18$	48.12 $\mu\text{m} \pm 3.66$	1.17 ± 0.09	Sub prolate	2.96 $\mu\text{m} \pm 0.20$	Microreticulate
320	<i>Cistus inflatus</i> Pourr. ex Demoly	This study	45.20 $\mu\text{m} \pm 6.88$	41.20 $\mu\text{m} \pm 5.31$	1.09 ± 0.06	Prolate spheroidal	2.45 $\mu\text{m} \pm 0.37$	Microreticulate with smooth supratectal elements
273	<i>Cistus inflatus</i> Pourr. ex Demoly	This study					2.5 μm	Microreticulate with smooth supratectal elements
321	<i>Cistus inflatus</i> Pourr. ex Demoly	This study						Microreticulate with smooth supratectal elements
323	<i>Cistus inflatus</i> Pourr. ex Demoly	This study						Microreticulate with smooth supratectal elements
	<i>Cistus inflatus</i> Pourr. ex Demoly (= <i>C. hirsutus</i> Lam.)	Jean and Pons 1963	48–50	45–48	1.05	Prolate spheroidal	4 μm	Reticulate
	<i>Cistus inflatus</i> Pourr. ex Demoly (= <i>C. psilosepalus</i> Sweet)	Márquez et al.1996	38.25–46.15	35.25–44.18	1.06	Prolate spheroidal	2.5–4 μm	Reticulate
	<i>Cistus inflatus</i> Pourr. ex Demoly (= <i>C. psilosepalus</i> Sweet)	Saenz de Rivas 1979	48	46	1.04	Prolate spheroidal	4.2 μm	Retipilate
	<i>Cistus ladanifer</i> L.	Jean and Pons 1963	50–61	50–59	1.02	Prolate spheroidal	4 μm	Reticulate
	<i>Cistus ladanifer</i> L.	Márquez et al.1996	31.29–39.47	28.4–38.21	1.06	Prolate spheroidal	2.8–3.2 μm	Reticulate
	<i>Cistus ladanifer</i> L.	Saenz de Rivas 1979	51	46	1.11	Prolate spheroidal	4.2 μm	Reticulate
333	<i>Cistus ladanifer</i> L.	This study					3–4 μm	Large reticulate
334	<i>Cistus ladanifer</i> L. ssp. <i>ladanifer</i> f. <i>albiflorus</i> (Dun.) Dans.	This study	43.80 $\mu\text{m} \pm 2.26$	41.52 $\mu\text{m} \pm 2$	1.06 ± 0.04	Prolate spheroidal	3.21 $\mu\text{m} \pm 0.35$	Large reticulate
307	<i>Cistus ladanifer</i> L. ssp. <i>mauritanicus</i> Pau & Sennen	This study					3–4 μm	Large reticulate
282	<i>Cistus ladanifer</i> L. ssp. <i>mauritanicus</i> Pau & Sennen	This study	62.3 $\mu\text{m} \pm 4.5$	58.1 $\mu\text{m} \pm 4.1$	1.07 ± 0.05	Prolate spheroidal	3–4 μm	Large reticulate
305	<i>Cistus ladanifer</i> L. ssp. <i>sulcatus</i> Demoly (= <i>C. palhinhae</i> Ingram)	This study					3–4 μm	Large reticulate
	<i>Cistus ladanifer</i> L. ssp. <i>sulcatus</i> Demoly (= <i>C. palhinhae</i> Ingram)	Saenz de Rivas 1979	50	46	1.09	Prolate spheroidal	4.2 μm	Reticulate

Table 3 continued

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
	<i>Cistus laurifolius</i> L.	Jean and Pons 1963	48–50	47–49	1.02	Prolate spheroidal	4–5 μ m	Reticulate
	<i>Cistus laurifolius</i> L.	Saenz de Rivas 1979	52	46	1.13	Prolate spheroidal	4.2 μ m	Reticulate
313	<i>Cistus laurifolius</i> L.	This study					2–3 μ m	Large reticulate
308	<i>Cistus laurifolius</i> L. ssp. <i>atlanticus</i> (Pitard) Sennen & Maurício	This study	48.94 μ m + 9.82	40.04 μ m + 6.41	1.22 + 0.10	Sub prolate	4.23 μ m $\pm$ 0.55	Large reticulate
	<i>Cistus libanotis</i> L.	Saenz de Rivas 1979	48	47	1.02	Prolate spheroidal	2.8 μ m	Striate
315	<i>Cistus libanotis</i> L.	This study	45.9 μ m $\pm$ 6.6	37.4 μ m $\pm$ 5.6	1.23 $\pm$ 0.12	Sub prolate	3.74 μ m $\pm$ 0.31	Striato-reticulate
357	<i>Cistus libanotis</i> L. f. <i>major</i> n.n.	This study	36.40 μ m $\pm$ 2.20	33.66 μ m $\pm$ 2.06	1.08 $\pm$ 0.04	Prolate spheroidal	3.22 μ m $\pm$ 0.22	Striato-reticulate
	<i>Cistus monspeliensis</i> L.	Jean and Pons 1963	45–50	43–50	1.02	Prolate spheroidal	3–4 μ m	Reticulate
	<i>Cistus monspeliensis</i> L.	Reille 1990					4 μ m	Reticulate
	<i>Cistus monspeliensis</i> L.	Saenz de Rivas 1979	55	51	1.08	Prolate spheroidal	4.2 μ m	Retipilate
331	<i>Cistus monspeliensis</i> L.	This study	37.04 μ m + 1.94	36.94 μ m + 2.02	1 $\pm$ 0.03	Spheroidal	2.71 μ m $\pm$ 0.31	Microreticulate with smooth supratectal elements
303	<i>Cistus monspeliensis</i> L.	This study	47.30 μ m + 4.27	47.05 μ m + 1.64	1.01 + 0.09	Prolate spheroidal	3.76 μ m $\pm$ 0.30	Microreticulate with smooth supratectal elements
375	<i>Cistus monspeliensis</i> L.	This study						Microreticulate with smooth supratectal elements
309	<i>Cistus munbyi</i> Pom.	This study	42.02 μ m + 2.31	37.14 μ m + 2.49	1 + 0.05	Spheroidal	2.21 μ m $\pm$ 0.10	Striato-reticulate
2248	<i>Cistus ochreatus</i> Chr. Sm.	This study	63.45 μ m + 3.46	57.20 μ m + 1.91	1.11 + 0.05	Prolate spheroidal	2.36 μ m $\pm$ 0.33	Microreticulate
311	<i>Cistus ochreatus</i> Chr. Sm.	This study					2–3 μ m	Microreticulate
2494	<i>Cistus osbeckifolius</i> ssp. <i>tomentosus</i> Bañares & Demoly	This study	61.60 μ m + 2.3	56.06 μ m + 1.8	1.10 + 0.03	Prolate spheroidal	2.27 μ m $\pm$ 0.16	Microreticulate
341	<i>Cistus osbeckifolius</i> Webb. ex Christ.	This study	47.18 μ m + 2.07	42.76 μ m + 1.76	1.10 + 0.04	Prolate spheroidal	2.28 μ m $\pm$ 0.41	Microreticulate
310	<i>Cistus osbeckifolius</i> Webb. ex Christ.	This study					2–3 μ m	Microreticulate
1989	<i>Cistus palmensis</i> Bañares & Demoly	This study	64.06 μ m + 2.51	59.66 μ m + 3.07	1.08 + 0.04	Prolate spheroidal	2.24 μ m $\pm$ 0.12	Microreticulate
316	<i>Cistus parviflorus</i> Lam.	This study	76.44 μ m + 4.66	66.15 μ m + 5.44	1.16 + 0.05	Sub prolate	5.17 μ m $\pm$ 0.53	Large reticulate
317	<i>Cistus parviflorus</i> Lam.	This study	48.69 μ m + 3.22	41.19 μ m + 4.30	1.19 + 0.08	Sub prolate	3.16 μ m $\pm$ 0.31	Large reticulate
360	<i>Cistus parviflorus</i> Lam.	This study					3 μ m	Striato-reticulate

Table 3 continued

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
322	<i>Cistus parviflorus</i> Lam.	This study					3–4 µm	Striato-reticulate
312	<i>Cistus parviflorus</i> Lam. (= <i>C. asperrimus</i>)	This study					3 µm	Large reticulate
	<i>Cistus populifolius</i> L.	Reille 1990					4–5 µm	Large reticulate
	<i>Cistus populifolius</i> L.	Saenz de Rivas 1979	57	53	1.08	Prolate spheroidal	4.2 µm	Reticulate
338	<i>Cistus populifolius</i> L.	This study	48.24 µm + 2.69	47.20 µm + 2.75	1.02 + 0.02	Prolate spheroidal	3 µm ± 0.13	Large reticulate
	<i>Cistus populifolius</i> L. ssp. <i>populifolius</i>	Jean and Pons 1963	40–50	40–46	1.05	Prolate spheroidal	4–6 µm	Reticulate
	<i>Cistus populifolius</i> L. ssp. <i>populifolius</i>	Márquez et al. 1996	37.25–44.90	36.28–44.96	1.01	Prolate spheroidal	2.88–4.44 µm	Reticulate
272	<i>Cistus populifolius</i> L. ssp. <i>populifolius</i> (= <i>C. narbonensis</i> Rouy & Foucaud)	This study	55.68 µm + 3.27	52.13 µm + 3.42	1.07 + 0.05	Prolate spheroidal	3.34 µm ± 1.12	Large reticulate
276	<i>Cistus populifolius</i> L. ssp. <i>populifolius</i> (= <i>C. narbonensis</i> Rouy & Foucaud)	This study					3–4 µm	Large reticulate
318	<i>Cistus populifolius</i> L. ssp. <i>major</i> (Dun.) Heywood	This study					3–4 µm	Large reticulate
306	<i>Cistus populifolius</i> L. ssp. <i>major</i> (Dun.) Heywood	This study	76.56 µm + 8.52	71.24 µm + 6.73	1.07 + 0.04	Prolate spheroidal	4.78 µm ± 0.19	Large reticulate
	<i>Cistus pouzolzii</i> Delile	Jean and Pons 1963	52–59	45–56	1.12	Prolate spheroidal	2–3 µm	Rugulate
351	<i>Cistus pouzolzii</i> Delile	This study	47.68 µm ± 3.91	42.58 µm ± 3.90	1.12 + 0.04	Prolate spheroidal	2.94 µm ± 0.33	Striato-reticulate
350	<i>Cistus pouzolzii</i> Delile	This study						Striato-reticulate
	<i>Cistus salvifolius</i> L.	Jean and Pons 1963	48–51	44–50	1.05	Prolate spheroidal	5 µm	
	<i>Cistus salvifolius</i> L.	Márquez et al. 1996	45.29–49.22	44.95–48.74	1.01	Prolate spheroidal	5 µm	Reticulate
	<i>Cistus salvifolius</i> L.	Reille 1990					5 µm	Reticulate
	<i>Cistus salvifolius</i> L.	Saenz de Rivas 1979	58	51	1.14	Prolate spheroidal	4.2 µm	Retipilate
383	<i>Cistus salvifolius</i> L.	This study	44.06 µm + 2.51	42.35 µm + 2.43	1.04 + 0.05	Prolate spheroidal	3 µm ± 0.07	Microechinate
335	<i>Cistus salvifolius</i> L.	This study						Microechinate
356	<i>Cistus sintenisii</i> de Lit.	This study	38.94 µm + 2.38	38.93 µm + 2.14	1 + 0.04	Spheroidal	2.76 µm ± 0.38	Striato-reticulate
325	<i>Cistus sintenisii</i> de Lit.	This study						Striato-reticulate
329	<i>Cistus symphytifolius</i> Lam.	This study	59.9 µm + 1.66	56.1 µm + 1.85	1.06 + 0.02	Prolate spheroidal	2.30 µm ± 0.16	Microreticulate
2462	<i>Cistus symphytifolius</i> Lam. (Pico de Cabras)	This study	59.80 µm + 2.86	55.18 µm + 2.45	1.08 + 0.04	Prolate spheroidal	2.29 µm ± 0.17	Microreticulate

Table 3 continued

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
1851	<i>Cistus symphytifolius</i> Lam. (Punta Gorda)	This study	58.65 μm + 2.85	56.72 μm + 3.01	1.04 + 0.04	Prolate spheroidal	2.26 μm $\pm$ 0.14	Microreticulate
2023	<i>Cistus symphytifolius</i> Lam. var. <i>canus</i> Demoly	This study	64.42 μm + 4.28	57.64 μm + 4.12	1.12 + 0.04	Prolate spheroidal	2.19 μm $\pm$ 0.51	Microreticulate
2319	<i>Cistus symphytifolius</i> Lam. var. <i>villosus</i> Demoly	This study	67.23 μm + 2.93	61.73 μm + 2.51	1.09 + 0.03	Prolate spheroidal	2.45 μm $\pm$ 0.38	Microreticulate
343	<i>Halimium antiatlanticum</i> Maire & Wilezek	This study	44.40 μm + 3.54	40.30 μm + 2.38	1.10 + 0.06	Prolate spheroidal	2.38 μm $\pm$ 0.26	Striato-reticulate
342	<i>Halimium antiatlanticum</i> Maire & Wilezek	This study	45.46 μm + 2.19	39.75 μm + 4.03	1.15 + 0.09	Sub prolate	2.42 μm $\pm$ 0.27	Striato-reticulate
271	<i>Halimium antiatlanticum</i> Maire & Wilezek	This study					2–3 μm	Striato-reticulate
275	<i>Halimium atlanticum</i> Humb. & Maire	This study					2–3 μm	Striato-reticulate
1587	<i>Halimium atlanticum</i> Humb. & Maire	This study	54.34 μm + 2.55	50.82 μm + 1.62	1.07 + 0.04	Prolate spheroidal	3.30 μm $\pm$ 0.27	Striato-reticulate
1595	<i>Halimium atlanticum</i> Humb. & Maire	This study	54.69 μm + 3.75	50.07 μm + 3.18	1.09 + 0.04	Prolate spheroidal	3.40 μm $\pm$ 0.24	Striato-reticulate
	<i>Halimium atriplicifolium</i> (Lam.) Spach	Jiménez-Albarrán 1984	57.29–55.83	53.56–52.19	1.06	Prolate spheroidal		Reticulate
	<i>Halimium atriplicifolium</i> (Lam.) Spach	Saenz de Rivas 1979	60	53	1.13	Prolate spheroidal	2.8 μm	Striate
290	<i>Halimium atriplicifolium</i> (Lam.) Spach	This study	53.80 μm + 3.83	45.52 μm + 4.60	1.19 + 0.08	Sub prolate	2.92 μm $\pm$ 0.24	Striato-reticulate
	<i>Halimium calycinum</i> (L.) K.Koch (= <i>H. commutatum</i> Pau)	Jiménez-Albarrán 1984	54.85–57.99	47.15–48.97	1.17	Sub prolate		Striato-reticulate
	<i>Halimium calycinum</i> (L.) K.Koch (= <i>H. commutatum</i> Pau)	Saenz de Rivas 1979	66	50	1.32	Sub prolate	2.8 μm	Striate
299	<i>Halimium calycinum</i> (L.) K.Koch (= <i>H. commutatum</i> Pau)	This study	59.16 μm + 3.51	45.22 μm + 2.52	1.31 + 0.08	Sub prolate	2.68 μm $\pm$ 0.32	Striato-reticulate
	<i>Halimium halimifolium</i> (L.) Willk.	Jean & Pons 1963	50–58	40–48	1.23	Sub prolate	4 μm	Striato-reticulate
	<i>Halimium halimifolium</i> (L.) Willk.	Jiménez-Albarrán 1984	49.98–49.42	44.1–42.92	1.14	Prolate spheroidal		Reticulate
	<i>Halimium halimifolium</i> (L.) Willk.	Saenz de Rivas 1979	43	42	1.02	Prolate spheroidal	2.8 μm	Striate
349	<i>Halimium halimifolium</i> (L.) Willk.	This study	48.70 μm + 3.06	43.94 μm + 2.46	1.11 + 0.07	Prolate spheroidal	3.46 μm $\pm$ 0.73	Striato-reticulate
347	<i>Halimium halimifolium</i> f. <i>lastocalycinum</i> (Boiss. & Reut.) Gross.	This study	45.90 μm + 2.24	40.25 μm + 2.38	1.14 + 0.06	Prolate spheroidal		Striato-reticulate
346	<i>Halimium halimifolium</i> f. <i>lastocalycinum</i> (Boiss. & Reut.) Gross.	This study						Striato-reticulate

Table 3 continued

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
345	<i>Halimium halimifolium</i> ssp. <i>multiflorum</i> auct. non Salzm.	This study	49.78 μm + 3.88	39.30 μm + 3.84	1.27 + 0.13	Sub prolate	1.94 μm $\pm$ 0.35	Striato-reticulate
1045	<i>Halimium halimifolium</i> ssp. <i>multiflorum</i> auct. non Salzm.	This study	54.21 μm + 2.83	48.09 μm + 3.00	1.13 + 0.05	Prolate spheroidal	2.50–2.80 μm	Striato-reticulate
344	<i>Halimium halimifolium</i> ssp. <i>halimifolium</i> (L.) Willk.	This study	44.4 μm + 2.33	37.22 μm + 2.50	1.20 + 0.08	Sub prolate	2.09 μm $\pm$ 0.22	Striato-reticulate
	<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Greuter & Burdet	Jean & Pons 1963	45–50	39–49	1.08	Prolate spheroidal	3 μm	Reticulate
	<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Greuter & Burdet	Jiménez-Albarrán 1984	49.39–51.94	44.44–47.74	1.11	Prolate spheroidal		Reticulate
	<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Greuter & Burdet	Márquez et al. 1996	44.55–49.79	43.36–51.05	1.00	Spheroidal	2.3–3.4 μm	Reticulate
	<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Greuter & Burdet	Saenz de Rivas 1979	50	44	1.14	Prolate spheroidal	2.8 μm	Striate
291	<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Greuter & Burdet	This study	47.83 μm + 2.99	40.17 μm + 2.99	1.19 + 0.07	Sub prolate	2.13 μm $\pm$ 0.21	Striato-reticulate
295	<i>Halimium lasianthum</i> ssp. <i>lasianthum</i> (Lam.) Spach	This study	53.52 μm + 3.74	45.56 μm + 2.75	1.18 + 0.06	Sub prolate	2.93 μm $\pm$ 0.32	Striato-reticulate
296	<i>Halimium lasianthum</i> ssp. <i>lasianthum</i> (Lam.) Spach	This study					2–3 μm	Striato-reticulate
394	<i>Halimium lasianthum</i> ssp. <i>lasianthum</i> (Lam.) Spach	This study					2–3 μm	Striato-reticulate
	<i>Halimium lasianthum</i> ssp. <i>alyssoides</i> (Lam.) Spach (= <i>H. alyssoides</i> (Lam.) Koch)	Jiménez-Albarrán 1984	52.58–53.73	42.24–50.3	1.08	Prolate spheroidal		Reticulate
348	<i>Halimium lasiocalycinum</i> ssp. <i>rhizophaeum</i> (Pau & Font Quer) Maire	This study	40.36 μm + 1.25	36.02 μm + 2.01	1.12 + 0.05	Prolate spheroidal	2.06 μm $\pm$ 0.17	Striato-reticulate
	<i>Halimium ocymoides</i> (Lam.) Willk.	Jiménez-Albarrán 1984	49.92–51.55	42.39–43.04	1.19	Sub prolate		Reticulate
	<i>Halimium ocymoides</i> (Lam.) Willk.	Márquez et al. 1996	40.36–45.83	38.47–45.88	1.02	Prolate spheroidal	1.9–3.3 μm	Reticulate
	<i>Halimium ocymoides</i> (Lam.) Willk.	Saenz de Rivas 1979	52	43	1.21	Sub prolate	2.8 μm	Striate
292	<i>Halimium ocymoides</i> (Lam.) Willk. f. <i>elongatum</i> (Vahl.) Gross.	This study					2–3 μm	Striato-reticulate
293	<i>Halimium ocymoides</i> (Lam.) Willk. f. <i>sampsucifolium</i> (Cav.) Gross.	This study	49.08 μm + 3.95	42.06 μm + 3.62	1.17 + 0.06	Sub prolate	2.16 μm $\pm$ 0.31	Striato-reticulate
	<i>Halimium umbellatum</i> (L.) Spach	Jean and Pons 1963	52–60	45–52	1.15	Sub prolate	3 μm	Reticulate
	<i>Halimium umbellatum</i> (L.) Spach	Jiménez-Albarrán 1984	50.6–51.27	46.96–48.08	1.07	Prolate spheroidal		Reticulate

Table 3 continued

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
	<i>Halimium umbellatum</i> (L.) Spach	Márquez et al.1996	43.16–51.35	41.51–48.37	1.05	Prolate spheroidal	3–4 µm	Striato-reticulate
298	<i>Halimium umbellatum</i> (L.) Spach	This study	55.68 µm + 5.80	46.64 µm + 3.91	1.20 + 0.10	Sub prolate	2.92 µm ± 0.28	Striato-reticulate
	<i>Halimium umbellatum</i> (L.) Spach ssp. <i>viscosum</i> (Willk.) Bolos & Vigo	Jiménez-Albarrán 1984	52–53.82	46.73–49.28	1.07	Prolate spheroidal		Reticulate
	<i>Halimium umbellatum</i> (L.) Spach ssp. <i>viscosum</i> (Willk.) Bolos & Vigo	Saenz de Rivas 1979	66	54	1.22	Sub prolate	2.8 µm	Striate
392	<i>Halimium umbellatum</i> (L.) Spach ssp. <i>viscosum</i> (Willk.) Bolos & Vigo	This study	45.00 µm + 3.38	38.70 µm + 2.57	1.16 + 0.06	Sub prolate	2.88 µm ± 0.39	Striato-reticulate
277	<i>Halimium umbellatum</i> (L.) Spach ssp. <i>viscosum</i> (Willk.) Bolos & Vigo	This study					2–3 µm	Striato-reticulate
2571	<i>Halimium voldii</i> Kit Tan, Perdetzoglou & Raus	This study	55.09 µm + 3.08	56.32 µm + 2.42	0.98 + 0.03	Oblate spheroidal	3.30 µm	Striato-reticulate
OUTGROUP								
	<i>Fumana ericoides</i> Pau subsp. <i>montana</i> (Pomel) Güemes & Muñoz	Saenz de Rivas 1979	68	68	1.00	Spheroidal	4.2 µm	Retipilate
	<i>Fumana ericoides</i> Pau subsp. <i>montana</i> (Pomel) Güemes & Muñoz	Jean and Pons 1963	52–58	59–65	0.89	Oblate spheroidal		Reticulate
	<i>Helianthemum caput-felis</i> Boiss.	Saenz de Rivas 1979	43	32	1.34	Prolate	2.8 µm	Striate
	<i>Helianthemum caput-felis</i> Boiss.	This study						Striato-reticulate
	<i>Helianthemum oelandicum</i> (L.) DC.	Jean and Pons 1963	42–50	30–35	1.41	Prolate	2 µm	Striate
	<i>Helianthemum oelandicum</i> (L.) DC. subsp. <i>incanum</i> (Willk.) López	This study						Striato-reticulate
	<i>Helianthemum oelandicum</i> (L.) DC. subsp. <i>incanum</i> (Willk.) López (= <i>H. canum</i> (L.) Hornem.)	Saenz de Rivas 1979	38	32	1.18	Sub prolate	2.8 µm	Striate
	<i>Helianthemum oelandicum</i> (L.) DC. subsp. <i>incanum</i> (Willk.) López (= <i>H. canum</i> (L.) Hornem.)	Márquez et al.1996	42.88–57.08	33.52–41.81	1.33	Sub prolate	1.22–2.39 µm	Striato-reticulate
	<i>Helianthemum syriacum</i> (Jacq.) Dum.-Cours.	This study						Striato-reticulate
	<i>Helianthemum syriacum</i> (Jacq.) Dum.-Cours. (= <i>H. lavandulaefolium</i> DC.)	Saenz de Rivas 1979	66	48	1.37	Prolate	2.8 µm	Striate
	<i>Helianthemum syriacum</i> (Jacq.) Dum.-Cours. (= <i>H. lavandulaefolium</i> DC.)	Jean and Pons 1963	50–65	45–50	1.21	Sub prolate	3 µm	Striate
	<i>Tuberaria guttata</i> (L.) Raf.	Saenz de Rivas 1979	52	38	1.37	Prolate	2.8 µm	Striate

Table 3 continued

No.	Taxon	Ref.	P	E	P/E	Shape	Exine thickness	Exine surface
	<i>Tuberaria guttata</i> (L.) Raf.	This study Jean and Pons 1963	43–45	40–42	1.07	Prolate spheroidal	3.5 µm	Striato-reticulate
	<i>Tuberaria guttata</i> (L.) Raf.							Striate

Pollen grains examined for this study were compared with previous studies (Ref.). For each sample are given, when known, the polar axis (P) and equatorial diameter (E), the P/E ratio, the shape of the pollen from P/E , and observations on exine thickness and surface. When pollen samples were examined by SEM, only an approximation of the thickness is given, on the basis of one measurement only. For outgroups, no SEM was carried out and interpretation is given only when our opinion differs from the literature. We have included some synonymy when the name found on the herbarium sample was different from the commonly accepted name and also in order to test pollen variation within the species

Pollen morphology of *Cistus*

The WWPC (Fig. 4) exhibited the highest diversity of exine ornamentation for all the clades discussed in this study, with some unique patterns not found elsewhere.

The large shrub-like species *Cistus ladanifer*, *C. laurifolius*, and *C. populifolius* had the unique, largely reticulate, exine ornamentation (Fig. 6a–h) shared by some samples of *C. parviflorus*, a much smaller species (Fig. 6I). The subspecies of *Cistus ladanifer* were examined for this study. *C. ladanifer* ssp. *mauritanus* (Fig. 6a–c), occurring in North Africa and southern Spain, showed no significant palynological difference from *C. ladanifer* subsp. *ladanifer* or from *C. ladanifer* subsp. *sulcatus*, which is regarded by some authors as a different species, *C. palhinhae* Ingram. To avoid any confusion about the pollen morphology of *C. ladanifer* subsp. *sulcatus* (*C. palhinhae*) we examined specimens collected by Ingram himself (pollen sample 305).

The pollen characters of *Cistus laurifolius* ssp. *atlanticus* (Fig. 6d–f) were morphologically comparable with those of the subspecies *C. laurifolius* ssp. *laurifolius*. The same observation was made for subspecies of *C. populifolius*. Although authors have agreed on the ornamentation for all species but *C. parviflorus*, we followed Reille (1990) for characterization of this type of sculpture and defined it as largely reticulate. The shape of most of the taxa observed was prolate spheroidal with minor variations. The exine was very thick, usually 3–4 µm. *Cistus parviflorus* was not examined in previous studies and had some interesting variation. Five samples of this species were studied, three had a very large reticulum (Fig. 6i–l), but samples 360 and 322 had a striato-reticulate exine (Fig. 6j, m–o). It could have been interesting to compare their DNA with those of other species but the two samples came from very old herbarium specimens (Table 1) and we only had one DNA accession. Four species of *Cistus* had a striato-reticulate exine but were not grouped together. Two species had not been studied before within this group, *Cistus munbyi* (Fig. 7a–b) and *C. sintenisii* (Fig. 7c–d). *Cistus pouzolzii* has been described as rugulate by Jean and Pons (1963). Our sample from North Africa (Fig. 7e) had a slightly more serrated exine that could be regarded by some authors as relaxed rugulate compared with the French sample (Fig. 7f), but we definitely regarded it as striato-reticulate exine.

Three species had supratectal ornamentation—*Cistus salviifolius* with microechinate elements (Fig. 1i) and *C. monspeliensis* and *C. inflatus* with smooth elements (Fig. 1g–h). For these species there was no difference in interpretation, we only used a different term to define their exine.

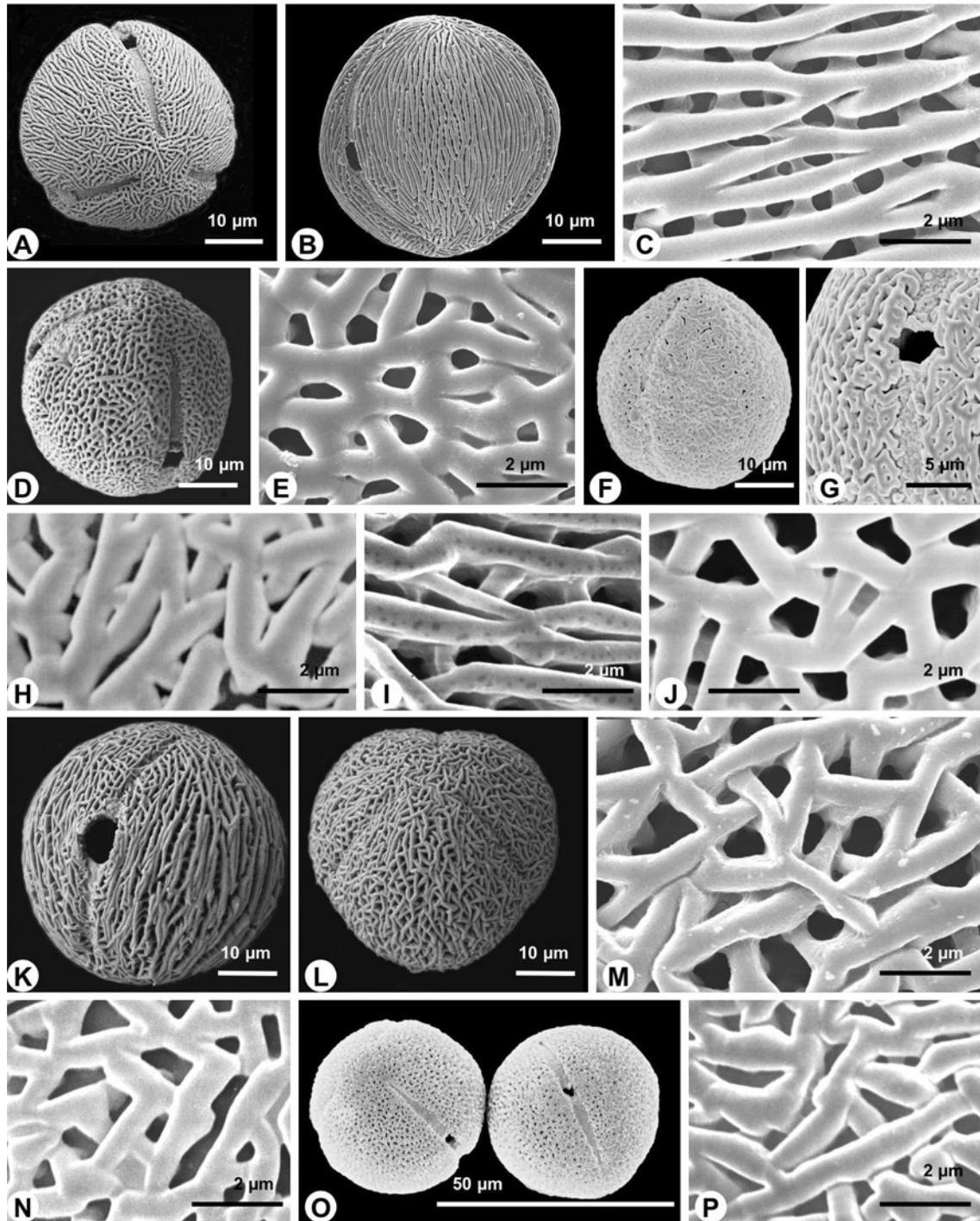


Fig. 5 SEM micrographs of pollen grains. **a–c** *Halimium atlanticum* 1595, **a** pollen grain in polar view, **b** equatorial view, **c** striato-reticulate exine. **d–e** *H. halimifolium* ssp. *multiflorum* 1045, **d** pollen grain in polar view, **e** striato-reticulate exine. **f–h** *H. lasiocalycinum* ssp. *rhypaeum* 348, **f** equatorial view, **g** aperture, **h** striato-reticulate exine. **i** *H. umbellatum* ssp. *viscosum* 392, striato-reticulate exine.

j *H. atriplicifolium* 290 striato-reticulate exine. **k–m** *H. veldii* 2571, **k** pollen grain in equatorial view, **l** polar view, **m** striato-reticulate exine. **n–o** *H. antiatlanticum* 271, **p** *H. antiatlanticum* 343, **n**, **p** striato-reticulate exine, **o** pollen grain in polar view and equatorial view

At the base of the “purple pink *Cistus* clade” (hereafter PPC), we found *Cistus crispus* and a clade formed by three species *C. albidus*, *C. creticus*, and *C. heterophyllus* with a

rugulate to microreticulate exine. *C. crispus* (Fig. 7g) and *C. albidus* L. (Figs. 1c, 7q) have a typically rugulate exine, except for Reille (microreticulate, 1990), with lumina

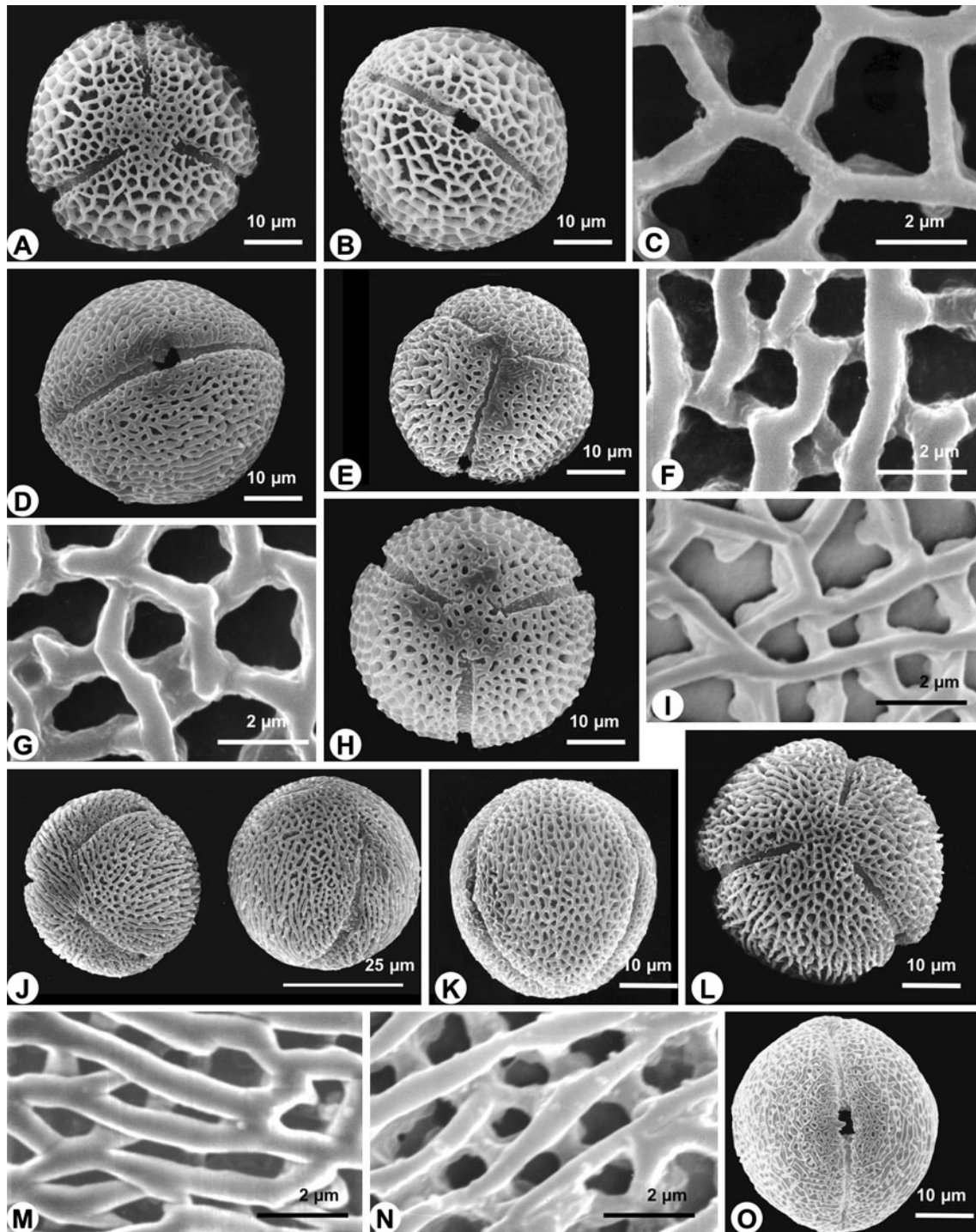


Fig. 6 SEM micrographs of pollen grains. **a–c** *Cistus ladanifer* var *petiolatus* 282, **a** prolate spheroidal pollen grain in polar view, **b** in equatorial view, **c** largely reticulate exine. **d–f** *C. laurifolius* ssp. *atlanticus* 308. **d** prolate spheroidal pollen grain in polar view, **e** equatorial view, **f** largely reticulate exine. **g–h** *C. populifolius* ssp. *major*. **g** 306 largely reticulate exine, **h** 318 pollen grain in polar view.

i–l *C. parviflorus* 316, **i** largely reticulate exine, **k** pollen grain in equatorial view, **l** polar view. **m–o** *C. parviflorus* 360. **m** striato-reticulate exine. **o** pollen grain in equatorial view. **j, n** *C. parviflorus* 322, **j** pollen grains in equatorial and polar view, **n** striato-reticulate exine

under 0.5 μm . We examined two taxa of *Cistus heterophyllus*, a North African sample (Fig. 7h) and the rare *C. heterophyllus* subsp. *carthaginensis* (Figs. 1b, 7i–j) from

Spain, both have a microreticulate exine. We examined five samples belonging to *C. creticus*, from Corsica (Fig. 7n–o) to others in Crimea and Morocco, the exine was rugulate in

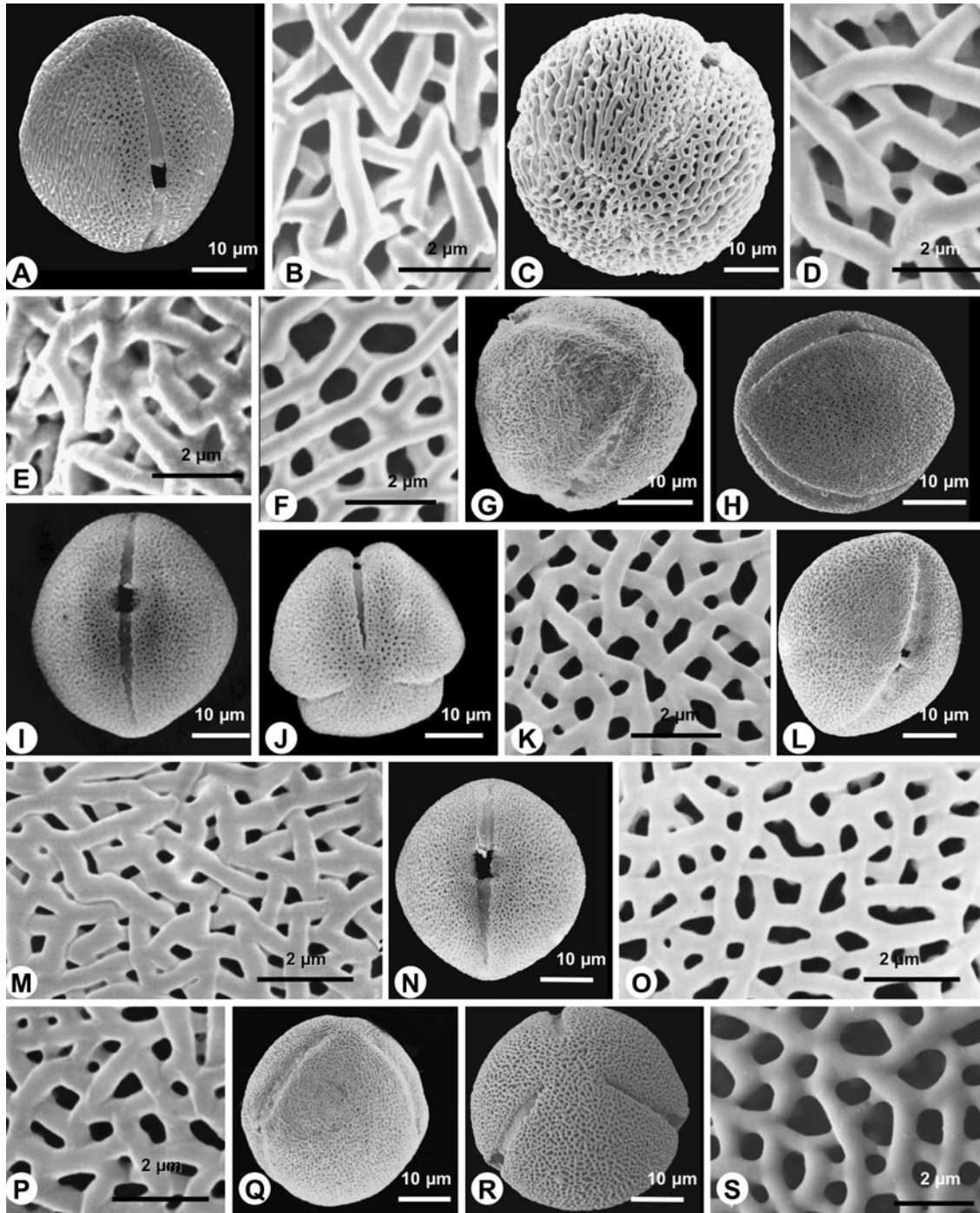


Fig. 7 SEM micrographs of pollen grains. **a–b** *Cistus munbyi* 309, **a** spheroidal pollen grain in equatorial view, **b** striato-reticulate exine. **c–d** *C. sintenisii* 325, **c** spheroidal pollen grain in polar view, **d** striato-reticulate exine. **e–f** *C. pouzolzii* striato-reticulate exine. **e** 351 from Morocco, **f** 350 from France. **g** *C. crispus* 336 pollen grain in polar view. **h** *C. heterophyllus* 314 pollen grain in equatorial view. **i–j** *C. heterophyllus* subsp. *carthaginensis* 281. **i** pollen grain in equatorial view, **j** polar view. **k** *C. heterophyllus* 314 microreticulate

exine. **l** *C. creticus* 359 prolate spheroidal pollen grain in equatorial view. **m** *C. creticus* 288 rugulate exine. **n–o** *C. creticus* var. *corsicus* 289, **n** prolate spheroidal pollen grain in equatorial view, **o** microreticulate exine. **p** *C. creticus* var. *tauricus* 280 microreticulate exine. **q** *C. albidus* 332 prolate spheroidal pollen grain in equatorial view **r–s** *C. asper* 1931 **r** prolate spheroidal pollen grain in polar view. **s** microreticulate exine

three cases (Fig. 7m) and microreticulate in the other two (Fig. 7o–p). There was no geographical pattern for exine ornamentation. Obviously, there is a continuum of state for

this character. Discrepancies between authors show that they had the same problem of continuum between the two states (Table 3). The shapes for these four species were not

very consistent and varied from prolate to prolate spheroidal with an exine thickness below 2.5 μm .

The Canarian taxa that formed a monophyletic group were also characterised by a micro-reticulate exine sometimes bordering on rugulate (Figs. 7, 8, 9). None of

these had been studied before. The exine thickness was usually less than 2.5 μm (Fig. 8d), except for *Cistus horrens* (No. 301, Table 3). The shape was prolate spheroidal, except for the same sample *Cistus horrens* (No. 301, Table 3).

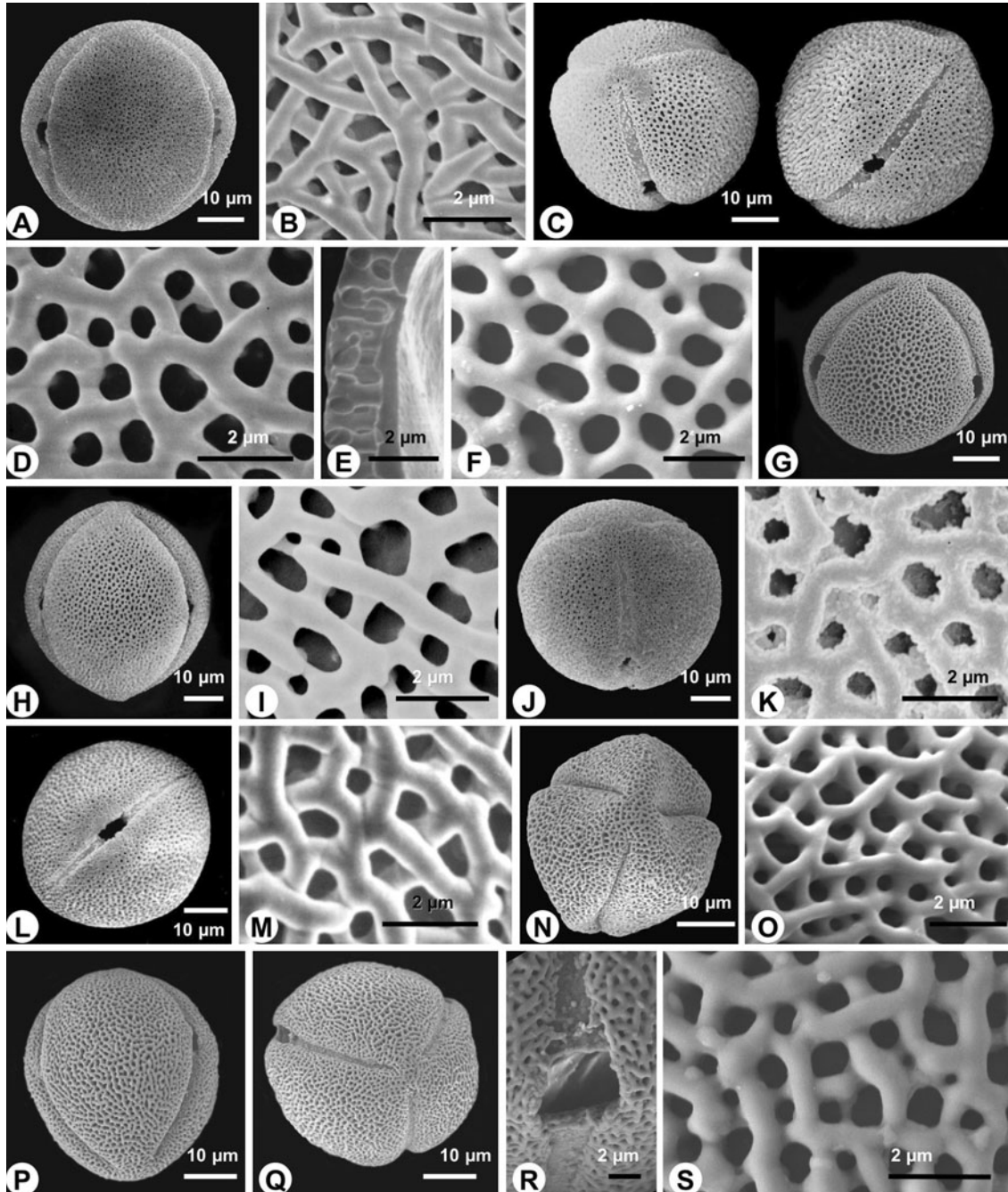


Fig. 8 SEM micrographs of pollen grains. **a–b** *Cistus chinamadensis* subsp. *chinamadensis* 391. **a** prolate spheroidal pollen grain in equatorial view. **b** microreticulate exine. **c–e** *C. chinamadensis* subsp. *gomeræ* 390. **c** pollen grains. **d** microreticulate exine. **e** exine thickness. **f–g** *C. chinamadensis* subsp. *ombriosus* 1922. **f** microreticulate exine. **g** pollen grain in equatorial view. **h–i** *C. horrens* 301. **h** subprolate pollen grain in equatorial view. **i** microreticulate exine.

j–k *C. ochreateus* 311. **j** pollen grain in polar view. **k** microreticulate exine. **l–m** *C. osbeckiifolius* subsp. *osbeckiifolius* 341. **l** pollen grain in equatorial view. **m** microreticulate exine. **n–o** *C. osbeckiifolius* subsp. *tomentosus* 2494. **n** pollen grain in polar view. **o** microreticulate exine. **p–s** *C. palmensis* 1989. **p** pollen grain in equatorial view. **q** pollen grain in polar view. **r** aperture. **s** microreticulate exine

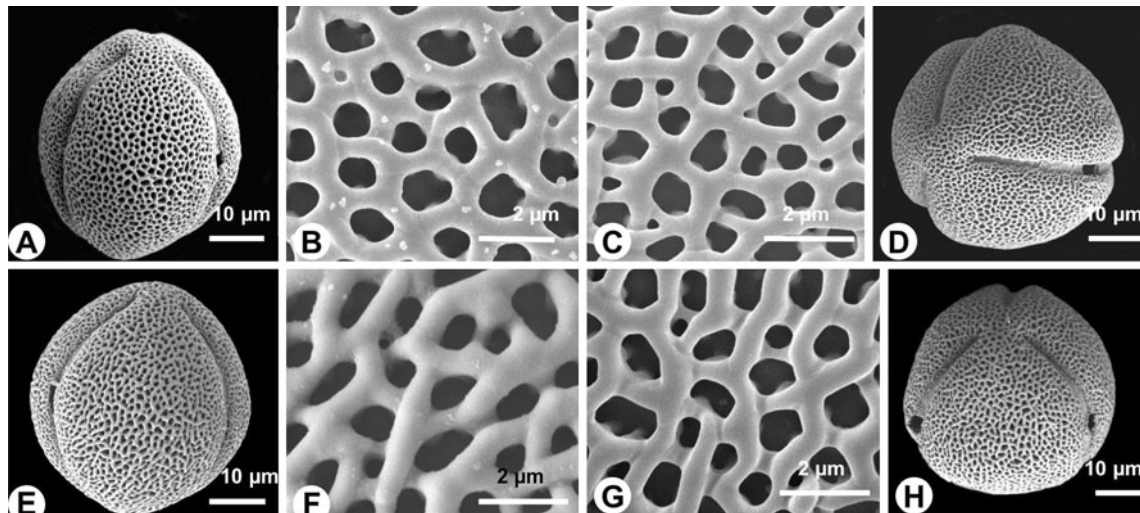


Fig. 9 SEM micrographs of *Cistus symphytifolius* pollen grains. **a–b** *C. symphytifolius* var. *canus* 2023. **a** prolate spheroidal pollen grain in equatorial view, **b** microreticulate exine. **c–d** *C. symphytifolius* (Pico de Cabra) 2462, **c** prolate spheroidal pollen grain in equatorial view,

d microreticulate exine. **e–f** *C. symphytifolius* (Punta Gorda) 1851, **e** prolate spheroidal pollen grain in equatorial view, **f** microreticulate exine. **g–h** *C. symphytifolius* var. *villosus* 2319, **g** microreticulate exine, **h** prolate spheroidal pollen grain

Discussion

Molecular analyses

If we compare the plastid region analysed, the phylogeny obtained with *trnS-G* was better resolved than that with only *trnL-F*. Some clades were present in both analyses with quite good support: the three *Halimium* clades, H 1, H 2, and H 3, and the PPC clade. The rest of the *Cistus* formed a clade with *trnS-G* only, not with *trnL-F*. Both plastid regions contributed to the better resolution of the strict consensus tree of the combined analysis. The *Halimium* species did not group together but instead formed three monophyletic groups, followed by a group of *Cistus*, mainly with white flowers, and the purple pink *Cistus* clade (Fig. 3). Petal colour, which was mapped on one of the single gene analyses (Fig. 2a), defined three monophyletic groups for the *Halimium* species, two yellow-flowered and one white-flowered. For *Cistus*, the two monophyletic groups were also quite well defined by their colour, the purple pink flowers being found grouped in one clade, and the only other pinkish species was very pale in comparison, and was grouped with the white species of *Cistus*.

There were several recognised monophyletic clades for the ingroup and all but one had high BS, the “white and whitish pink *Cistus*” group being the weakest. Neither of the two genera examined formed a monophyletic group. The two clades of *Cistus* never grouped together and the situation was even worse for the *Halimium* species, where the very long length of the branches prevented any grouping.

Systematics

The white and whitish pink *Cistus* clade had the weakest support, and had some interesting particularities. It includes some subspecies or varieties on both sides of the Gibraltar strait and is very heterogeneous with regard to sections, no fewer than six sections have been defined within this group. We examined the species with varieties or subspecies distributed on both sides of the Gibraltar strait. *Cistus ladanifer* subsp. *mauritanicus* Pau and Sennen, *Cistus laurifolius* L. ssp. *atlanticus* (Pitard) Sennen and Mauricio, *Cistus populifolius* var. *major* Dun. are the Moroccan counterpart subspecies or varieties of the European species. Within each species, the sequences were identical for all taxa except for *Cistus populifolius* var. *major* Dun. which had two mutations not present on *Cistus populifolius* L. var. *populifolius*. There were two small clades with good BS, *Cistus parviflorus* Lam. and *Cistus sintenisii* de Lit., which are two species from the east of the Mediterranean, and *Cistus clusii* Dunal with *Cistus* aff. *Munbyi*.

The six generic sections were Ledonella Dun., Stephanocarpus (Spach.) Gren. and Godr., Stephanocarpoidea Rouy. and Foucaud, Ledonia Dun., Ladanium (Spach.) Gren. and Godr., and Halimioides Wilk.. The section Ledonella was monospecific with *C. parviflorus* only. This species was grouped by Dansereau (1939) with the other pink-flowered species (subgenus Erythrocistus), but this is not supported by our analyses and cannot be maintained. The section Stephanocarpus comprised two species, *C. monspeliensis* and *C. sintenisii*, but this grouping was not supported either. These two sections seemed to be quite

artificial. Moreover, *C. parviflorus* and *C. sintenisii* formed a well supported group in the combined analysis (Fig. 3). These two species are eastern Mediterranean species and fertile hybrids between the two are easily produced. The two species share, with *C. monspeliensis* also, a stephanocapsule. *C. pouzolzii* belonged to the monospecific section Stephanocarpoidea and was grouped with *C. monspeliensis* but with no support. A long style characterizes *C. pouzolzii*, and this character is found in the purple species also, but, because no hybrids have been recorded between these two groups, they are probably genetically distant. It seems for the moment that this is simple convergence. The section Ledonia, which comprises three species, *C. salviifolius*, *C. inflatus*, and *C. populifolius*, was paraphyletic in our study. Two of the tallest species of Cistaceae, *C. ladanifer* and *C. laurifolius*, belong to the section Ladanium, sharing a reduced number of sepals (3). Again this section was not monophyletic, because of the position of *C. salviifolius* at the base of the clade formed by the subspecies of *C. ladanifer*. The character common to *C. salviifolius* and the other two species of the section Ladanium is the paucity of the numbers of flowers per inflorescence; in *C. ladanifer* they are even completely unifloral. However, Dansereau (1941) noted that two forms of *C. salviifolius* can be found in the wild, some trifloral (pure *C. salviifolius*) and some with many more flowers per inflorescence (probably introgressed with *C. monspeliensis*). The last section of this group of *Cistus* is Halimioides with the remaining three species *C. libanotis*, *C. clusii*, and *C. munbyi*. *C. libanotis* was not grouped with any other species and the other two taxa, *C. clusii* and *C. aff. munbyi*, had an intermediate position between the *Halimium* and the rest of the Cistaceae.

Within the purple pink *Cistus* clade, it was easy to distinguish two groups belonging to two *Cistus* sections. First we found the four species of the section *Cistus* which were paraphyletic (*C. crispus*, *C. creticus*, *C. albidus*, and *C. heterophyllus*), and second a monophyletic group of Canarian taxa which all belonged to the section *Macrostyliia* Willk. The four species of the subgenus *Cistus* and those of the subgenus *Macrostyliia* do not hybridize freely with any other section of *Cistus*. When artificial hybridization is carried out, the progeny is sterile and very weak, and does not survive for long (Demoly 1996).

Comparison of *Cistus albidus* and *C. creticus*

Within the section *Cistus*, the distribution of *Cistus albidus* and *Cistus creticus* is rather interesting. These species overlap very little around the Mediterranean. *Cistus creticus* occurs mainly in the eastern part of the Mediterranean basin whereas *C. albidus* is found only in the western part.

However, the two species can be found in sympatry in some parts of Italy and Morocco. There is some evidence that the distribution of *C. creticus* might not have always been the same in the past and has been different from what we find now. When the work for *Flora iberica* (Demoly and Montserrat 1993) was started, some very old herbarium specimens of *Cistus creticus* from Portugal and Catalonia were discovered. Most of those stations have now disappeared but one relictual population of *C. creticus* has since been found in the Albacete province and is now under protection.

For some authors *C. creticus* comprises three subspecies, *Cistus creticus* subsp. *eriocephalus*, *C. creticus* subsp. *corsicus*, and *C. creticus* subsp. *creticus* (Greuter et al. 1984) with some geographical structuring, but the areas of these taxa are not separated (Falchi et al. 2009; Paolini et al. 2009).

It can be wondered whether *C. creticus* and *C. albidus* might be two sub-units of a large ancestral species that has been fragmented into two geographical entities with a limited outcrossing barrier between them, or whether they are two ancient species that are now differentiated enough to be able to remain distinct, even when they are in sympatry. Their sequences were very similar, with few differences, and it would be useful to see how they behave where they grow in sympatry. Ecologically, these two species share some interesting similarities. They are the only taxa of the purple pink clade able to grow in chalky soil. On the whole, most *Cistus* species are found on acidic rather than basic soil. Some ecological differences can be found between the two species, *C. albidus* being less dynamic in acidic soil and it is also more sensitive to cold. In Morocco it is found only up to 1,100 m whereas *C. creticus* can reach 2,100 m (Raynaud 1992). In Spain it has been collected up to 1,300 m (Grant et al. 2006) and in Europe its altitudinal limit is 1,400 m (Tutin et al. 1968). When these two species are in sympatry, some fertile hybrids can be found (*C. × canescens* Sweet), but they do not take the place of their parents, nor do they seem to backcross preferentially with one of the parents, producing a large hybrid population with a wide spectrum of intermediate characters (Dansereau 1940). Despite these hybridization events, which are a frequent phenomenon within Cistaceae, these two taxa are considered as two distinct species and the hybrids do not take over because they are probably less adapted to their environment than the two parents.

In Corsica and Sardinia the differentiation of *C. creticus* is correlated with geology. The distribution of haplotypes separates them into two groups, one found on granite and the other on schist. The accumulation of mutations between these two groups indicates that this species has been there for a long time (Falchi et al. 2009). Heterogeneity of

habitat also seems to be involved in the genetic diversity of *C. albidus* (Grant et al. 2006).

The Canarian taxa

The purple pink-flowered *Cistus* reach their maximum endemism in the Canary Islands. Only four purple pink-flowered taxa are found outside this archipelago. The Canary Islands form a volcanic archipelago, with seven main islands, which have been dated from 20 to 21 million years (Fuerteventura) to less than 1 MA (El Hierro) (Emerson 2003; Garcia-Talavera 1999). *Cistus* species are only present on the five western islands. Fuerteventura and Lanzarote, which are among the older Islands and also the closest to the continent, do not have any *Cistus* species on them. They also lack forest habitats, and are of low elevation. In contrast, Gran Canaria, which has both forest and high elevation, and is also the oldest, ranging in age from 14 to 16 MA (Emerson 2003; Hoernle et al. 1991), is the first where *Cistus* species are encountered. Four species occur on Gran Canaria, *C. ochreatus*, the ubiquitous *C. monspeliensis*, which is also found on all five islands (Barquín-Diez and Voggenreiter 1988), a new recently described species *C. grancanariae* Marrero-Rodr., Almeida & C. Ríos (Marrero-Rodríguez et al. 2008), belonging to the subgenus *Leucocistus* (not included in our sampling), and *C. horrens* which was the sister species to the other Canarian taxa on the combined molecular tree. The plant

called *C. symphytifolius* var. *leucophyllus* (Spach), which is restricted to Gran Canaria, has been given species status and is now *C. ochreatus*. Previous studies showed, on the basis of allozymes, that it was already distinct from other *C. symphytifolius* varieties (Batista et al. 2001). Of the Canarian taxa, *C. horrens* seems to be the species “best adapted” to xeric conditions (Demoly 2004). Two polytomies, A and B, were found (Fig. 10). The taxa present in polytomy B were mainly from the south of the three islands on which they occur (La Gomera, Tenerife, and La Palma) with the exception of *C. symphytifolius* Puntagorda which occurs on the north-eastern part of La Palma. The members of the polytomy A (Fig. 10) were mainly from the north, east, or west of three islands (El Hierro, Tenerife, and Gran Canaria). *Cistus symphytifolius* is a widespread species (Batista et al. 2001). We sampled five *C. symphytifolius* taxa showing some variability and they did not group together, even on the same island, Tenerife, which is not surprising, because previous studies showed that little gene flow occurred between populations (Batista et al. 2001). *Cistus chinamadensis* comprises three subspecies, each constituted by a single population on a single island, and each of which is critically endangered (Bañares et al. 2008; Moreno 2008). They did not group together; the same pattern was found by Batista et al. (2001) with the two subspecies known at that time. The two subspecies of *C. osbeckiifolius* occurring on Tenerife were also in different clades. Because we only used chloroplast data further investigations are needed.

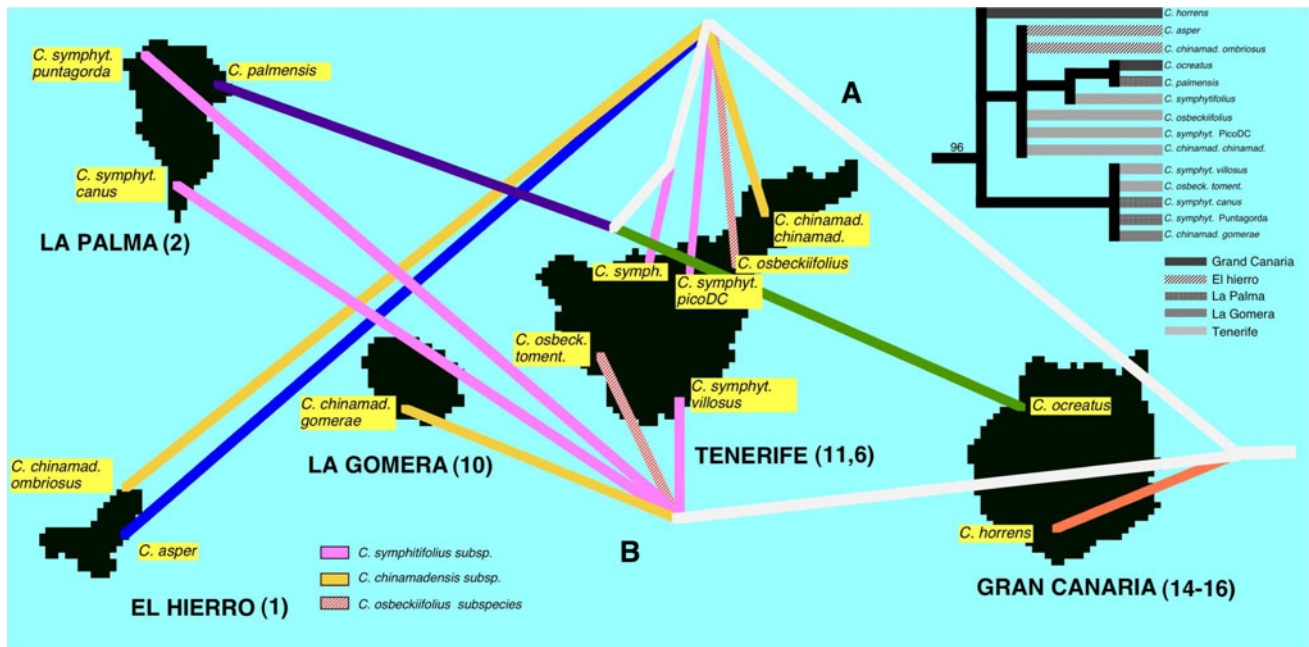


Fig. 10 The combined molecular tree, restricted to Canarian taxa, is mapped on the Canary Islands. The branches of the tree end at the place from which plants of group A or group B were collected.

Numbers in brackets refer to geological ages estimated in millions of years (Emerson 2003)

In the combined analysis, clade H 1 was between the PPC clade and the WWPC clade, but this position was not supported at all by bootstrap. Two species belonged to this clade: *Halimium umbellatum* and the recently described species *H. voldii* from Greece (Tan and Iatrou 2001); both are members of the section *Halimium* of that genus. It is the only group of *Halimium* with white flowers and it shares some morphological characters with *Cistus* of the section *Halimioides*: white flowers, linear leaves with an inwardly rolled margin, and an umbellate inflorescence (also present in *C. laurifolius* and *C. libanotis*).

Clades H 2 and H 3 were characterised by only yellow flowers and two sections of the genus *Halimium*, *Chrysorhodon* and the monospecific section *Commutati* (with only *H. calycinum*). Because of the position of *Halimium atlanticum* grouped with *H. calycinum*, the section *Chrysorhodon* was not monophyletic and either *H. atlanticum* should be transferred to the section *Commutati* or only a single section should be kept. In H 2, two well-supported groups can be morphologically distinguished. The two species *H. atriplicifolium* and *H. lasianthum* have homogeneous leaves whereas the rest of the group have two kinds of leaves depending on whether the shoot is sterile or fertile. Sterile stems have petiolated and trinerved leaves. On shoots bearing flowers, *H. halimifolius*, *H. lasiocalycinum*, and *H. halimifolium* ssp. *multiflorum* have sessile and uninerved leaves, and *H. ocymoides* has petiolated and one-nerved leaves. *H. lasiocalycinum* is often considered by botanists as a distinct species but there is only one base pair of divergence with *H. halimifolius* and nothing with *H. halimifolium* ssp. *multiflorum*. Both *H. lasiocalycinum* and *H. halimifolium* ssp. *multiflorum* should be regarded as varieties or subspecies of *H. halimifolius* as already suggested by some authors (Ball 1877; Grosser 1903). *Halimium atlanticum* in group H 3, has always been regarded as a close relative of *H. lasianthum*, but this was not confirmed by our analyses. There are 24 mutations between *H. atlanticum* and *H. calycinum*. These two species have a very divergent ecology, *H. atlanticum* is found above 1,500 m in the Rif in northern Morocco whereas the second is a species found on coastal sand dunes in Portugal, Spain, and the north of Morocco. Their distributions never overlap. They share three morphological characters: revolute leaf margins, pauciflorous inflorescences, and small size, but many other characters distinguish them. We included two samples of *H. atlanticum*, *H. atl.* 1587 and *H. atl.* 1595 collected on two different mountains. They were hardly distinguishable but they differed by three mutations, which is a lot more than between some species, for example *C. osbeckiifolius* or *C. chinamadensis*.

Morocco is the place where the most species are found for both *Cistus* (12) and *Halimium* (7) (Fennane et al. 1999). This country has a complex geography with numerous mountains that originated from paleo-islands within the Tethys (Dercourt et al. 1992), lately connected when the Mediterranean sea was formed. Because of its diverse environments and complex palaeohistory, Morocco is a species-rich country with a high percentage of endemic taxa. The common ancestor of the Canarian species probably originated from Morocco, first because it is the nearest continental land to the Canary Islands and, second, because all the closest three relatives (*Cistus heterophyllus*, *C. albidus*, and *C. creticus*) of the Canarian section *Macrostyliia* still occur in mainland Morocco.

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