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Historical biogeography and character evolution of Cistaceae (Malvales) based on analysis of plastid *rbcL* and *trnL-trnF* sequences

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Abstract

Cistaceae consist of eight genera and about 180 species. Some taxonomic limits and relationships within the family remain unresolved when relying exclusively on morphological data. In the present study, a phylogeny was reconstructed and divergence times were estimated for 47 species representing various groups in Cistaceae and using coding (*rbcL*) and spacer (*trnL-trnF*) sequences of plastid DNA. The firm set of morphological synapomorphies that indicates the monophyly of the family is supported by both Bayesian and parsimony analyses. Five major lineages can be distinguished within the Cistaceae: (1) an early-diverging lineage containing *Fumana* species; (2) the New World *Lechea* clade; (3) the *Helianthemum* s.l. clade, containing two sister groups, one of species from the New World (*Crocanthemum, Hudsonia*) and the other with species from the Old World (*Helianthemum* s. str.); (4) the *Tuberaria* clade; and (5) a cohesive complex consisting of *Halimium* and *Cistus* species. Evolutionary shifts in 12 key characters of Cistaceae are inferred based on the most plausible phylogenetic hypothesis. Reconstructing the evolution of ovule position supports anatropous ovules as the ancestral condition within the Cistaceae, which is currently found only in *Fumana*. The *Cistus*-*Halimium* assemblage is consistently obtained as a natural clade and further supported by a cytological synapomorphy (chromosome number $n = 9$). Optimisation of ancestral distribution areas and estimates of divergence times reveal an early divergence (10.17–18.51 Ma) of the Mediterranean-European genera, which may be related to subtropical vegetation, as complemented by paleobotanical data. In addition, the occurrence of multiple, independent migration events from the Old World to America between the Middle Miocene (8.44–14.7 Ma; *Lechea*) and the Upper Miocene (5.15–9.20 Ma; *Crocanthemum*/*Hudsonia*), and to the Canary Islands in the Pleistocene is inferred. We argue that the Mediterranean basin has been the main centre of differentiation of Cistaceae.

Keywords: Biogeography; Centre of differentiation; Character reconstruction; Malvales; *rbcL*; *trnL-trnF*

Introduction

Concerning the phylogenetic position of Cistaceae within the angiosperms, no full consensus has been reached in the last decades (Bixales, Takhtajan 1987; Violales, Cronquist 1988; Malvales, Dahlgren 1989; Violales, Thorne 1992; Cistales, Takhtajan 1997). Although Cistaceae share several vegetative and seed characters with other families (Alverson et al. 1998; Nandi 1998a, 1998b; Kubitzki and Chase 2003; Horn 2004), synapomorphies are difficult to be found. Given the difficulties in finding key morphological characters, it is necessary to use molecular phylogenetics to propose

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relationships within the order. In this context, molecular analyses based on plastid and nuclear sequences have confirmed the inclusion of Cistaceae within Malvales, forming the dipterocarpalean clade together with two tropical families (Dipterocarpaceae, Sarcolaenaceae) (Savolainen et al. 2000; Soltis et al. 2000). Close relationships between these three families had been stressed earlier by Nandi (1998b), which are supported by the presence of the peculiar bixoid chalazal region of the seed coat – a character previously found in Cistaceae, Bixaceae and Cochlospermaceae (Corner 1976; Takhtajan 1992) – and by wood-anatomical similarities between Dipterocarpaceae (subfam. Monotoideae) and Cistaceae (Baas and Werker 1981).

Cistaceae are a medium-sized family (eight genera, 180 species) typically consisting of heliophyte shrubs, subshrubs and herbs occurring in open areas on poor soils. Distributed in temperate and subtropical regions of the northern hemisphere, Cistaceae show the highest genus and species diversity in the Mediterranean floristic region. In fact, five of the eight genera (*Cistus*, *Fumana*, *Halimium*, *Helianthemum*, *Tuberaria*) are native to this region while the remaining three (*Crocanthemum*, *Hudsonia*, *Lechea*) inhabit temperate regions in America. The eight genera share hermaphroditic, actinomorphic and hypogynous flowers with three or five sepals (usually with the outer sepals smaller than the inner ones) opposite to petals (when petals are present). Cleistogamous flowers are common in certain genera (*Fumana*, *Lechea*, *Helianthemum, Crocanthemum*). The androecium has numerous fertile stamens, except for the outer, sterile stamens of *Fumana*. The gynoecium is formed by a three- or five-carpellate ovary (although *Cistus ladanifer* displays 6–12 carpels) and a solitary style with a single capitate or discoid stigma, except for the three free stigmas of *Lechea* (Table 1).

The taxonomy of Cistaceae has been based primarily on vegetative (growth form, leaf arrangement and attachment) and reproductive (sepal number, petal number and colour, style length, stamen fertility, number of fruit valves) characters (Table 1). Some taxonomic treatments of the family have been available since the 18th century, including various generic and infrageneric classifications (Tournefort 1718; Linnaeus 1753; Dunal 1824; Spach 1836; Willkomm 1856; Grosser 1903; Ponzo 1921; Martín Bolaños and Guinea 1949) (Table 2). Eight genera were recognised using morphological and molecular characters in the latest taxonomic assessment (Arrington and Kubitzki 2003). A particular point of disagreement between previous classifications of Cistaceae is the recognition of the genus *Crocanthemum* as an independent taxonomic entity. The circumscription within the family of the New World species of *Crocanthemum* is one of the most problematic, resulting in some authors arguing for a separate genus (Martín Bolaños and Guinea 1949; Arrington and Kubitzki 2003), whereas others proposed its placement in *Helianthemum* (Fernald 1917; Calderón de Rzedowski 1992). In addition, *Halimium* has formed a non-monophyletic group in previous analyses based on DNA sequence data (Guzmán and Vargas 2005, 2009). In particular, one species of *Halimium* and all species of *Cistus* formed a natural group (Guzmán and Vargas 2005). This result, as well as the sharing of some morphological and karyological characters, suggested the need for an in-depth study including all *Halimium* species.

In addition, a well-supported phylogeny of Cistaceae has not been proposed to date. In the present study, we utilise DNA sequence data from the plastid *rbcL* gene and the *trnL-trnF* spacer to (1) test the monophyly of Cistaceae; (2) identify monophyletic groups in Cistaceae and relate them to generic circumscriptions; (3) infer sister-group relationships within the Cistaceae; and (4) offer new insights into evolutionary changes in key characters.

Material and methods

Sampling strategy and DNA sequencing

A total of 54 individuals representing all species of *Cistus* (21), three of *Crocanthemum* (about 20 taxonomically recognised), three of *Fumana* (9 recognised), all of *Halimium* (8), eight of *Helianthemum* (about 90 recognised), one of *Hudsonia* (3 recognised), one of *Lechea* (17 recognised) and two of *Tuberaria* (12 recognised) were sampled for *trnL-trnF* and *rbcL* sequencing (Arrington and Kubitzki 2003; Table 3). A particular effort was made to include representatives of most infrageneric taxa (i.e. species representing most of the subgenera and sections). Accordingly, we have sequenced one representative of each of the three *Fumana* subgenera (*Fumana, Pomelina, Fumanopsis*), one of two sections of *Lechea* (sect. *Lechea*), three species of *Crocanthemum* section *Lecheoides* (but none of the three species in section *Spartioides*), one species representing the small genus *Hudsonia* (three species), six species representing four out of seven sections of *Helianthemum*, and one species of each of the two sections of *Tuberaria* (*Tuberaria, Xolantha*) (Table 2). Total genomic DNA was extracted from material collected in the field, from plants in the living collections of R. Page, O. Filippi and the Royal Botanic Garden of Madrid, and from eight herbarium specimens (MA). Field-collected material was dried in silica gel. DNA was extracted using Kneasy Plant Mini Kit (QIAGEN Inc., California) and amplified using the PCR (Polymerase Chain Reaction) on a Perkin-Elmer PCR System 9700 (California) or an MJ Research (Massachusetts) thermal cycler. Standard primers were used for direct amplification

Table 1. Morphological characters and character states on which the taxonomy of the Cistaceae genera has been mostly based; data from Martín Bolaños and Guinea (1949), Calderón de Rzedowski (1992), Demoly and Montserrat (1993), Ukraintseva (1993), Nandi (1998a, 1998b), Arrington and Kubitzki (2003), and our own observations.

	No. of sepals	No. of petals	Petal colour	Staminodes	No. of stamens	No. of stigmas	Style length
Cistus L.	3–5	5	white, purple	no	many (50–200)	1	sessile, short, elongate
Crocanthemum Spach	5	5	yellow	no	few to many	1	short
Fumana (Dunal) Spach	5	5	yellow	yes	numerous (26–40)	1	elongate
Halimium (Dunal) Spach	3(5)	5	white, yellow	no	numerous to many (20–100)	1	sessile, short
Helianthemum Mill.	5	5	white, yellow, purple	no	few to many (7–100)	1	elongate
Hudsonia L.	5	5	yellow	no	few to numerous (10–30)	1	elongate
Lechea L.	5	3	dark red	no	few to numerous (3–25)	3	sessile
Tuberaria (Dunal) Spach	5	5	yellow	no	few to numerous (10–50)	1	sessile, short

	No. of carpels	Ovule position	Embryo shape	Pollen type	Flower type
Cistus L.	5–12	orthotropous	circinate	*Cistus*	chasmogamous
Crocanthemum Spach	(2)3	orthotropous	curved	*Crocanthemum*	chasmogamous, cleistogamous
Fumana (Dunal) Spach	3	anatropous	curved	*Fumana, Helianthemum*	chasmogamous
Halimium (Dunal) Spach	3	orthotropous	curved to circinate	*Cistus*	chasmogamous
Helianthemum Mill.	3	orthotropous	simple plicate, biplicate	*Helianthemum*	chasmogamous, cleistogamous
Hudsonia L.	3	orthotropous	curved	*Hudsonia*	chasmogamous
Lechea L.	3	orthotropous	linear, slightly curved	*Lechea*	chasmogamous, cleistogamous
Tuberaria (Dunal) Spach	3	orthotropous	triangular	*Cistus*	chasmogamous

	Leaf attachment	Leaf arrangement on vegetative branches	Gametophytic number of chromosomes (n =)	Life form
Cistus L.	exstipulate	opposite	9	shrubs
Crocanthemum Spach	exstipulate	alternate	10	shrubs
Fumana (Dunal) Spach	exstipulate, stipulate	alternate, opposite	16	dwarf shrubs
Halimium (Dunal) Spach	exstipulate	opposite	9	shrubs, suffruticoses
Helianthemum Mill.	exstipulate, stipulate	opposite	10(5, 12, 20)–11	shrubs, subshrubs, herbs
Hudsonia L.	exstipulate	alternate	10	low shrubs
Lechea L.	exstipulate	alternate	–	perennial suffruticoses
Tuberaria (Dunal) Spach	exstipulate, stipulate	opposite, alternate	12, 18, 24	annual or perennial herbs

Table 2. Historical overview of Cistaceae classification.

Present study	Tournefort (1718)	Linnaeus (1753–1756)	Dunal (1824)	Spach (1836)
Cistus L.	*Cistus* Tourn.	*Cistus* L.	Genus *Cistus* Tourn. Sect. I. *Erythrocistus* Dunal Sect. II. *Ledonia* Dunal Genus *Helianthemum* Tourn. Sect. I. *Halimium* Dunal	Tribus I. *Cisteae* Spach Sect. II. *Cistineae* Spach Subdivisio 2. *Cistoideae* Spach Genus *Ladanium* Spach Genus *Rhodocistus* Spach Genus *Cistus* (Tourn.) Spach Sect. I. *Rhodopsis* Spach Sect. II. *Eucistus* Spach Sect. III. *Ledonella* Spach Genus *Stephanocarpus* Spach Genus *Ledonia* Spach Genus *Halimium* Dunal
Crocanthemum Spach	*Helianthemum* Tourn.	*Cistus* L.	Genus *Helianthemum* Tourn. Sect. II. *Lecheoides* Dunal	Tribus I. *Cisteae* Spach Sect. II. *Cistineae* Spach Subdivisio 3. *Heteromerineae* Spach Genus *Crocanthemum* Spach Genus *Heteromeris* Spach Genus *Taeniostema* Spach
Fumana (Dunal) Spach	*Helianthemum* Tourn.	*Cistus* L.	Genus *Helianthemum* Tourn. Sect. VII. *Fumana* Dunal	Tribus I. *Cisteae* Spach Sect. I. *Fumanieae* Spach Genus *Fumana* Dunal
Halimium (Dunal) Spach	*Helianthemum* Tourn.	*Cistus* L.	Genus *Helianthemum* Tourn. Sect. I. *Halimium* Dunal	Tribus I. *Cisteae* Spach Sect. II. *Cistineae* Spach Subdivisio 2. *Cistoideae* Spach Genus *Halimium* Dunal
Helianthemum Mill.	*Helianthemum* Tourn.	*Cistus* L.	Genus *Helianthemum* Tourn. Sect. IV. *Macularia* Dunal Sect. V. *Brachypetalum* Dunal Sect. VI. *Eriocarpum* Dunal Sect. VIII. *Pseudocistus* Dunal Sect. IX. *Euhelianthemum* Dunal	Tribus I. *Cisteae* Spach Sect. II. *Cistineae* Spach Subdivisio 1. *Helianthemoideae* Spach Genus *Helianthemum* (Tourn.) Spach Sect. I. *Aphananthemum* Spach Sect. II. *Eriocarpum* Dunal Sect. III. *Euhelianthemum* Dunal Sect. IV. *Argyrolepis* Spach Genus *Rhodax* Spach
Hudsonia L.	not included	*Hudsonia* L.	Genus *Hudsonia* L.	Genus anomalum Genus *Hudsonia* L.
Lechea L.	not included	*Lechea* L.	Genus *Lechea* L.	Tribus II. *Lechidieae* Spach Genus *Lechea* (L.) Spach Genus *Lechidium* Spach
Tuberaria Dunal	*Helianthemum* Tourn.	*Cistus* L.	Genus *Helianthemum* Tourn. Sect. III. *Tuberaria* Dunal	Tribus I. *Cisteae* Spach Sect. II. *Cistineae* Spach Subdivisio 1. *Helianthemoideae* Spach Genus *Tuberaria* (Dunal)

Present study	Willkomm (1856)	Grosser (1903)	Ponzo (1921)
Cistus L.	Subfam. I. Cistoideae Willk. Tribus I. Normales Willk. Subtribus 1. Cisteae Willk. Genus *Cistus* Tourn. Subgenus I. *Erythrocistus* Dunal Sect. 1. *Macrostylia* Willk. Sect. 2. *Brachystylia* Willk. Sect. 3. *Astylia* Willk. Subgenus II. *Leucocistus* Willk. Sect. 4. *Stephanocarpus* Spach Sect. 5. *Ledonia* Spach Sect. 6. *Ladanium* Spach Sect. 7. *Halimioides* Willk.	Genus *Cistus* L. Group A. Sect. 1. *Rhodocistus* (Spach) Gross. Sect. 2. *Eucistus* Spach Sect. 3. *Ledonella* Spach Group B. Sect. 4. *Stephanocarpus* (Spach) Willk. Sect. 5. *Ledonia* Dunal Group C. Sect. 6. *Ladanium* (Spach) Willk. Sect 7. *Halimioides* Willk.	*Cistus* L.

Table 2. (*continued*)

Present study	Willkomm (1856)	Grosser (1903)	Ponzo (1921)
Crocanthemum Spach	Subfam. I. Cistoideae Willk. Tribus I. Normales Willk. Subtribus 1. Cisteae Willk. Genus *Crocanthemum* Spach	Genus *Halimium* (Dunal) Willk. Group B. Sect. 3. *Lecheoides* Dunal	*Heteromeris* Spach
Fumana (Dunal) Spach	Subfam. I. Cistoideae Willk. Tribus I. Normales Willk. Subtribus 2. Fumaneae Willk. Genus *Fumana* Spach Sect. 1. *Helianthemoides* Willk. Sect. 2. *Eufumana* Willk.	Genus *Fumana* (Dunal) Spach.	*Fumana* (Dunal) Spach
Halimium (Dunal) Spach	Subfam. I. Cistoideae Willk. Tribus I. Normales Willk. Subtribus 1. Cisteae Willk. Genus *Halimium* Willk. Sect. 1. *Oligospermia* Willk. Sect. 2. *Polyspermia* Willk.	Genus *Halimium* (Dunal) Willk. Group A. Sect. 1. *Spartioides* Gross. Group B. Sect. 2. *Euhalimium* Gross.	*Halimium* (Dunal) Willk.
Helianthemum Mill.	Subfam. I. Cistoideae Willk. Tribus I. Normales Willk. Subtribus 1. Cisteae Willk. Genus *Helianthemum* Willk. Subgenus I. *Ortholobum* Willk. Sect. 1. *Brachypetalum* Dunal Sect. 2. *Eriocarpum* Dunal Sect. 3. *Euhelianthemum* Dunal Sect. 4. *Polystachyum* Willk. Subgenus II. *Plectolobum* Willk. Sect. 5. *Chamaecistus* Willk.	Genus *Helianthemum* Adans. Subgen. I. *Ortholobum* Willk. Group A. Sect. 1. *Polystachyum* Willk. Sect. 2. *Euhelianthemum* Dunal Sect. 3. *Pseudomacularia* Gross. Group B. Sect. 4. *Eriocarpum* Dunal Sect. 5. *Brachypetalum* Dunal Subgen. II. *Plectolobum* Willk.. Sect. 1. *Chamaecistus* Willk. Sect. 2. *Macularia* Dunal	*Helianthemum* Willk.
Hudsonia L.	Subfam. I. Cistoideae Willk. Tribus I. Normales Willk. Subtribus 1. Cisteae Willk. Genus *Hudsonia* L.	Genus *Hudsonia* L.	*Hudsonia* L.
Lechea L.	Subfam. II. Lechidioideae Willk. Genus *Lechea* (L.) Spach Genus *Lechidium* Spach	Genus *Lechea* Kalm. Sect. 1. *Eulechea* Robins Sect. 2. *Lechidium* Torr	*Lechea* L.
Tuberaria Dunal	Subfam. I. Cistoideae Willk. Tribus I. Normales Willk. Subtribus 1. Cisteae Willk. Genus *Tuberaria* Spach Sect. 1. *Eutuberaria* Willk. Sect. 2. *Scorpioides* Willk.	Genus *Tuberaria* (Dunal) Spach Sect. 1. *Eutuberaria* Willk. Sect. 2. *Scorpioides* Willk.	*Tuberaria* (Dunal) Spach

of the *trnL*(UAA)-*trnF*(GAA) spacer (Taberlet et al. 1991), whereas the *rbcL* exon was amplified in two overlapping segments using a combination of primers 1F-724R and 636F-1460R (Savolainen et al. 2000). After 1–3 min pretreatment at 94 °C, PCR conditions for *rbcL* and *trnL-trnF* amplification were: 24–28 cycles of 1 min at 94 °C, 1 min at 48–50 °C and 2–4 min at 72 °C. A volume of 1 μl of dimethyl-sulfoxide (DMSO) was included in each 25 μl reaction. Amplified products were cleaned using spin filter columns (PCR Clean-up kit, MoBio Laboratories, California) following the manufacturer's protocols. Cleaned products were then directly sequenced using dye terminators (Big Dye Terminator v. 2.0, Applied Biosystems, Little Chalfont, UK) following the manufacturer's protocols, and run on polyacrylamide electrophoresis gels (7%) using an Applied Biosystems Prism model 3700 automated sequencer. PCR primers were used for cycle sequencing of the *trnL-F* spacer and the *rbcL* exon. Sequenced data were assembled and edited using the Seqed programme (Applied Biosystems, California). The limits of the regions were determined by positions of flanking primers. IUPAC (International Union of Pure and Applied Chemistry) symbols were used to represent nucleotide ambiguities.

Molecular analysis

DNA sequence variation was used to reconstruct phylogenetic relationships using Bayesian Inference (BI) and maximum parsimony (MP). A combination of the *rbcL* and *trnL-trnF* data sets of Cistaceae and eight representatives of other families in Malvales was

Table 3. Cistaceae taxa studied (taxonomy after Savolainen et al. 2000; Guzmán and Vargas 2005), sources of material, voucher data, and GenBank accessions (sequences of Bixaceae, Dipterocarpaceae, Muntingiaceae, Thymelaeaceae, Sarcolaenaceae, Sphaerosepalaceae obtained from GenBank).

Taxon	Locality/source	Voucher	Accession no.	
			trnL-trnF	*rbcL*
Cistus L.				
Cistus albanicus E.F. Warb. ex Heywood	cultivated	R.G. Page 8cBGA04 (MA)	DQ093030	FJ225880
Cistus albidus L	Spain, Madrid, Aldea del Fresno	P. Vargas 25PV03 (MA)	DQ093021	FJ492042
Cistus chinamadensis Bañares et Romero	Canary Islands, La Gomera	Á. Fernández & J. Leralta 44BGA04 (MA)	DQ093033	FJ225866
Cistus clusii Dunal	Spain, Málaga, Mijas	R.G. Page 8bBGA04 (MA)	DQ093056	FJ225879
Cistus creticus L.	Greece, Olympus	P. Vargas 209PV04 (MA)	DQ093025	FJ225862
Cistus crispus L.	Spain, Córdoba, Posadas	B. Guzmán 58BGA04 (MA)	DQ093060	FJ225882
Cistus heterophyllus Desf.	Morocco, Beni-Hadifa	B. Guzmán 99BGA04 (MA)	DQ093036	FJ225868
Cistus horrens Demoly	Canary Islands, Gran Canaria, Ayacata	B. Guzmán 2BGA05 (MA)	FJ492018	FJ492045
Cistus ladanifer L.	Spain, Madrid, Boadilla del Monte	B.Guzmán 7BGA03 (MA)	DQ093043	FJ225872
Cistus laurifolius L.	Spain, Jaén, Sierra de Segura	B. Guzmán 13BGA03 (MA)	DQ093052	FJ225876
Cistus libanotis L.	Spain, Córdoba	R.G. Page 149BGA04 (MA)	DQ093040	FJ225870
Cistus monspeliensis L.	Portugal, Sagres	B. Guzmán 35BGA04 (MA)	DQ093059	FJ225881
Cistus munbyi Pomel	Morocco	O. Filippi 4BGA04 (MA)	DQ093053	FJ225877
Cistus ochreatus C. Sm. ex Buch	Canary Islands, Gran Canaria	R.G. Page 8BGA04 (MA)	DQ093032	FJ492043
Cistus osbeckiifolius Webb ex Christ	Canary Islands, Tenerife	P. Escobar 48/05 (MA)	FJ492017	FJ492044
Cistus parviflorus Lam.	Greece, Crete	O. Filippi 6BGA04 (MA)	DQ093023	FJ225861
Cistus populifolius L.	Portugal, Ourique	B. Guzmán 20BGA04 (MA)	DQ093049	FJ225875
Cistus pouzolzii Delile	France	R.G. Page 8tBGA04 (MA)	DQ093054	FJ492046
Cistus psilosepalus Sweet	Spain, Ávila, Arenas de San Pedro	P. Vargas 7PV03 (MA)	DQ093041	FJ225871
Cistus salviifolius L.	Spain, Ávila, Arenas de San Pedro	P. Vargas 6PV03 (MA)	DQ093037	FJ225869
Cistus symphytifolius Lam.	Canary Islands, La Palma, La Cumbrecita	B. Guzmán 143BGA04 (MA)	DQ093057	FJ225877
Crocanthemum Spach				
Crocanthemum argenteum (S.Watson) Janch.	Mexico, Guanajuato	J. Rzedowski (MA527770)	FJ492000	FJ492026
Crocanthemum chihuahuense S.Watson	Mexico, Michoacán	G. Calderón (MA527771)	FJ491999	FJ225856
Crocanthemum pringlei (S.Watson) Janch.	Mexico, Guanajuato	G. Calderón (MA527767)	FJ491998	FJ225855
Fumana (Dunal) Spach				
Fumana ericoides Pau	Spain, Almería, Cabo de Gata	B. Guzmán 3BGA06 (MA)	FJ491992	FJ492020
Fumana fontanesii Clauson ex Pomel	cultivated	J. Güemes 121BGA04 (MA)	FJ491993	FJ492021
Fumana thymifolia (L.) Spach ex Webb	Portugal, Ferrerías	B. Guzmán 53BGA04 (MA)	DQ093015	FJ225850
Halimium (Dunal) Spach				
Halimium atlanticum Humbert & Maire	Morocco, Tazzeka (1)	RDG14/2006/5	FJ492006	FJ492032
Halimium atlanticum Humbert & Maire	Morocco, Bab-Taza (2)	J. Molero et al. (RDG14/2006/1)	FJ492007	FJ492033
Halimium atriplicifolium (Lam.) Spach	Spain, Granada, Sierra Almizara (1)	J.M. Martínez 7BGA07 (MA)	FJ492008	FJ492034
Halimium atriplicifolium (Lam.) Spach	Spain, Málaga, Coín (2)	R.G. Page 155bBGA05 (MA)	FJ492009	FJ225859
Halimium calycinum (L.) K. Koch	Portugal, Cabo Sardao (1)	B. Guzmán 49BGA04 (MA)	DQ093020	FJ492039
Halimium calycinum (L.) K. Koch	Portugal, Cabo de San Vicente (2)	B. Guzmán 37BGA04 (MA)	FJ492014	FJ492038
Halimium halimifolium (L.) Willk. *halimifolium*	Spain, Málaga, Marbella	A. Segura (MA 580185)	FJ492015	FJ492040
Halimium halimifolium (L.) Willk. *multiflorum* (Salzm. Ex Dunal) Marie	Portugal, Pegoes	E. Monasterio et al. (MA 459452)	FJ492016	FJ492041
Halimium lasianthum (Lam.) Spach *lasianthum*	Spain, Málaga, Parque Nacional Alcornocales	P. Vargas 3PV06 (MA)	FJ492004	FJ492030
Halimium lasianthum (Lam.) Spach *alyssoides* (Lam.) Greuter	Portugal, Algarve	L. Medina et al. (MA690834)	FJ492005	FJ492031

Halimium lasiocalycinum (Boiss. & Reut.) Gross ex Engl. *riphaeum* (Pau & Font Quer) Maire	Morocco, Bab-Berred	P. Escobar 665/04 (MA)	FJ492013	FJ492037
Halimium ocymoides (Lam.) Willk.	Portugal, Coimbra (1)	R.G. Page 158BGA04 (MA)	FJ492011	FJ492035
Halimium ocymoides (Lam.) Willk.	Spain (2)	R.G. Page 158bBGA04 (MA)	FJ492010	FJ225858
Halimium umbellatum (L.)	Spain, Madrid, Tres Cantos	P. Vargas 71BGA04 (MA)	DQ093014	FJ225857
Halimium umbellatum (L.)	Spain, Ciudad Real, S^{a} Morena	L. Serra (MA705587)	FJ492012	FJ492036
Helianthemum Mill.				
Helianthemum aegyptiacum (L.) Mill.	Spain, Madrid, Rivas VaciaMadrid	P. Vargas 200PV04 (MA)	FJ491996	FJ492024
Helianthemum almeriense Pau	Spain, Granada, Calahonda	B. Guzmán 80BGA04 (MA)	FJ491997	FJ492025
Helianthemum kahiricum Delile	Morocco	P. Escobar 93/04 (MA)	FJ492003	FJ492029
Helianthemum ledifolium (L.) Mill.	Spain, Madrid, Tres Cantos	P. Vargas 185PV05 (MA)	FJ491995	FJ492023
Helianthemum marifolium (L.) Mill.	Portugal, Sagres	B. Guzmán 31BGA04 (MA)	FJ492002	FJ492028
Helianthemum oelandicum (L.) Dum. Cours.	–	J.M. Martínez 8BGA07 (MA)	FJ492001	FJ492027
Helianthemum scopulicolum L.	cultivated	B. Guzmán 67BGA04 (MA)	DQ093017	FJ225852
Helianthemum squamatum (L.) Dum. Cours.	cultivated	B. Guzmán 70BGA04 (MA)	DQ093016	FJ225851
Hudsonia L.				
Hudsonia tomentosa Nutt.*	USA, MI, dunes N. of Luddington	Chase & Fay 14587	FJ491991	FJ492019
Lechea L.				
Lechea tripetala (Moc. & Sessé ex Dunal) Britton	Mexico, Guanajuato	J. Rzedowski (MA527766)	–	FJ492022
Tuberaria Dunal				
Tuberaria guttata (L.) Fourr.	Portugal, Vila do Bispo	B. Guzmán 44BGA04 (MA)	DQ093018	FJ225853
Tuberaria globulariifolia (Lam.) Gallego	Spain, Orense, Sierra de Xures	J. Martínez 269JM04 (MA)	FJ491994	FJ225854
Bixaceae				
Diegodendron Capuron				
Diegodendron humbertii Capuron	Madagascar	Fay et al. (1998)	–	Y15138
Dipterocarpaceae				
Anisoptera Korth.				
Anisptera costata Korth.	–	Yuwa-amornpitak, T. et al. (unpublished data)	DQ157291	–
Anisoptera marginata Korth.	–	Fay et al. (1998)	–	Y15144
Hopea Roxb.				
Hopea hainanensis Merr. & Chun	–	Cho et al. (unpublished data)	–	AJ247623.1
Monotes A.D.C.				
Monotes madagascariensis Humbert	–	Gamage et al. (2006)	AB246543.1	–
Muntingiaceae				
Muntingia L.				
Muntingia calabura L.	–	Fay et al. (1998)	–	Y15146
Thymelaeaceae				
Aquilaria Lam.				
Aquilaria beccariana Tiegh.	–	Fay et al. (1998)	–	Y15149
Sarcolaenaceae				
Sarcolaena Thouars				
Sarcolaena multiflora Thou.	–	Ducousso et al. (2004)	–	AY157715
Sphaerosepalaceae				
Rhopalocarpus Coger				
Rhopalocarpus sp.	–	Fay et al. (1998)	–	Y15148

* Plant material from The Royal Botanic Gardens, Kew, DNA Bank (www.rbgkew.org.uk/data/dnaBank/homepage.html).

analysed. We chose six of the most closely related families as the outgroup: Bixaceae, Dipterocarpaceae, Muntingiaceae, Thymelaeaceae, Sarcolaenaceae and Sphaerosepalaceae (Alverson et al. 1998; Soltis et al. 2005) (Table 3). Sequences were aligned using Clustal X 1.62b (Thompson et al. 1997), with further adjustments by visual inspection.

For the Bayesian Inference (BI) analysis, the simplest model of sequence evolution that best fits the sequence data was determined using the Hierarchical Likelihood Ratio Test (hLRT) and the Akaike Information Criterion (AIC). Both tests were conducted separately for each data set using MrModeltest 1.1b (Posada and Crandall 1998; Nylander 2002). The optimal models of evolution found were GTR+G for *trnL-trnF*, and GTR+I+G for *rbcL*. Bayesian Inference analysis was conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003), with each model substitution fitted to each molecular partition. Four chains were run twice for ten million generations (chain temperature = 0.2; sample frequency = 100). In both runs probabilities converged on the same stable value approximately after generation 1,000,000. A 50% majority–rule consensus tree was calculated using the *sumt* command to yield the final Bayesian estimate of phylogeny.

Parsimony analyses were conducted using Fitch parsimony as implemented in PAUP* (Swofford 2002), with equal weighting of all characters and of transitions/transversions. We performed 100,000 random-addition replicates of heuristic searches with TBR, holding 100 trees per replicate and the options Multrees (keeping multiple, shortest trees) and Steepest Descent in effect. Internal support was assessed using 5,000,000 bootstrap replicates (fast stepwise addition; Mort et al. 2000).

Evolutionary patterns of fourteen morphological characters considered to be taxonomically important in Cistaceae were traced on one of the most parsimonious trees chosen based on congruence with the BI analysis using MacClade 4.06 (Maddison and Maddison 2000). Exploration of character changes was made using ACCTRAN optimisation and assuming Fitch parsimony, equal weighting of all characters, transitions among all states equally probable, and characters as unordered. Character states were determined from the literature and personal observations. Because we did not sample all species for the DNA-based phylogeny, we chose species representing sections, subgenera and genera and coded them either as having a particular character state of the taxon, when the latter is monomorphic, or as having multiple character states, when the taxon is polymorphic. For instance, flower colour in *Helianthemum* was coded as polymorphic because three flower colours are known in the genus and our sampling was too limited to resolve the ancestral state in the genus.

To obtain a dated phylogeny, the combined data set of *rbcL* and *trnL-F* sequences set was analysed using a relaxed Bayesian approach as implemented in BEAST version 1.4.6 (Drummond and Rambaut 2007). This method allows the likely probability distribution for node ages to be modelled as priors, and uncertainty about the dates of nodes used for calibration, as well as tree uncertainty, to be incorporated into the analysis (Drummond et al. 2006). A Yule process speciation prior and an uncorrelated log-normal model of rate variation were implemented. The analysis was run in the absence of topological constraints, except where these were necessary to ensure resolution of the calibration node. Node constraints were assigned a uniform distribution model. Posterior probability distributions of node ages were obtained for the combined data set in a concatenated and partitioned framework (model parameters were unlinked across partitions). The respective optimal models of nucleotide substitution for each data set were as identified above for part of the phylogenetic analyses using MrModelTest. Posterior distributions for each parameter were obtained using a Monte Carlo Markov Chain (MCMC), which was run for 15 million generations (burn-in 20%) and sampled every 1,000th generation. Tracer v1.4 was then used to measure the effective sample size of each parameter (all resulting effective sample sizes exceeded 700, in most cases by a large margin) and to calculate the mean and the upper and lower bounds of the 95% highest posterior density interval (95% HPD) for divergence times (Rambaut and Drummond 2007). Trees were summarised as maximum clade credibility trees using the TreeAnnotator, and visualised using FigTree version 1.0 (Rambaut 2006). To calibrate the BEAST analysis, we used the minimum and maximum age constraints of the Dipterocarpaceae/Cistaceae divergence from Wikström et al. (2001), one macrofossil age (Palibin 1909), and two pollen records (Naud and Suc 1975; Menke 1976), as in Guzmán and Vargas (2009).

Results

Phylogenetic relationships

The characteristics of the two sequence data sets are summarised in Table 4. The combined data matrix of Cistaceae plus outgroup sequences consists of 1,884 characters, of which 544/339 are variable/parsimony-informative. The heuristic search resulted in 32,689 equally parsimonious trees, each with a length of 944 steps, a Consistency Index (CI) of 0.64 and a Retention Index (RI) of 0.85. The BI analysis of the combined data matrix (*rbcL*, *trnL-F*) displays better resolution (Fig. 1) than the strict consensus tree (results not shown). Both analyses show the family Cistaceae as monophyletic

Table 4. Characteristics of the *trnL-trnF* and *rbcL* sequences obtained for Cistaceae + outgroup (see text).

	trnL-trnF	*rbcL*
Total aligned length (bp)	519	1,405
Length range Cistaceae + outgroup (bp)	399–409	1,405
Length range ingroup (bp)	324–460	1,405
Total number of characters	519	1,365
Variable/parsimony-informative characters	181/118	157/108
Maximum sequence divergence (GTR)	25.8%	4.47%
Mean G + C content	32.0%	54.00%

(100 PP, 72% BS) and as sister to the tropical families Dipterocarpaceae and Sarcolaenaceae (Fig. 1). *Fumana* consistently branches off first in the Cistaceae, followed by *Lechea*. Only BI strongly supports (100 PP) the close relationship of the New World genera *Crocanthemum* and *Hudsonia* to the Old World genus *Helianthemum*. A very close relationship between two of the American genera can be inferred from the strong grouping of *Hudsonia tomentosa* and *Crocanthemum argenteum* (100 PP, 94% BS). The phylogenetic analyses reveal that all accessions of *Helianthemum* form a well-supported monophyletic lineage (100 PP, 79% BS). *Tuberaria* appears to be monophyletic (100 PP, 99% BS) and sister to *Halimium* plus *Cistus*, although the monophyly of the latter two genera has low support (73 PP; 66%BS). Both BI and MP indicate monophyly of the respective conspecific accessions of *Halimium atlanticum* (100 PP; 99% BS), *H. calycinum* (100 PP; 96% BS), *H. umbellatum* (100 PP; 100% BS), and *H. ocymoides* (100 PP; 94% BS). *Halimium* species are grouped into three ladderised subclades, but relationships among subclades are weakly supported in the BI and not resolved in the MP analysis. *Cistus* accessions form a weakly supported monophyletic group as long as *H. umbellatum* is included. Only two well-supported clades of *Cistus* species are retrieved: the purple-flowered (subgenus *Cistus,* excluding *C. parviflorus*) (100 PP, 93% BS) and the white-flowered (subgenera *Leucocistus* and *Halimioides*) (100 PP, 87% BS) species. The BI analysis depicts *Halimium umbellatum* as sister to the white-flowered lineage of *Cistus* (89 PP), whereas the MP analysis gives low support.

Patterns of character evolution

The most relevant results from the historical reconstructions can be described as follows.

1. Ovule position (Fig. 2A). The reconstruction of character states reveals *Fumana* as the only genus in Cistaceae retaining the plesiomorphic state (anatropous ovules), whereas the other genera acquired orthotropous ovules once.
2. Pollen type (Fig. 2B). The *Tuberaria-Halimium-Cistus* (*Cistus*-type) has a single origin whereas the *Helianthemum*-type could have arisen independently twice as this pollen type is also found in *Fumana*.
3. Chromosome number (Fig. 2C). A haploid number of $n = 10$ is common within a basal grade of Cistaceae (*Crocanthemum, Helianthemum, Hudsonia*), including a shift to $n = 11$. Interestingly, the number is higher in *Fumana* ($n = 16$), but lower in the crown-group including all the species of the *Cistus-Halimium* complex ($n = 9$).
4. Distribution (Fig. 2D). Based on the data provided here, an Old World ancestry of Cistaceae is inferred from optimising the distribution of the species. In addition, the reconstruction shows two independent migration events of Cistaceae to America. Our results agree with a major centre of diversification in the Mediterranean region and Europe. Character reconstruction together with the occurrence of endemic species indicate at least three independent introductions to the Canarian archipelago: one of the white-flowered *Cistus* (*C. monspeliensis*), one of the purple-flowered *Cistus* (five species), and at least one of *Helianthemum*.
5. Life form (not figured). The character is revealed as very homoplastic within the family. Plants in the outgroup (Dipterocarpaceae, Sarcolaenaceae) are mostly trees, which makes outgroup comparison difficult. A shrub form appeared up to five times in the *Cistus-Halimium* complex, whereas the herbaceous form (perennial and annual herbs) arose independently in three genera (*Crocanthemum*, *Helianthemum*, *Tuberaria*). Subshrub forms appear to be plesiomorphic and, then, retained at least in some species of all eight genera of Cistaceae.
6. Petal colour (not figured). The character state reconstruction shows yellow flowers as plesiomorphic. Purple flowers evolved twice in *Cistus*, whereas only one change from yellow to white flowers was inferred in the *Cistus-Halimium* assemblage. Red flowers are an autapomorphy of the American genus *Lechea*.
7. Sepal number (not figured). A calyx with five sepals has been mostly maintained in Cistaceae. Evolution of this character has, however, been dynamic in the *Tuberaria-Halimium-Cistus* group. For instance, character optimisation suggests acquisition of three sepals at least twice independently in *Cistus*.
8. Carpel number (not figured). An increase in carpel number was clearly observed in Cistaceae. It cannot be decided, however, whether the 5-carpellate ovary evolved twice in *Cistus* or whether the 3-carpellate ovary in *Halimium umbellatum* is a reversal to the ancestral condition (Guzmán and Vargas 2005).

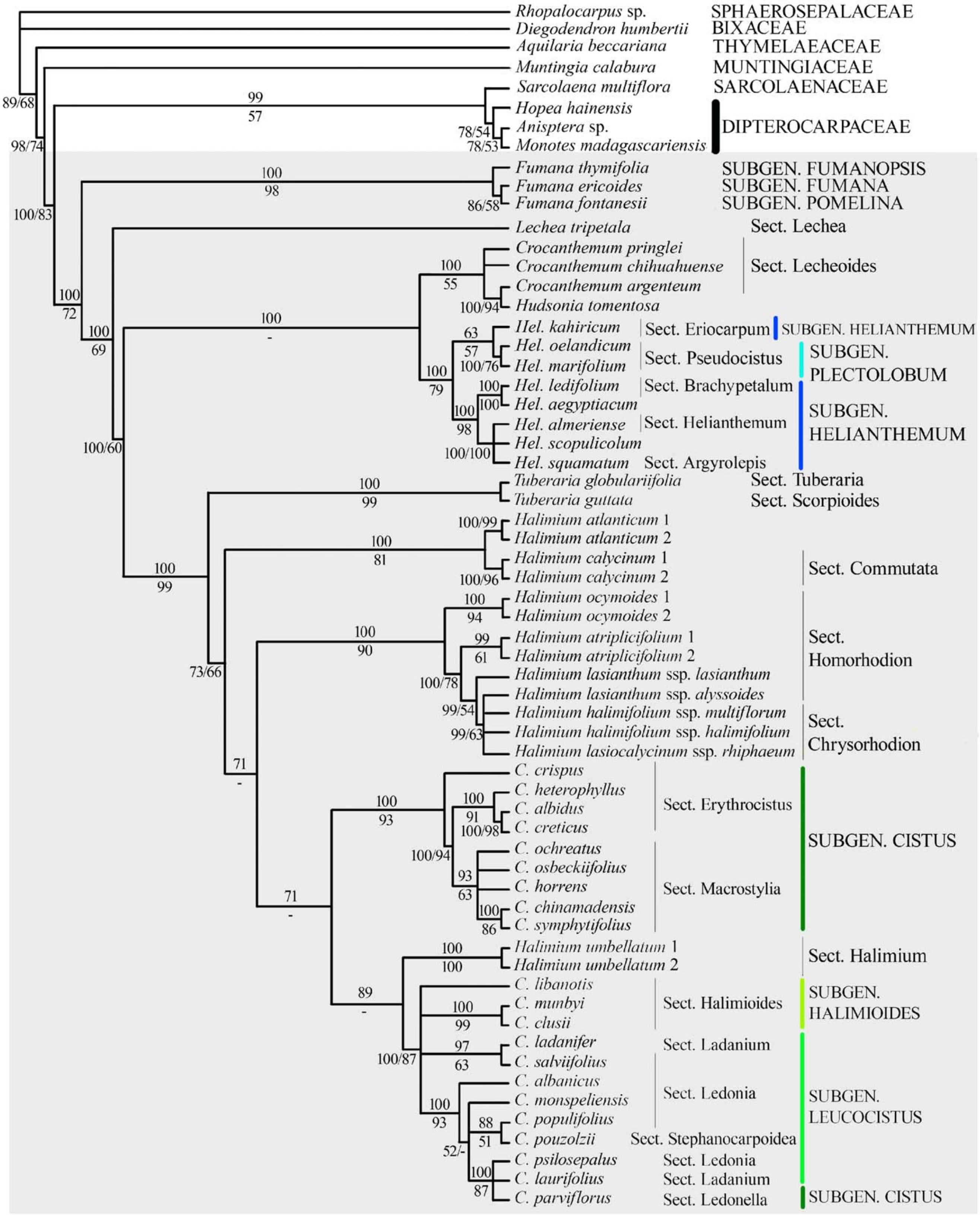

Fig. 1. Phylogeny of Cistaceae based on plastid *rbcL* and *trnL-F* sequences and on Bayesian Inference (BI). Numbers above branches show posterior probabilities. Numbers below branches show bootstrap support for clades recovered by maximum parsimony analysis and in agreement with the BI. Taxonomy follows Demoly and Montserrat (1993) in *Cistus*, Nogueira et al. (1993) in *Halimium*, Willkomm (1856) in *Tuberaria*, López (1993) and Grosser (1903) in *Helianthemum*, Arrington and Kubitzki (2003) and Calderón de Rzedowski (1992) in *Crocanthemum*, Güemes and Molero (1993) in *Fumana*.

Ovaries with 5–12 carpels are only found in *C. ladanifer*.

9. Embryo shape (not figured). The basalmost lineage (*Fumana*) shares the ancestral curved embryo with two of the American genera (*Crocanthemum* and *Hudsonia*) (Arrington and Kubitzki 2003). Embryo shape is equivocal for the *Halimium-Cistus* ancestor, although *Cistus* displays only circinate embryos.
10. Staminodes (not figured). The presence of staminodes in the periphery of the androecium is extensively distributed in Malvales. In Cistaceae, this character state is found only in the basalmost genus *Fumana*.
11. Leaf arrangement (not figured). Historical reconstruction shows alternate leaf arrangement to be plesiomorphic. Independent acquisition of opposite leaves once in *Tuberaria-Halimium-Cistus* and once in *Helianthemum* is unequivocal.
12. Petal macule (not figured). Recurrent acquisition of notched petals in *Helianthemum, Halimium* and *Tuberaria* can be inferred from the character reconstruction. In *Cistus*, a marked macule is only displayed in some populations of *C. ladanifer*.

Leaf attachment (stipulate, exstipulate) and leaf base (petiolate, sessile) were also reconstructed (not figured), but evolutionary changes were extremely difficult to interpret due to the large variation of these characters even within a single species.

Divergence times

The data indicate a Miocene-Pliocene divergence of the genera of Cistaceae (Fig. 3). Respective ages between 14.7 and 8.44 millions of years (Ma) were obtained for the split of New World genera from Old (node 3 in Table 5). A pre-Pliocene split (9.20–5.15 Ma; Table 5) appears to have resulted in the formation of the *Crocanthemum-Hudsonia-Helianthemum* clade, and a Miocene-Pliocene divergence (5.30–4.22 Ma) in the formation of the *Tuberaria-Halimium-Cistus* clade.

Discussion

Phylogenetic relationships in Cistaceae

Our plastid sequence analysis provides the first available phylogenetic framework for relationships within the family (but see Arrington and Kubitzki 2003). Both the BI and MP analyses recognise Cistaceae as a well-supported monophyletic group. This group is also defined by some morphological characters (parietal placentation, lack of mucilage and/or resin canals, presence of multipapillate epidermal cells on petals, stigmas with multicellular papillae, dimorphic and no wing-like sepals) (Nandi 1998b; Kubitzki and Chase 2003).

None of the classifications proposed (Table 2) are fully congruent with the phylogenetic hypothesis presented here, because the genera *Crocanthemum* and *Halimium* are not monophyletic. The historical division of the Old World *Helianthemum* (highly supported as monophyletic; Fig. 1) in two new infrageneric taxa (Table 2) is supported by a biphyletic topology (Fig. 1), provided that some species are recircumscribed. *Helianthemum* subgenus *Helianthemum* is paraphyletic, because *Helianthemum* subgenus *Plectolobum* originated from a most recent common ancestor shared with *Helianthemum kahiricum* (subgenus *Helianthemum*) (Fig. 1). Neither historical nor recent classifications (Table 2) recognise a division of *Halimium* in three clades that would correspond to the three monophyletic groups retrieved by us (Fig. 1). Only Jiménez's (1981) delimitation of three sections agrees with our results (although African species were not included in her study): sect. *Halimium* (*H. umbellatum*); sect. *Chrysorhodion* Spach (*H. atriplicifolium*, *H. halimifolium*, *H. lasianthum*, *H. ocymoides*); sect. *Commutatae* (*H. calycinum*). Additionally, our phylogenetic hypothesis indicates that polyphyly also affects the accessions of the two subspecies of *Halimium lasianthum* (Fig. 1). The status of the *Cistus-Halimium* assemblage as a natural clade suggested in previous studies (Guzmán and Vargas 2005) is confirmed here, which partly supports that parallel evolution with occasional junctions (and, may be, character exchanges) has occurred in *Cistus* and *Halimium*, as pointed out by Dansereau (1939).

Extensive sampling work is certainly required to fully elucidate the phylogenetic relationships within the Cistaceae. Lack of resolution in some clades necessitates more extensive DNA sequencing, particularly of nuclear genes, to generate a more consistent evolutionary hypothesis.

Nandi's hypothesis of *Fumana* as a basal lineage

Two studies analysed in detail the evolution of ontogenetic patterns in Cistaceae and related Malvales (Nandi 1998a, 1998b). Comparison of flower development among Malvales revealed similarities with only one genus of Cistaceae (*Fumana*). For instance, the ovule position is anatropous in *Fumana*, as in related Malvales, whereas it is orthotropous in the rest of Cistaceae. Moreover, *F. procumbens* shows a stigma form similar to that found in floral buds of some Sarcolaenaceae (Nandi 1998b). In *Fumana* and the rest of Cistaceae, only the seed is the diaspore, except for *F. procumbens* and *F. baetica*, in which fruits are dispersed with the contribution of the calyx, as in Dipterocarpaceae (Hegi 1925; Ashton 2003). Based on these floral morphologies Nandi (1998a, 1998b)

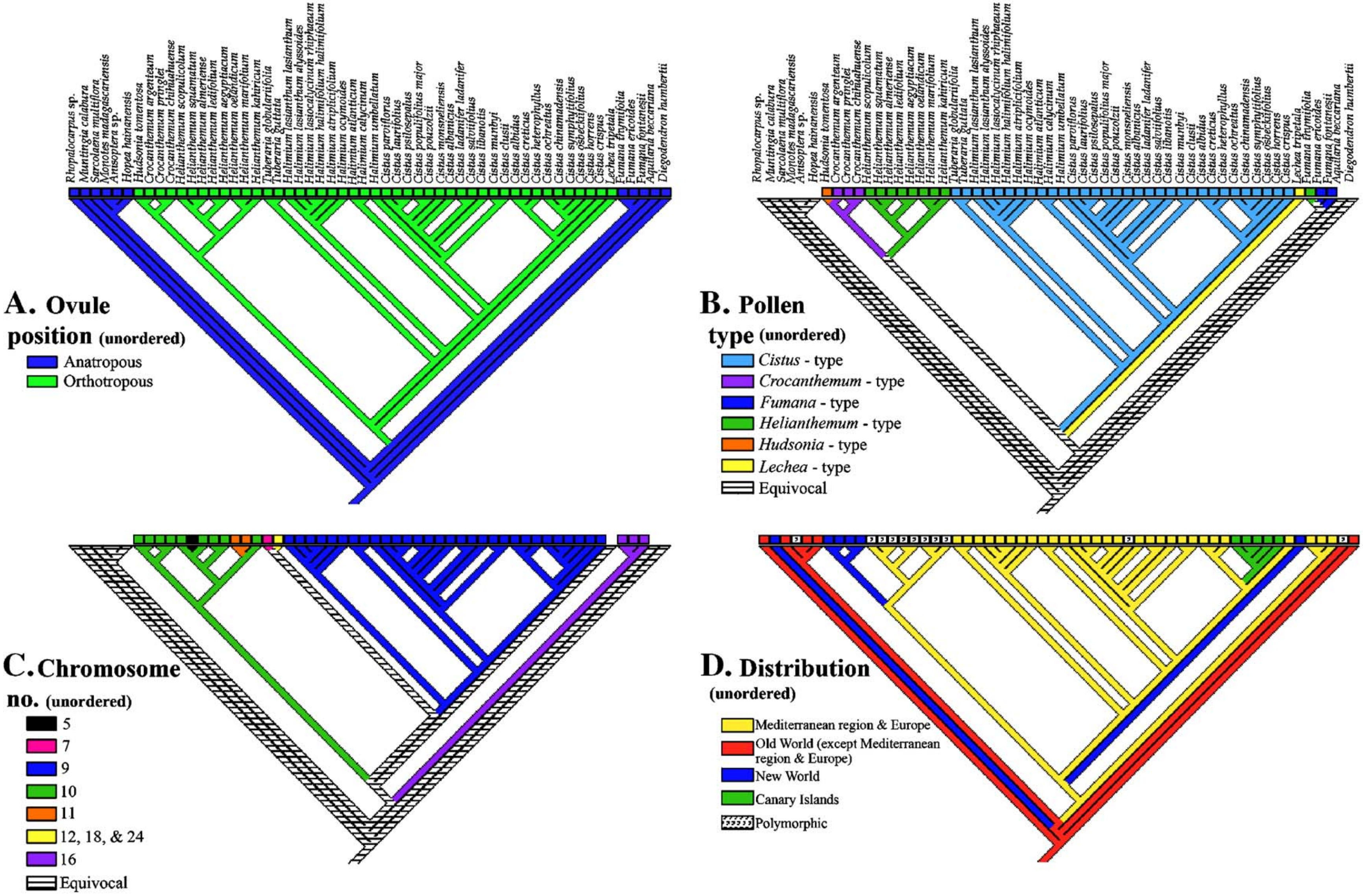

Fig. 2. Selected characters mapped onto the tree hypothesis obtained in the maximum parsimony analysis of *rbcL* and *trnL-trnF* sequences combined, showing "all parsimonious states" as implemented in MacClade (Maddison and Maddison 2000); topology congruent with that of BI tree in Fig. 1. Character optimisation of (A) ovule position, (B) pollen type, (C) gametophytic chromosome number, and (D) geographical distribution.

Table 5. Estimated dates and 95% confidence intervals for the most important constrained and unconstrained nodes of Cistaceae clades based on BEAST analysis of combined *rbcL*/*trnL-trnF* data sets.

Node	Estimated date	95% confidence interval
1	23.95	23.0–27.60
2	14.20	10.17–18.51
3	11.76	8.44–14.70
4	9.30	7.43–11.00
5	7.60	5.15–9.20
6	5.10	4.22–5.30
7	4.62	3.20–5.03
8	1.78	0.79–3.34

Nodes numbered as in Fig. 3.

suggested "an isolated position of *Fumana* at the base of Cistaceae".

Historical inference of character state transformations allows us to test Nandi's hypothesis of character evolution (Fig. 2). The inference concerning the anatropous ovule position does support character retention in *Fumana* (Fig. 2A). Nandi's hypothesis was also based on Ukraintseva's (1993) Cistaceae pollen classification. Ukraintseva had described six types of pollen primarily congruent with the generic subdivision of the family. Only *Tuberaria, Halimium* and *Cistus* share the same pollen type, whereas *Crocanthemum*, *Helianthemum*, *Hudsonia* and *Lechea* have particular types (Fig. 2B). In addition, *Fumana* has two pollen types (*Fumana*-type, *Helianthemum*-type). The polymorphic state of the character in *Fumana*, together with the placement of this genus in Cistaceae, calls for the analysis of additional species. Sharing a character state with some relatives does not necessarily imply a single origin from a common ancestor. Indeed, Nandi (1998a) pointed out that the presence of staminodes in the periphery of the androecium in *Fumana* (Cistaceae), *Xyloolaena* (Sarcolaenaceae) and *Dipterocarpus* (Dipterocarpaceae) may be considered as parallelism. Our character state optimisation (results not figured) confirms the presence of staminodes as a synapomorphy of *Fumana* in Cistaceae, but recurrent in Malvales. Similarly, staminodes evolved independently at least 14 times within the Rosidae (Walker-Larsen and Harder 2000).

In conclusion, Nandi's hypothesis of an isolated position of *Fumana* at the base of Cistaceae based on morphological characters is a remarkable prediction supported by our results based on DNA sequence data, provided that the addition of other species has not significantly altered the spine of the tree. Ontogeny of stigma and diaspore traits should also be investigated in a wider number of genera and species to infer whether ancestral states of these two characters are also retained in *Fumana*.

Biogeography and differentiation in the Mediterranean Cistaceae

The incomplete sampling of the American genera (*Lechea* section *Lechidium*, *Crocanthemum* sect. *Spartioides*) and lack of resolution in the topology at some levels of the MP tree for the *Crocanthemum*-*Hudsonia* and *Helianthemum* relationships prevent us from addressing a key issue in the biogeography of Cistaceae: the number of migration events between the New World and the Old. However, our sampling strategy (representatives of most supraspecific taxa) and results (monophyletic groups considering both taxonomy and geographical distribution of genera) give insight into biogeographical patterns of Cistaceae.

Historically, the respective locations of the most ancestral forms have been used to infer the geographical origin of a taxon (Platnick 1981). The basalmost lineage found so far (*Fumana*; Fig. 1) is exclusive to the Mediterranean (although *F. procumbens* reaches central Europe and Euroasiatic regions; Güemes and Molero 1993) and has retained ancestral characters (see above). *Cistinocarpum roemeri*, a Middle Oligocene macrofossil from Germany described as an ancestor of the extant Cistaceae (Palibin 1909), and *Tuberaria* pollen found in Pliocene formations of Germany (Menke 1976) indicate an ancient presence of the family in Europe, but fall outside of the current centre of diversification, the Mediterranean region (Guzmán and Vargas 2005). An ancient occurrence of Cistaceae in Europe is further supported by our optimisation of species distribution (Fig. 2D). As in many other Mediterranean taxa, it has been hypothesized that Cistaceae differentiated in tropical areas occurring in the Tertiary (Herrera 1992). Fossil evidence, the Eocene-Oligocene split between the Sarcolaenaceae-Dipterocarpaceae and Cistaceae lineages (Wikström et al. 2001), and a dominant tropical vegetation in Europe (Palamarev 1989) suggest a Mid-Tertiary origin of the Old World Cistaceae. An ancient but more recent occurrence of Cistaceae in Europe is further supported by our dated phylogeny (Fig. 3; Table 5), in which the divergence of *Fumana* appears to have taken place in the Miocene (18.51–10.17 Ma). Moreover, the Mediterranean Basin harbours not only the highest number of species (97) and genera (5), but also all major lineages of Cistaceae, which can be used as an additional argument for ancient diversification in this floristic region (Forest et al. 2007). The origin of the family, however, can be inferred with caution only, given the missing taxa in our analysis and the solid general reasoning concerning centres of origin and of diversification (Bremer 1992). In any case, the biogeographical reconstruction and the divergence dates presented here (Figs. 2D, 3) are consistent with an early differentiation process of Cistaceae in the Mediterranean (18.51–10.17 Ma).

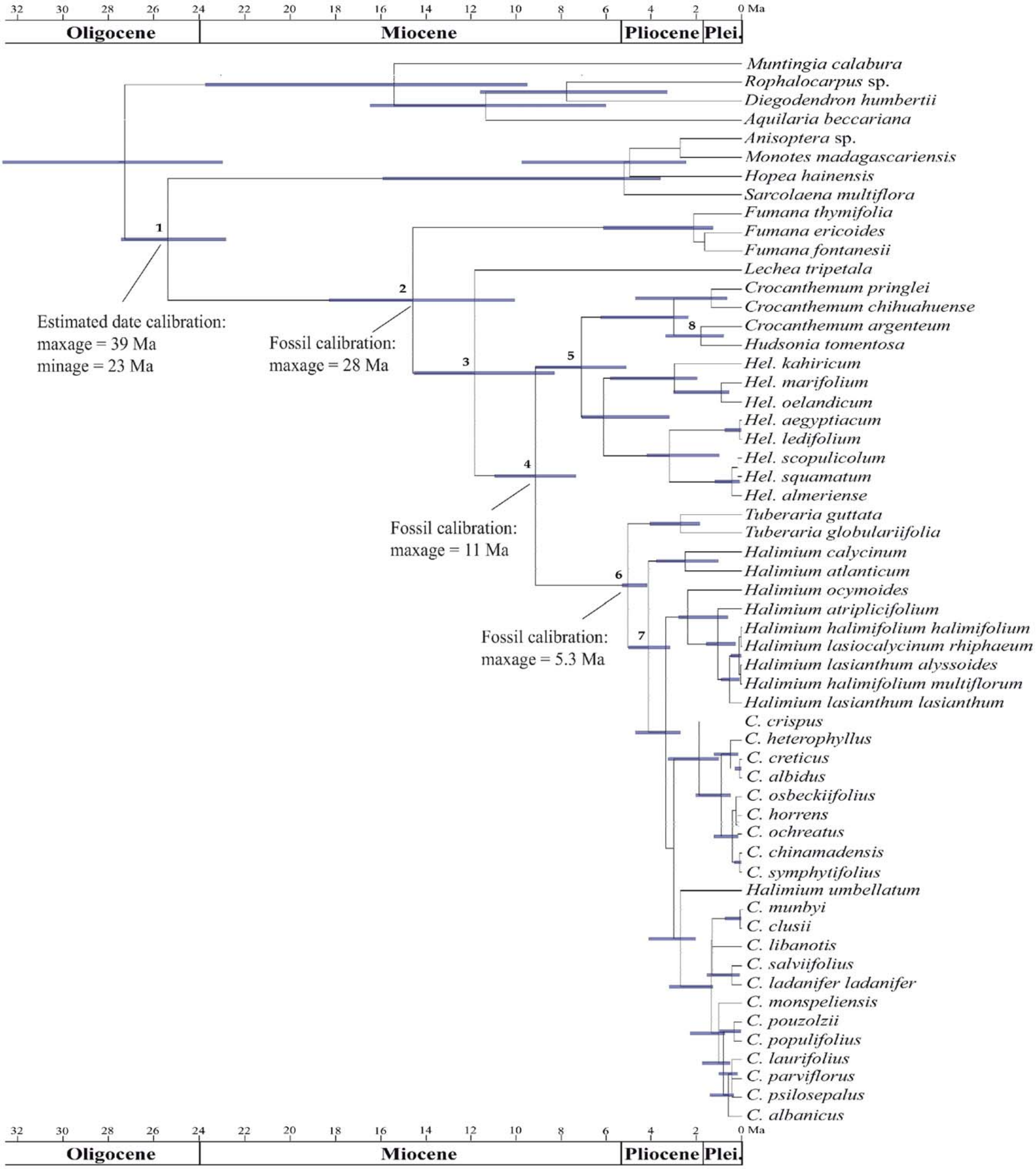

Fig. 3. Maximum Clade Credibility chronogram of *rbcL*/*trnL-trnF* sequences inferred using BEAST. Time scale in millions of years before present (Ma). Error bars (blue) represent 95% posterior credibility intervals and are given only for nodes present on more than 70% of sampled trees. Three fossils and the estimated date of the Dipterocarpaceae-Cistaceae divergence (Wikström et al. 2001) were used to calibrate the analyses.

Considering an Old World origin of Cistaceae, at least two independent migration events from Europe to America (Fig. 2D) occurred between the Middle to Upper Miocene (14.7–8.44 Ma; *Lechea*) and the Upper Miocene (9.20–5.15 Ma; *Crocanthemum*/*Hudsonia*) (Fig. 3). The first colonisation of America is remarkably coincident with other angiosperm disjunctions between the Old World and the New, such as within tribe

Antirrhineae (Scrophulariaceae) in the Upper Miocene (Vargas et al. 2004), between western North American *Styrax platanifolius*-*S. redivivus* and eastern Mediterranean *S. officinalis* in the Middle to Upper Miocene (Fritsch 1999), among semiarid species of *Platanus* from western North America and Europe in the Middle Miocene (Feng et al. 2005), and in some eastern Asian-eastern North American lineages in the Middle to Upper Miocene (*Mitchella undulata*/*M. repens*; *Pachysandra axillaris*/*P. procumbens*; *Podophyllum emodi*/*P. peltatum*; *Phryma leptostachya* var. *asiatica*/*P. leptostachya* var. *leptostachya*; Xiang et al. 2000). The question remains as to whether similar biogeographical patterns and divergence times (congruence) in different groups of angiosperms are the result of stochastic processes or of similar historical causes.

The close relationship among three Old World genera (*Tuberaria*, *Halimium*, *Cistus*), as previously recognised (Ukraintseva 1993; Nandi 1998a), is one of the most robust findings of the present study (Fig. 1). Coupled with previous analyses of plastid (*trnL*-*trnF*, *trnK*-*matK*) and nuclear (ITS) sequences, our data show a congruent topology in which *Cistus* is embedded in *Halimium* (Guzmán and Vargas 2005). A cohesive evolutionary history of the *Cistus*-*Halimium* complex may have occurred primarily in the Mediterranean region, since two of the three genera, and 29 of the c. 45 species and basalmost lineages, are currently exclusive to the Mediterranean Basin. Multiple shifts in chromosome number reflect active cytological differentiation in Cistaceae (Fig. 2C), but differentiation in the *Cistus*-*Halimium* complex has not been accompanied by a change in chromosome number ($n = 9$) or in the predominant self-incompatibility mechanism (Carrio et al. 2003). In this group, historical reconstruction of petal colour recognises character sharing between the only white-flowered *Halimium* (*H. umbellatum*) and the white-flowered *Cistus*, in contrast to acquisition and maintenance of mauve or reddish petals in the purple-flowered lineage (results not figured). Interestingly, retention of petal colour in these two species-rich lineages of *Cistus*-*Halimium* is inferred in spite of the intense selection pressure on flower colour often found in entomophilous angiosperms (Irwin and Strauss 2005).

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