

Phylogenetic reconstruction of the genus *Helianthemum* (Cistaceae) using plastid and nuclear DNA-sequences: Systematic and evolutionary inferences

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Abstract *Helianthemum* is the largest, most widely distributed and most taxonomically complex genus of the Cistaceae. To examine the intrageneric phylogenetic relationships in *Helianthemum*, we used sequence data from plastid DNA (*ndhF*, *psbA-trnH*, *trnL-trnF*) and the nuclear ITS region. The ingroup consisted of 95 species and subspecies (2 subgenera, 10 sections) from throughout the range of *Helianthemum*, while the outgroup was composed of 30 species representing all the genera in the Cistaceae (*Cistus Crocanthemum*, *Fumana*, *Halimium*, *Hudsonia*, *Lechea*, *Tuberaria*) plus *Anisoptera thurifera* subsp. *polyandra* (Dipterocarpaceae). To infer phylogenetic relationships, we analysed three different matrices (cpDNA, nrDNA, cpDNA+nrDNA concatenated) using maximum likelihood and Bayesian inference, and performed molecular dating to estimate the ages of origin of the main clades using a Bayesian approach. The cpDNA+nrDNA concatenated dataset provided the highest Bayesian posterior probabilities and bootstrap support values, and the results supported the monophyly of the genus *Helianthemum* and its sister relationship to a clade consisting of all species of *Cistus*, *Crocanthemum*, *Halimium*, *Hudsonia* and *Tuberaria*. This result means that we did not retrieve the sister relationship between *Helianthemum* and *Crocanthemum* (plus *Hudsonia*) that could be expected according to previous published studies. Despite their different statistical support, the topology of the inner branches of all the consensus trees showed that *Helianthemum* is characterized by the emergence of three major clades in agreement with above-species taxonomy, although unresolved polytomies still remain towards the tips of the trees (species and subspecies). Clade I (mainly distributed in Mediterranean and alpine environments in European and western Asiatic mountain chains) fully coincided with subg. *Plectolobum*, whereas subg. *Helianthemum* was retrieved in clade II (arid and semi-arid environments from Macaronesia, the Mediterranean, subtropical northern Africa, Anatolia and central Asia) and clade III (Mediterranean ecosystems around the Mediterranean Basin). The burst of diversification during the Plio-Pleistocene detected in the three main clades of *Helianthemum* is concomitant with the Messinian salinity crisis, the onset of Mediterranean climatic conditions, and Quaternary glaciations, as found in many other groups of Mediterranean plants. Thus, the general lack of resolution in the trees can be attributed to rapid species diversification and events of reticulate evolution. A series of further taxonomic and evolutionary inferences can be drawn from our analyses: (i) no species occupied an early-diverging position with regard the rest of the species; (ii) a close relationship between *H. caput-felis* and subg. *Plectolobum*; (iii) an unexpected close relationship between *H. squamatum*/*H. syriacum* (and *H. motae*), *H. lunulatum*/*H. pomeridianum* and among *H. songaricum*/*H. antitauricum*/*H. germanicopolitanum*; (iv) a close relationship between incertae sedis species and sect. *Eriocarpum*; and (v) the existence of a monophyletic lineage consisting of Canary Islands species formerly ascribed to sect. *Argyrolepis* or sect. *Lavandulaceum* within sect. *Helianthemum*.

Keywords Cistaceae; *Helianthemum*; Mediterranean flora; nrDNA; phylogeny; plastid DNA

Supplementary Material Electronic Supplement (Appendix S1; Figs. S1–S4) and DNA sequence alignment files are available in the Supplementary Data section of the online version of this article at <http://ingentaconnect.com/content/iapt/tax>

■ INTRODUCTION

The Cistaceae is a small family consisting of 8 genera and ca. 170 heliophytic shrub, subshrub and annual species found almost exclusively in Mediterranean, temperate and subtropical

regions of the Northern Hemisphere (Arrington & Kubitzki, 2003). Originating from the western Mediterranean Basin (Guzmán & Vargas, 2009a), plastid *rbcL* DNA molecular phylogenies support the family's inclusion in the Dipterocarpacean clade of the Malvales, along with the Sarcocaulaceae (trees and

shrubs endemic to Madagascar) and Dipterocarpaceae (resinous trees from evergreen pantropical forests); the main synapomorphy of this Dipoterocarpacean clade is the bixoid-type chalazal region of the seed coat (Kubitzki & Chase, 2003; APG, 2009).

Phylogenetically, Arrington & Kubitzki (2003) (see also Biver & al., 2016) have argued that *Fumana* (Dunal) Spach and *Lechea* L. constitute early-diverging groups in the Cistaceae, while *Helianthemum* Mill. is the monophyletic sister lineage to a clade of *Cistus* L., *Crocانthemum* Spach, *Halimium* (Dunal) Spach, *Hudsonia* L. and *Tuberaria* (Dunal) Spach. However, Guzmán & Vargas (2009a) distinguished five evolutionary lineages within the Cistaceae: two early-diverging lineages each represented by *Fumana* and *Lechea* species, a cohesive clade including all *Halimium* and *Cistus* species, the *Tuberaria* clade, and the *Helianthemum* s.l. clade, in which a sister relationship between the Old World *Helianthemum* s.str. and the New World *Crocانthemum* plus *Hudsonia* species was retrieved. Indeed, *Helianthemum* s.l. is a taxonomically complex group in which generic delimitation has historically been unclear. Dunal (1824) accepted that *Helianthemum* included all New and Old World species; however, Spach (1836) described three different genera (*Crocانthemum*, *Heteromeris* Spach, *Taeniostema* Spach) to accommodate the American species, which were all subsequently ascribed to *Halimium* by Grosser (1903). Although Janchen (1925) delimited the current generic boundaries of the family, with *Helianthemum* restricted to the Old World and all American species to *Crocانthemum* (and *Hudsonia*), controversy still reigns since some authors accept Dunal's (1824) position (e.g., Fernald, 1941; Daoud & Wilbur, 1965; Wilbur & Daoud, 1967; Calderón de Rzedowski, 1992; Christenhusz, 2009), while others support Janchen (1925; e.g., Arrington & Kubitzki, 2003; Sorrie, 2015). While some American species originally described as *Helianthemum* have been formally transferred to *Crocانthemum* (Sorrie, 2011, 2015) others have not. For a historical overview of the classification of Cistaceae see Guzmán & Vargas (2009a).

In this paper we focus on the intrageneric phylogenetic relationships of *Helianthemum* s.str. (hereafter *Helianthemum*; Janchen, 1925), the most diverse and widespread genus in the Cistaceae. *Helianthemum*, restricted to the Old World, consists of about 136 species and subspecies found from Cape Verde to Central Asia in a variety of habitats (xerophytic, Mediterranean, temperate, alpine) that show remarkable life history and functional trait diversity. It includes therophytes and woody-fruticose and suffruticose plants whose breeding and mating systems vary from cleistogamy to self-incompatibility with different levels of outcrossing (Herrera, 1992; Rodríguez-Pérez, 2005; Aragón & Escudero, 2009; Agulló & al., 2015). It is also notable that only some *Helianthemum* species have large geographical distribution areas (e.g., *H. apenninum* Mill., *H. cinereum* Pers., *H. kahiricum* Delile, *H. ledifolium* (L.) Mill., *H. lippii* (L.) Dum.Cours., *H. nummularium* Mill., *H. oelandicum* (L.) DC., *H. salicifolium* (L.) Mill., or *H. stipulatum* C.Ch.) whereas most have restricted ranges or are endemic to very small regions (cf., Davis, 1965; Proctor & Heywood, 1968; Greuter & al., 1984; López-González, 1993; Thulin, 1993; Tzvelev, 2006).

The taxonomic and nomenclatural complexity of *Helianthemum* is recognisable in both the older (Dunal, 1824; Spach, 1836; Willkomm & Lange, 1880; Grosser, 1903; Janchen, 1925) and the more recent botanical literature (Guinea, 1954; Quezel & Santa, 1962; Proctor & Heywood, 1968; Greuter & al., 1984; López-González, 1993; Fennane & al., 1999). Species delimitation is often challenging given the instability of the main diagnostic features (habit; leaf arrangement, shape and size; presence and size of stipules; presence and type of indumentum; inflorescence type; flower bud shape and size; petal colour) due to the lability of reproductive barriers (hybridization and introgression), phenotypic plasticity and local adaptation and convergence (Soubani, 2010; Soubani & al., 2014a; Widén, 2015). In fact, the most common species (e.g., *H. apenninum*, *H. cinereum*, *H. nummularium*, and *H. oelandicum*) are species complexes that contain an array of morphological forms (usually grouped into subspecies) that even coexist in certain areas of their ranges (Soubani & al., 2014a).

All modern taxonomic treatments (e.g., Font Quer & Rothmaler, 1934; Quezel & Santa, 1962; Proctor & Heywood, 1968; Jafri, 1977; Pottier-Alapetite, 1979; Pignatti, 1982; López-González, 1993; Fennane & Ibn Tattou, 1998; Arrington & Kubitzki, 2003; Tzvelev, 2006; see also Electr. Suppl.: Appendix S1) agree in recognizing *Helianthemum* to be primarily subdivided into two taxa at subgenus rank, *H.* subg. *Helianthemum* (= subg. *Ortholobum* Willk.) and subg. *Plectolobum* Willk., and rule out the segregation of subg. *Plectolobum* into the separate genus *Rhodax* Spach. Subgenus *Helianthemum* is characterised by its central simple-plicate embryos with straight cotyledons, alternate or opposite stipulate leaves, stamens reaching the height of the style or less, straight styles that may be slightly sigmoid at the base, and a somatic chromosome number of $2n = 20$ (very rarely $2n = 10, 40$); subg. *Plectolobum* has peripheral conduplicated embryos with curved cotyledons, opposite and often stipulate leaves, stamens longer than the style, styles that are sigmoid at the base, and a somatic chromosome number of $2n = 22$ (very rarely $2n = 24$) (cf. Rice & al., 2015). Within subg. *Helianthemum*, the following sections have traditionally been recognized (e.g., Proctor & Heywood, 1968): sect. *Argyrolepis* Spach (= sect. *Polystachium* Willk.), sect. *Brachypetalum* Dunal, sect. *Eriocarpum* Dunal, sect. *Helianthemum* and sect. *Pseudomacularia* Grosser. However, López-González (1993) proposed splitting sect. *Argyrolepis* into three monospecific sections: sect. *Argyrolepis* (*H. squamatum* Pers., $2n = 10$), sect. *Caput-felis* G.López (*H. caput-felis* Boiss., $2n = 24$) and sect. *Lavandulaceum* G.López (*H. syriacum* (Jacq.) Dum.Cours., $2n = 20$). Within subg. *Plectolobum*, most authors recognize sect. *Pseudocistus* Dunal (= sect. *Chamaecistus* Willk.) and sect. *Macularia* Dunal, the latter including only *H. lunulatum* DC. López-González (1993) suggested that sect. *Atlantenum* (Raynaud) G.López & al. should accommodate *H. sanguineum* (Lag.) Lag. ex Dunal, the only therophytic species in this subgenus, which was formerly included in sect. *Brachypetalum* or even in the monospecific genus *Atlantenum* Raynaud (see Table 1 for historical taxonomic assignment of controversial species, and Aparicio & Albaladejo, 2017 for a discussion of

the identity of *H. mathezii* Dobignard). Finally, a few species from NE Africa have never been explicitly assigned to any particular section or, in some cases, authors have expressed doubts regarding their correct assignment (e.g., Gillett, 1954); these species will be initially considered *incertae sedis* in this study.

Molecular systematic studies in *Helianthemum* to date have only been presented for the species found in the Iberian Peninsula, one of its main centres of diversity with around 46 taxa, 20 of them endemic (López-González, 1993). The ITS-based phylogenetic hypothesis in Parejo-Farnés & al. (2013) provided strong support for the monophyly of the genus, for the above-species systematics (sections and subgenera) suggested

by López-González (1993) (i.e., for sect. *Argyrolepis*, sect. *Atlantemum*, sect. *Caput-felis* and sect. *Lavandulaceum*), and for the convergent evolution of the therophytic habit in three different lineages (sect. *Atlantemum*, sect. *Brachypetalum*, sect. *Helianthemum*). Nevertheless, this phylogenetic analysis retrieved most species and subspecies in large polytomies with poor resolution and branch support. Furthermore, Parejo-Farnés & al. (2013) admitted that their phylogenetic hypothesis was preliminary since it left out sect. *Eriocarpum* (also sect. *Macularia* and sect. *Pseudomacularia*), a large group of mostly Saharo-Arabian and Irano-Turanian species that are absent from the Iberian Peninsula. From an evolutionary perspective,

Table 1. History of taxonomic assignment of controversial species of *Helianthemum*.

	Dunal (1824)	Spach (1836)	Willkomm (1856)	Boissier (1867)	Grosser (1903)	Janchen (1925)	Bornmüller (1930)	Schwartz (1939)	Gillett (1954)	Quézel & Santa (1962)	Davis & Coode (1965)	Verdcourt (1966)	Proctor & Heywood (1968)	Yuzepchuk (1974)	Meikle (1977)	Marrero (1992)	López-González (1993)	Cao & al. (2002)	Ghazanfar (2002)	Thulin (2002)	Santos-Guerra (2014)	This study
<i>H. squamatum</i> Pers.	Psc	Arg	Pol		Arg								Arg				Arg					Arg
<i>H. sanguineum</i> (Lag.) Lag. ex Dunal	Bra												Bra				Atl					Atl
<i>H. caput-felis</i> Boiss.			Pol	Pol	Arg								Arg				Cap					Cap
<i>H. argyreum</i> Baker							Hel															Eri
<i>H. cylindrifolium</i> Verdc.												D										Eri
<i>H. somalense</i> Gillett									D													Eri
<i>H. speciosum</i> Thulin																				na		Eri
<i>H. citrinum</i> Ghaz.																			na			Eri
<i>H. ordosicum</i> Y.Z.Zhao & al.																		Psm				Eri
<i>H. aegyptiacum</i> Mill.	Bra	Bra	Bra	Bra	Bra	Bra				Bra		Bra	Bra	Bra			Hel					Hel
<i>H. gonzalezferreri</i> Marrero Rodr.																Arg						Lav Hel
<i>H. inaguae</i> Marrero Rodr. & al.																						Lav Hel
<i>H. juliae</i> Wildpret																Hel						Lav Hel
<i>H. teneriffae</i> Coss.																Hel						Lav Hel
<i>H. tholiforme</i> J.Ortega & B.Navarro																Hel						Lav Hel
<i>H. bramwelliorum</i> Marrero Rodr.																Arg						Lav Hel
<i>H. bystropogophyllum</i> Svent.																Arg						Lav Hel
<i>H. syriacum</i> (Jacq.) Dum.Cours.	Euh	Euh	Pol	Pol	Pol	Arg							Arg	Arg			Lav					Lav
<i>H. lunulatum</i> DC.	Mac		D	Mac	Mac								Mac									Mac
<i>H. pomeridianum</i> Dunal			Eri	Eri					Cha													Mac
<i>H. germanicopolitanum</i> Bornm.							Pol															Psm
<i>H. songaricum</i> Fisch. & C.A.Mey			Euh	Psm	Psm									Psm			Psm					Psm
<i>H. antitauricum</i> P.H.Davis & Coode										Hel												Psm

Last column shows to which of the currently recognized subgenera and sections these species are more closely related in this study. In bold, sections of subg. *Plectolobum*; otherwise, all sections belong to subg. *Helianthemum*. Arg, *Argyrolepis* Spach; Atl, *Atlantemum* (Raynaud) G.López & al.; Bra, *Brachypetalum* Dunal; Cap, *Caput-felis* G.López; Cha, *Chamaecistus* Willk. = Psc; D, doubtful; Eri, *Eriocarpum* Dunal; Euh, “*Euhelianthemum*” Dunal = Hel; Hel, *Helianthemum*; Lav, *Lavandulaceum* G.López; Mac, *Macularia* Dunal; na, not assigned; Pol, *Polystachium* Willk. = Arg; Psc, *Pseudocistus* Dunal; Psm, *Pseudomacularia* Grosser.

it has been speculated that *H. squamatum*, which has the lowest chromosome number in the genus and a particular set of morphological features, is probably a relatively ancient lineage with no close relatives, and that *H. caput-felis* is the only extant representative of another ancient lineage that is intermediate between subg. *Helianthemum* and subg. *Plectolobum* (López-González, 1992). Su & al. (2011) considered *H. songaricum* Schrenk ex Fisch. & C.A.Mey. to be a relict species from the ancient Tethys region in Central Asia, while Casazza & al. (2005) regarded *H. lunulatum* as a taxonomically isolated and relict element from the Tertiary palaeoflora with a refugium in the Maritime Alps. Gillett (1954) described *H. somalense* Gillett from NE Africa as a relict species from an ancient wider distribution of *Helianthemum* in tropical Africa, rather than a Mediterranean-derived floristic element.

Polytomies in the phylogenetic hypothesis for *Helianthemum* by Parejo-Farnés & al. (2013) leave open the question of whether hybridization and rapid diversification, unsuitable sampling and/or the particular mode of evolution of molecular markers are responsible for the lack of phylogenetic resolution. The goal of the present study is therefore to test if extended taxonomic and molecular coverage increases phylogenetic resolution and to establish as-solid-as-possible intrageneric phylogenetic relationships in *Helianthemum*. To do so, we performed a phylogenetic reconstruction on an extensive sample that includes all intrageneric taxa of *Helianthemum* using nuclear (ITS) and three plastid (*ndhF*, *psbA-trnH*, *trnL-trnF*) DNA sequences. These data along with the estimation of divergence times for the main clades can provide a better understanding of the evolutionary history of *Helianthemum*. We specifically addressed the following questions: (1) is *Helianthemum* a monophyletic lineage (Guzmán & Vargas,

2009a; Parejo-Farnés & al., 2013)? (2) are *Helianthemum* and *Crocantemum* sister taxa that radiated independently in the Old and New World, respectively (Dunal, 1824; Janchen, 1925; Guzmán & Vargas, 2009a)? (3) is the retrieved tree topology consistent with the current systematic subdivision (subgenera, sections) of *Helianthemum* (e.g., López-González, 1992)? (4) do *H. squamatum* and/or *H. caput-felis* represent ancient evolutionary lineages within the genus (López-González, 1992)? and (5) what are the phylogenetic relationships of the incertae sedis species?

■ MATERIALS AND METHODS

Taxon sampling. — Our phylogenetic analyses had a wide taxonomic and geographical scope, including all intrageneric taxa of *Helianthemum* (2 subgenera, 10 sections) and representing the entire distribution range (Fig. 1): overall the in-group included 95 taxa (72 species and 24 subspecies, 140 accessions) that represent about 70% of all taxa recognised in the genus (Table 2; Appendix 1), while the outgroup included 30 species (33 accessions) belonging to all other genera of Cistaceae (*Cistus*, *Crocantemum*, *Fumana*, *Halimium*, *Hudsonia*, *Lechea*, *Tuberaria*) plus *Anisoptera thurifera* subsp. *polyandra* (Blume) P.S.Ashton (Dipterocarpaceae).

DNA extraction, PCR amplification and sequencing. — We extracted total genomic DNA from dried leaves using the Invisorb Spin Plant Mini Kit (Invitex, Berlin, Germany) following the supplier's instructions. We focused on the nrDNA ITS region (ITS1+5.8S+ITS2) and three plastid regions: the intergenic spacers *trnL-trnF*, *psbA-trnH* and a portion of the coding *ndhF* gene. We used the ITS sequences of Parejo-Farnés

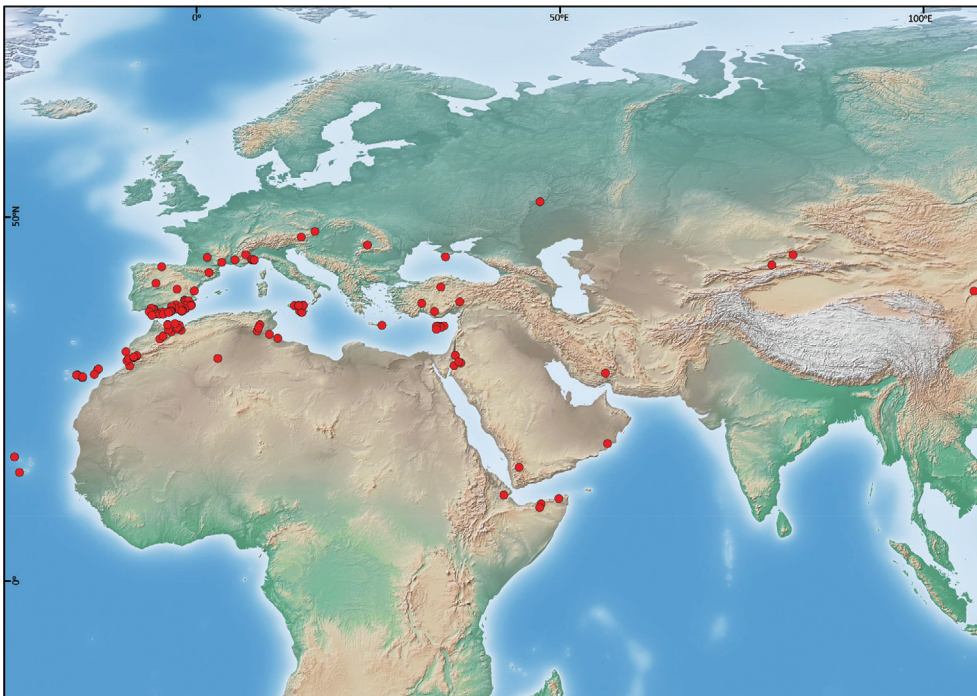


Fig. 1. Geographical location of the samples of *Helianthemum* used in this study.

& al. (2013) but added new sequences of ITS and the plastid regions for the rest of the taxa in this study. Primers P1A and P4 were used for amplification of ITS (Fuertes-Aguilar & al., 1999). For certain difficult samples with poor DNA quality, including some herbarium specimens, we amplified the ITS1 and ITS2 regions separately with the internal primers 5.8S-R and 5.8S-F from Fernández-Mazuecos & al. (2013) in conjunction with primers P1A and P4, respectively. Universal primers for the amplification of the *trnL-trnF* spacer were taken from Taberlet & al. (1991) and for the *psbA-trnH* spacer from Whitlock & al. (2010). Primers for the partial *ndhF* gene were modifications of primers 803 and 1318R from Olmstead & Sweere (1994) with sequences 5'-CAA TGG TAG CAG CGG GAA TTT TTC-3' and 5'-ATA GAT CCG ACA CAT ATA AAA TGC GGT-3', respectively.

We performed PCR in 25 µl reaction volumes, containing 16.8 µl of sterile water, 5 µl of polymerase reaction buffer, 1 µl (10 µM) of each primer, 0.2 µl (5 U/µl) of the fast MyTaqRed DNA polymerase (Bioline, London, U.K.) and 1 µl of template total DNA. For the successful amplification of the ITS region, the addition of 2 µl of DMSO was necessary (this volume was subtracted from the amount of sterile water in the amplification mix). The PCR cycling conditions used were as follows: 35 cycles of denaturation at 95°C for 15 s, annealing at 51°C–54°C for 15 s and extension at 72°C for 10 s, followed by a final extension step at 72°C for 1 min. We purified PCR products with Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, Massachusetts, U.S.A.) following the manufacturer's protocol. Purified products were directly sequenced on an ABI 3730 with the ABI Big Dye terminator kit (PE Applied Biosystems, Foster City, California U.S.A.) at the Unidad de Genómica (UCM, Madrid, Spain).

Phylogenetic analyses. — We assembled the sequences with Sequencher v.4.3 (Gene Codes, Ann Arbor, Michigan, U.S.A.), aligned them in MAFFT v.5 (Kato & al., 2005) and checked them visually for minor corrections. We analysed three molecular matrices: nrDNA (ITS), cpDNA (*trnL-trnF*, *psbA-trnH*, *ndhF*) and the cpDNA+nrDNA combined matrix (i.e., the concatenated dataset). The plastid genome behaves as a single linkage group (Escobar-García & al., 2012), so plastid markers were concatenated a priori using FASconCAT v.1.0 (Kuck & Meusemann, 2010). However, prior to concatenation of the cpDNA and nrDNA matrices, we checked the congruency between the nrDNA and cpDNA trees by visually inspecting incongruent placements of individual accessions or whole clades (Pirie, 2015) with Bayesian posterior probabilities ≥ 0.95 and bootstrap support $>70\%$ (Hillis & Bull, 1993; Salichos & al., 2014); after removal of the incongruent accessions the cpDNA and nrDNA matrices were concatenated and analysed. In all cases, only accessions with three or more sequenced regions (see Appendix 1) were included for concatenation of the cpDNA and cpDNA+nrDNA matrices.

We selected the best-fitted nucleotide substitution model (GTR+G+I in the ITS and plastid aligned matrices) according to the Akaike information criterion values using Modeltest v.3.06 (Posada & Crandall, 1998). Phylogenetic reconstructions for the three datasets were performed under both Bayesian inference (BI) and maximum likelihood (ML) approaches. For the Bayesian inference we used MrBayes v.3.2.6 (Ronquist & al., 2012) on XSEDE via the CIPRES Science Gateway (<http://www.phylo.org/>) with four simultaneous runs, each with four parallel Markov chains (three hot and one cold), and sampled every 10,000 trees to obtain a total of 25 million generations. The first 10% of trees of each run were discarded

Table 2. Overview of the intrageneric systematics of *Helianthemum* following Grosser (1903), Proctor & Heywood (1968) and López González (1993), and approximate number of species and subspecies also considering the results of this study.

Subgenus	Section	Somatic chromosome number	Distribution area	Approximate no. of species (subspecies)
<i>Helianthemum</i> Mill.	<i>Argyrolepis</i> Spach	10	Iberian Peninsula, N Algeria	1
	<i>Brachypetalum</i> Dunal	20 (40)	Mediterranean, Macaronesia	5
	<i>Eriocarpum</i> Dunal	20	Saharo-Arabian, Irano-Turanian, Macaronesia (Mediterranean)	24 ^a
	<i>Helianthemum</i>	20	Mediterranean, Eurosiberian, Macaronesia	43 (21)
	<i>Lavandulaceum</i> G.López	20	Mediterranean	2 ^b
	<i>Pseudomacularia</i> Grosser	20 (40)	Irano-Turanian	4 ^c
<i>Plectolobum</i> Willk.	<i>Atlantemum</i> (Raynaud) G.López & al.	22	Mediterranean	1 ^d
	<i>Caput-felis</i> G.López	24	Tyrrhenian	1 ^e
	<i>Macularia</i> Dunal	22	Maritime Alps	2 ^f
	<i>Pseudocistus</i> Dunal	22	Mediterranean, Eurosiberian	17 (23)

a, Including incertae sedis species; — b, Excluding Canary Islands species grouped in sect. *Helianthemum*; — c, Including *H. antitauricum* P.H.Davis & Coode and *H. germanicopolitanum* Bornm.; — d, Excluding *H. mathezii* Dobignard (Aparicio & Albaladejo, 2017); — e, Traditionally included in subg. *Helianthemum*; — f, Including *H. pomeridianum* Dunal

(burn-in); successful convergence of runs were assessed with Tracer v.1.6 (<http://tree.bio.ed.ac.uk/software/tracer/>) and 50% majority-rule consensus trees were constructed. Maximum likelihood analyses were conducted with RAxML using the available RAxML BlackBox server (<http://embnet.vital-it.ch/raxml-bb/>, last accessed 25 Jan 2017) (Stamatakis & al., 2008). The GAMMA model of rate heterogeneity was employed for all partitions. The “Maximum likelihood search” and “Estimate proportion of invariable sites” boxes were selected, and a total of 100 bootstrap replicates were performed.

Lineage divergence times. — We performed a relaxed-clock Bayesian MCMC approach as implemented in BEAST v.1.8.0 (Drummond & al., 2012) using the cpDNA+nrDNA combined matrix because of its higher resolution (see Results). The birth-death process was selected as the tree prior, with an uncorrelated lognormal (UCLN) model for rate variation within branches. The GTR+I+G substitution model was selected as described above. We employed a combination of a fossil and a molecular estimate (N1) and applied a normal prior distribution and three minimum-age fossil constraints (N2, N3, N4) using lognormal prior distributions to calibrate the following nodes, following the strategy of previous studies of Cistaceae (Guzmán & Vargas, 2009a; Fernández-Mazuecos & Vargas, 2010). The Dipterocarpaceae/Cistaceae divergence (N1) was implemented using both a recent estimate obtained from the analysis of angiosperm families (Bell & al., 2010) and the macrofossil *Cistinocarpum roemeri* Conis, a Middle Oligocene reproductive structure from Germany described as an ancestor of extant Cistaceae (Palibin, 1909) (mean = 42.0, standard deviation = 5.5). The stem node of *Tuberaria* (N2) was calibrated with pollen from Pliocene formations in Germany (Menke, 1976) (offset = 2.58, mean = 5.33 and standard deviation logarithm = 1.0). The stem node of *Helianthemum* (N3) was calibrated with pollen found in Upper Miocene formations in France (Naud & Suc, 1975) (offset = 5.33, mean = 11.63 and standard deviation logarithm = 1.0). Finally, the crown node of sect. *Helianthemum* (N4) was calibrated with pollen found in Middle Pleistocene formations, specifically from the Elsterian Glaciation in Poland (Hrynowiecka & Winter, 2016) (offset = 0.32, mean = 0.4 and standard deviation logarithm = 1.5). The offset of all nodes corresponds to the minimum age of the period (millions of years, myr) to which the fossil was assigned; the mean parameter represents the end of that period (myr) since there are no upper limits to divergence times of the calibration points since the actual lineages could have been in existence well before the appearance of the fossil used for calibration (Ho & Phillips, 2009).

Four Markov chain Monte Carlo (MCMC) analyses with 25 million generations each and a sample frequency of 10,000 were run through the CIPRES Science Gateway (Miller & al., 2010). Parameter analysis in Tracer v.1.5 (Rambaut & Drummond, 2007) showed MCMC convergence within chains, with effective sample sizes (ESS) above 200 samples. Chains were combined using LogCombiner v.1.8.4 after discarding the first 10% of sampled generations as burn-in. Trees were summarized in a maximum clade credibility (MCC) tree obtained in TreeAnnotator v.1.8.4 and visualized in FigTree v.1.4.2.

(<http://tree.bio.ed.ac.uk/software/figtree/>). To assess the sensitivity of the results to the ingroup calibrations (N3, N4), we performed two additional molecular dating analyses, in which one or the other of the two calibration points were removed to check whether or not the estimated age was similar without the respective calibration point. Both *Helianthemum* fossil constraints were found to be suitable (results not shown).

Supplementary analyses. — Aiming at assessing discrepancies among studies of the phylogenetic relationships within Cistaceae and seeking further support for our data or to detect possible incongruences among datasets (see Discussion below) we performed two additional analyses (plastid and nuclear) for 85 taxa of Dipterocarpaceae, Sarcolaenaceae, Cistaceae and Bixaceae representing the whole Dipterocarpacean clade of the Malvales (Kubitzki & Chase, 2003; APG, 2009) for which ITS plus *rbcL* and *psbA-trnH* sequences were available in GenBank (last accessed Jan 2017). To root the analyses, accessions of Malvaceae, Muntingiaceae and Thymelaeaceae were also included following the same criterion. The methodology for the phylogenetic analyses is the same described in the Phylogenetic Analyses section.

■ RESULTS

Sequences. — The aligned nrDNA data matrix of 171 sequences (124 taxa: 29 outgroup+95 ingroup) consisted of 865 characters, of which 404/197 were variable (with/without outgroup taxa) and 289/139 were parsimony informative. In the plastid regions, *trnL-trnF* consisted of 606 characters, of which 229/87 were variable and 150/34 parsimony informative. The *psbA-trnH* region was 383 bp long and harboured 213/120 variable and 178/93 parsimony-informative sites. The partial *ndhF* region (475 pb) was the least variable region with 127/68 variable positions and 99/41 parsimony-informative sites. The plastid matrix of 168 concatenated sequences (120 taxa: 28 outgroup+92 ingroup) was 1464 bp long and contained 569/246 variable and 427/167 parsimony-informative sites. Finally, the concatenated cpDNA+nrDNA matrix of 165 sequences (118 taxa: 28 outgroup+90 ingroup) consisted of 2321 characters, of which 968/440 were variable and 707/306 were parsimony-informative. GenBank accession numbers for the ITS and the three plastid regions are given in Appendix 1.

Trees. — The 50% majority-rule consensus trees obtained from the Bayesian analyses of the nuclear (nrDNA) and plastid (cpDNA) datasets in this study were biologically meaningful and had congruent topologies except for the placement of three accessions in clades with Bayesian posterior probabilities (PP) ≥ 0.95 and bootstrap support values (BS) $> 70\%$: *H. lumulatum* 324, *H. oelandicum* subsp. *alpestris* 184 and *H. ruficomum* 110 (see Electr. Suppl.: Figs. S1, S2). After their removal, the cpDNA+nrDNA concatenated tree was also meaningful and showed the highest Bayesian and bootstrap support values showing that in *Helianthemum* nuclear and plastid DNA regions had similar evolutionary histories at the above-species level (Olmstead & Sweere, 1994; Pelter & al., 2010). Therefore, only the cpDNA+nrDNA concatenated tree is here shown (Figs.

2, 3), and the individual nrDNA and cpDNA trees are included in the Electronic Supplement (Figs. S1, S2). Unless otherwise stated, sample size and branch support reported refers to the cpDNA+nrDNA concatenated tree.

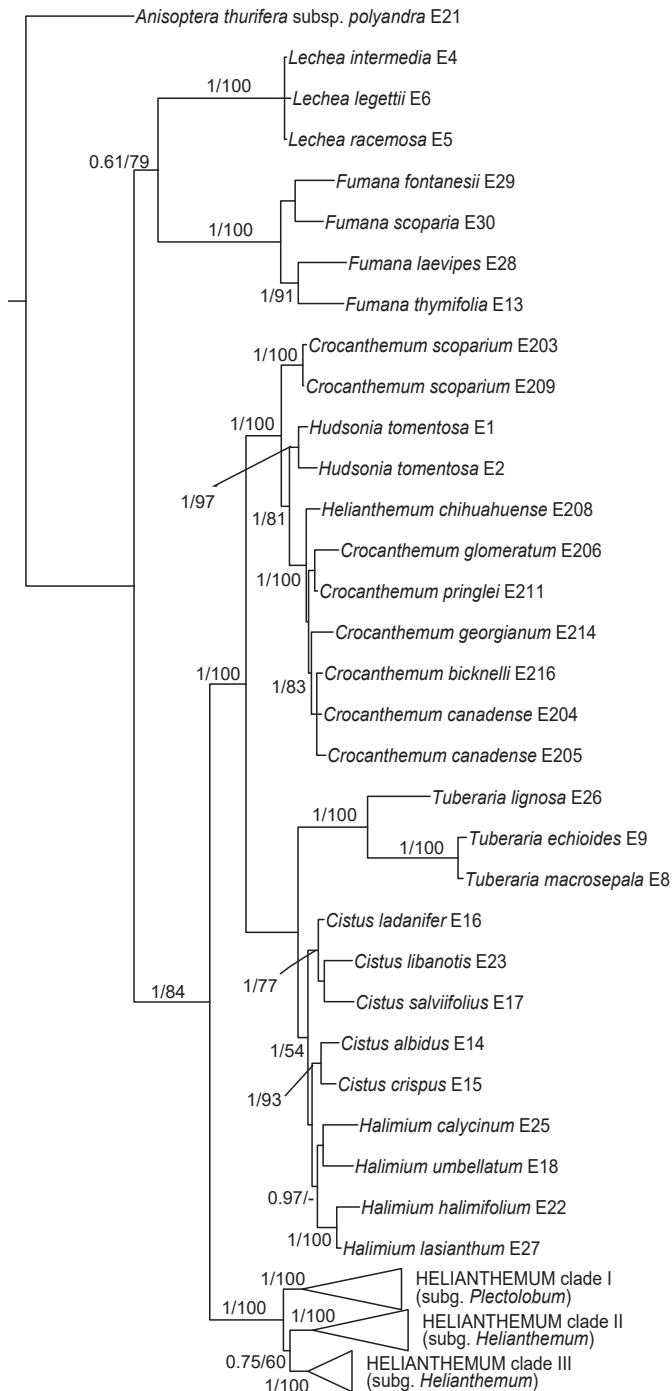
At the family level (Fig. 2), the analyses retrieved a trichotomy represented by *Fumana*, *Lechea* (PP = 0.61, BS = 79%) and a strongly supported clade (PP = 1, BS = 84%) containing the remaining genera of Cistaceae. Within this latter lineage, the analyses also retrieved two highly supported sister sub-lineages, one (PP = 1, BS = 100%) including *Cistus*, *Halimium*

and *Tuberaria* plus the New World genera *Crocanthemum* and *Hudsonia*, the other (PP = 1, BS = 100%) containing all species of *Helianthemum* whose monophyly is very well supported (notice that Arrington & Kubitzki, 2003 and Sorrie, 2011 & 2015 transferred all North American species of *Helianthemum* to *Crocanthemum* and that *Helianthemum chihuahuense* S.Watson and *H. patens* Hemsl. from Central America have never been formally combined in *Crocanthemum*). Therefore, none of the analyses performed provided support for a close or sister relationship between New World *Crocanthemum* and Old World *Helianthemum*. The analyses consistently showed the existence of three *Helianthemum* clades, well supported in the concatenated tree (Figs. 2, 3A–C), and no *Helianthemum* species occupied a solitary, early-diverging or intermediate position among the three clades. The relationships among these three clades remained unresolved.

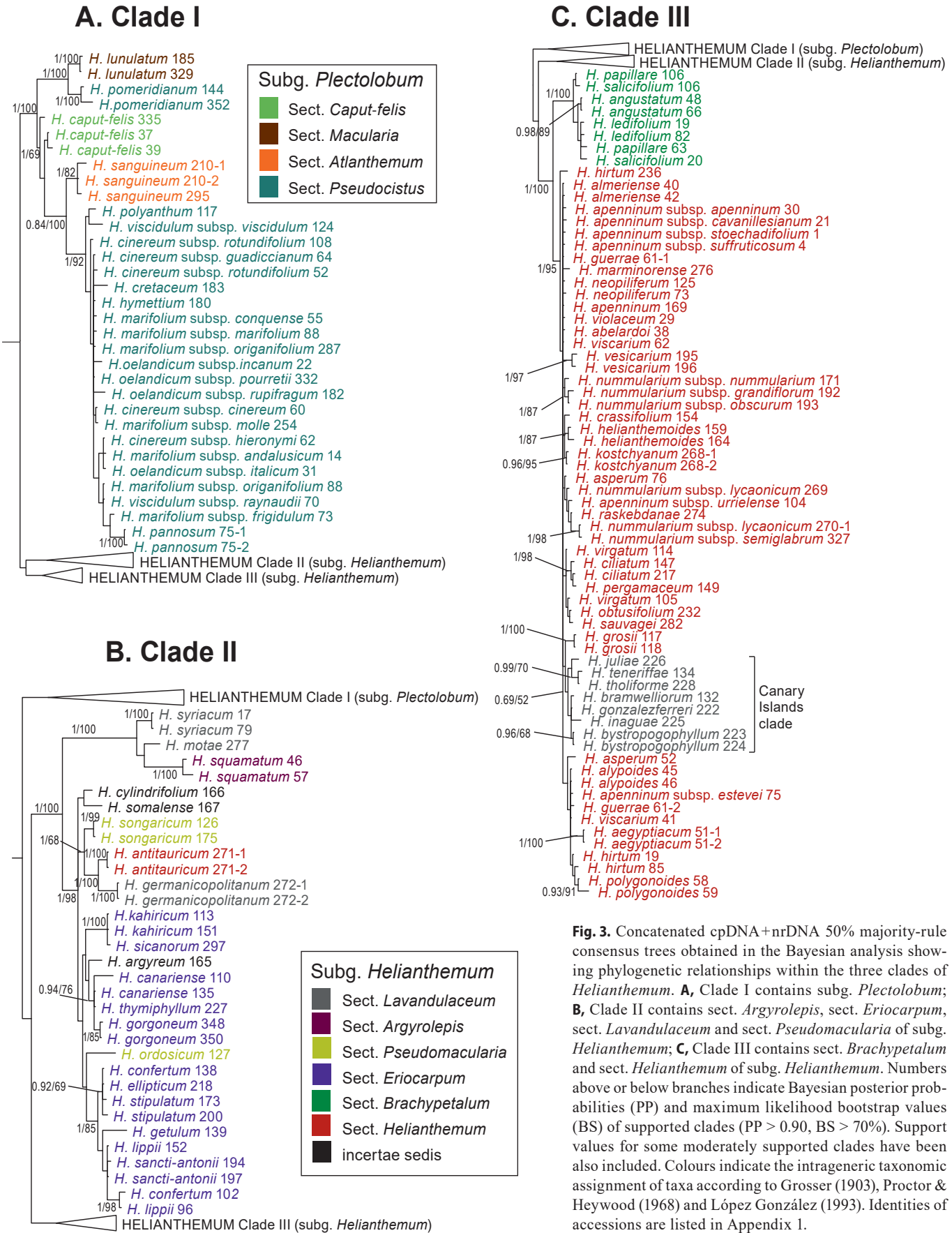
Clade I (PP = 1, BS = 100%, Fig. 3A) contained all 24 taxa (12 species and 13 subspecies, 33 accessions) representing subg. *Plectolobum* (sect. *Atlantemum*, sect. *Macularia*, sect. *Pseudocistus*) plus sect. *Caput-felis*. Interestingly, (i) our results confirm the close relationship between *H. caput-felis* and this subgenus, (ii) the accessions of *H. pomeridianum* Dunal grouped with those of *H. lunulatum*, and (iii) sect. *Atlantemum* was supported. However, within the branch representing sect. *Pseudocistus* the accessions formed a large unresolved polytomy.

Clade II (PP = 1, BS = 100%, Fig. 3B) contained 21 taxa (21 species, 33 accessions) of subg. *Helianthemum* belonging to sect. *Argyrolepis*, sect. *Eriocarpum*, sect. *Lavandulaceum* and sect. *Pseudomacularia*, plus the incertae sedis species. Of note is (i) that sect. *Eriocarpum* was retrieved as non-monophyletic, (ii) that sect. *Argyrolepis* and sect. *Lavandulaceum* form a monophyletic sister clade to sect. *Eriocarpum* and sect. *Pseudomacularia* and the species included as incertae sedis, (iii) the well-supported clade (cpDNA PP = 1 BS = 67%; concatenated PP = 1, BS = 68%) containing the Irano-Turanian *H. germanicopolitanum* Bornm., *H. antitauricum* P.H.Davis & Coode and *H. songaricum*, (iv) the position of *H. ordosicum* Y.Z.Zhao & al., which is not closely related to this clade, (v) the well-supported clade (PP = 0.94, BS = 76%) consisting of the Macaronesian species *H. canariense* Pers., *H. thymiphyl-lum* Svent. and *H. gorgoneum* Webb, (vi) the well-supported (PP = 1, BS = 85%) clade consisting mostly of north African species, and (vii) the proximity of *H. sicanorum* Brullo & al. to *H. kahiricum*, two morphologically very similar species.

Clade III (PP = 1, BS = 100%, Fig. 3C) contained 45 taxa (36 species and 9 subspecies, 68 accessions) belonging to subg. *Helianthemum* sect. *Helianthemum* and sect. *Brachypetalum*.



◀ **Fig. 2.** Concatenated cpDNA+nrDNA 50% majority-rule consensus tree obtained in the Bayesian analysis. Phylogenetic relationships within the three clades of *Helianthemum* (collapsed) are shown in Fig. 3. Numbers above, below or next to branches indicate Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) of supported clades (PP > 0.90, BS > 70%). Support values for some moderately supported clades have been also included. Identities of accessions are listed in Appendix 1.



In this clade, (i) sect. *Helianthemum* and sect. *Brachypetalum* are highly supported sister groups (PP = 1, BS = 100%), (ii) *H. aegyptiacum* Mill. falls into sect. *Helianthemum*, and (iii) a geographically relevant – albeit only moderately supported (nrDNA PP = 0.72, BS = 72%, concatenated PP = 0.69, BS = 52%) – “Canary Islands clade” that contained endemic species from the Canary Islands that were previously ascribed to sect. *Argyrolepis* or sect. *Lavandulaceum* (see Tables 1, 2) was retrieved.

Lineage divergence times. — The ages recovered for the main well-supported clades were: 14.10 myr (95% highest posterior density (HPD) intervals: 7.07–23.86) for the divergence between *Helianthemum* and the sister clade consisting of *Cistus*, *Crocianthemum*, *Halimium*, *Hudsonia* and *Tuberaria* (stem node of *Helianthemum*), 7.80 myr (95% HPD: 3.56–14.08)

for the early diversification of *Helianthemum* (crown node), 4.62 myr (95% HPD: 1.97–8.89) for the start of diversification of clade I, 4.37 myr (95% HPD: 1.65–8.57) for the start of diversification of clade II and 3.76 myr (95% HPD: 1.39–7.47) for the start of diversification of clade III (Fig. 4). Within this clade III, the estimated age for divergence of the Canary Islands clade was 1.28 myr (95% HDP: 0.48–2.61) (collapsed in Fig. 4).

Supplementary analyses. — The results of the plastid and nuclear supplementary analyses of the Dipterocarpacean clade of Malvales (Electr. Suppl.: Figs. S3, S4) were largely congruent among them and with our results, always retrieving *Crocianthemum* (plus *Hudsonia*) in a well-supported clade with *Cistus*, *Halimium* and *Tuberaria* (nrDNA: PP = 0.94, BS = 72%; cpDNA: PP = 1, BS = 98%) sister to *Helianthemum* (nrDNA: PP = 1, BS = 63%; cpDNA: PP = 1, BS = 100%).

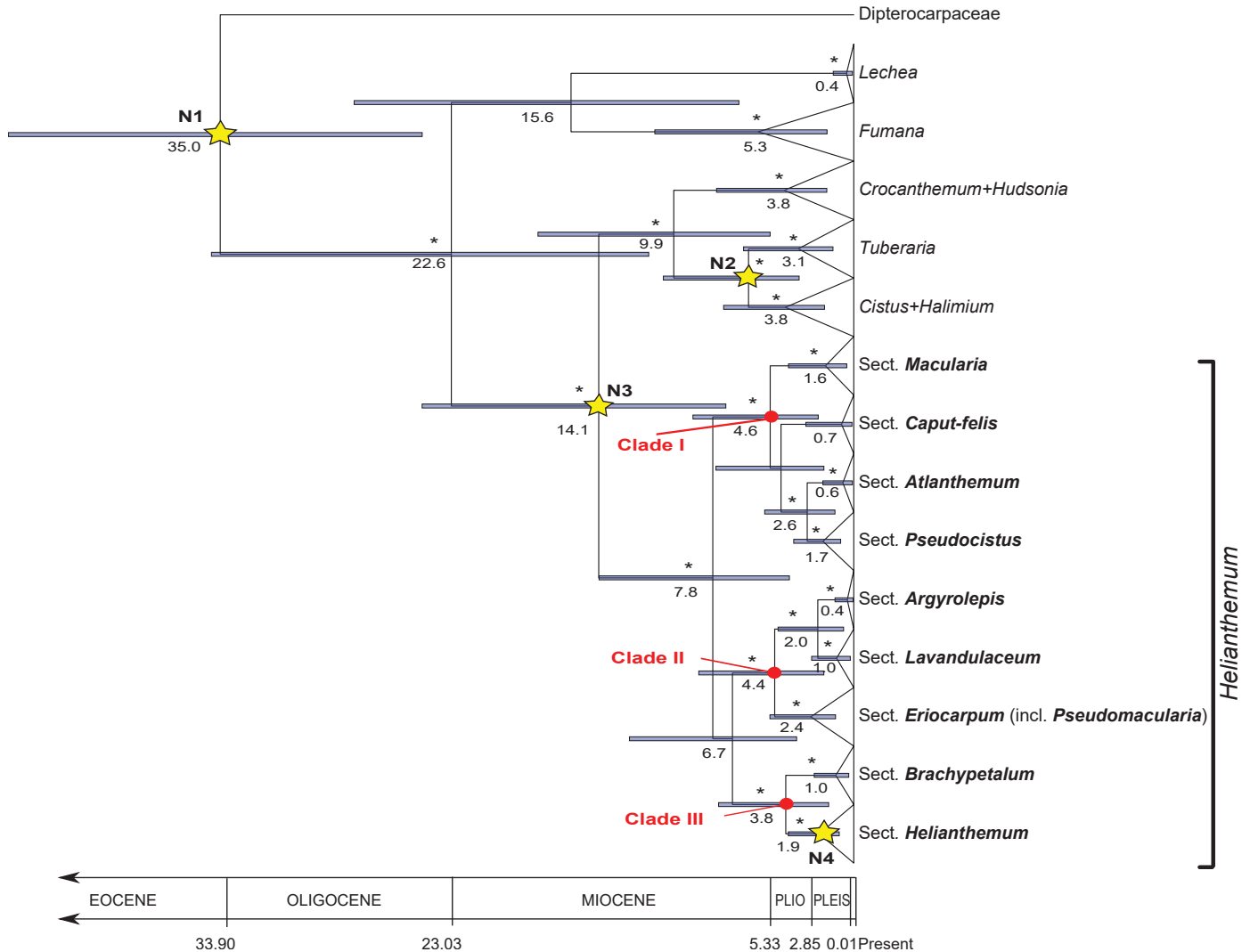


Fig. 4. Maximum clade credibility (MCC) chronogram from the relaxed molecular-clock analysis of concatenated cpDNA + nrDNA sequences inferred using BEAST. Clades are collapsed at genus level in the outgroup and to sections in *Helianthemum*. Time scale is in million years (myr). Numbers below branches correspond to the medium age of divergence time estimates and the blue bars represent 95% posterior credibility intervals. Asterisks denote clades with Bayesian posterior probabilities higher 0.95. Four fossil calibration points (yellow stars) were used (see Lineage divergence times section of Material and Methods for more details).

DISCUSSION

Our phylogenetic reconstruction provides compelling evidence that *Helianthemum* is a monophyletic group which started to diversify about 7.8 million years ago (mya). Although the analyses retrieved the above-species classification of the genus, they were unable to resolve most phylogenetic relationships at the species and subspecies level (Parejo-Farnés & al., 2013). The evolutionary history of *Helianthemum* has probably been driven by the major palaeoclimatic events that impacted the Mediterranean Basin since the Upper Miocene (i.e., the Messinian salinity crisis, the establishment of a Mediterranean-type climate and the range contraction-expansion cycles during the Pleistocene), so the general lack of resolution provided by this set of DNA markers in *Helianthemum* can be attributed to rapid and recent species diversification and to reticulate evolution (Calviño & al., 2008; Garamszegi, 2014; Hilpold & al., 2014; Volkova & al., 2016). Therefore, any further attempt to disentangle phylogenetic relationships at species and subspecies level in this genus will require a battery of high-power resolution markers and the use of massive gene-sequencing techniques on a genome-wide scale (Emerson & al., 2010; Eaton & Ree, 2013; Gonen & al., 2015).

All analyses consistently showed the existence of three clades in agreement with the above-species classification of *Helianthemum* since clade I fully coincides with subg. *Plectolobum* and clades II and III represent subg. *Helianthemum*, and no species occupied a solitary, early-diverging or intermediate position among these three clades. However, our trees (Fig. 3; Electr. Suppl.: Figs. S1, S2) show that controversies in the taxonomic assignment of many species to sections (Table 1), resulted from evolutionary convergence of traits usually used for the delimitation of sections (e.g., habit, number of stamens, presence of stipules, inflorescence type) in different lineages (López-González, 1992; Parejo-Farnés & al., 2013). We conclude that these apparent discrepancies are not indicative of a polyphyletic or paraphyletic evolutionary history of some lineages, but indicate that a comprehensive revision of the intrageneric classification of *Helianthemum* is necessary. This is probably the case for all therophytic species historically assigned to sect. *Brachypetalum*, for the species assigned to sect. *Argyrolepis* or sect. *Lavandulaceum* based on their shrubby habit and the ramose inflorescences, for *H. pomeridianum*, for the incertae sedis species, or for the relationships detected among *H. antitauricum*, *H. germanicopolitanum* and *H. songaricum* (see Fig. 3, Table 1 and further discussion below).

Phylogeny of Cistaceae. — Although phylogenetic analysis of the Cistaceae lies beyond the scope of this paper since a different sampling and sequencing strategy would be necessary, all our analyses coincided in retrieving a trichotomy of three clades: *Fumana*, *Lechea* and the remaining genera of Cistaceae (Fig. 2). As stated before, this last clade is strongly supported and is composed of two subclades comprising *Cistus*, *Crocانthemum*, *Halimium*, *Hudsonia* and *Tuberaria* sister to *Helianthemum*. This means that the existence of the *Helianthemum* s.l. clade consisting of two allopatric lineages that radiated independently in the Old (*Helianthemum*) and

the New World (*Crocانthemum* plus *Hudsonia*) during the Upper Miocene as suggested by Guzmán & Vargas (2009a) (see also Dunal, 1824; Wen & Ickert-Bond, 2009; Vargas & al., 2014) is not supported by our nrDNA, cpDNA or concatenated datasets. The results of the two supplementary analyses for the whole Dipterocarpacean clade of Malvales designed to assess discrepancies among studies (Electr. Suppl.: Figs. S3, S4) were largely congruent among them and with the results presented here, and always retrieved *Crocانthemum* (plus *Hudsonia*) in a well-supported clade with *Cistus*, *Halimium* and *Tuberaria* sister to *Helianthemum*. Since the phylogenetic relatedness among these five genera (*Cistus*, *Crocانthemum*, *Halimium*, *Hudsonia*, *Tuberaria*) has also been shown by other studies using different sets of DNA markers (Arrington & Kubitzki, 2003; Guzmán & Vargas, 2009b; Biver & al., 2016), we think that all current evidence supports *Helianthemum* and *Crocانthemum* as two separate geographic, taxonomic and phylogenetic entities. Indeed, *Helianthemum* and *Crocانthemum* are readily differentiated by the arrangement of their leaves, the presence of stipules, and their pollen, style, funicle and embryo shapes, which are the features used by Arrington & Kubitzki (2003) and Sorrie (2011, 2015) to transfer all North American species of *Helianthemum* to *Crocانthemum*.

Phylogeny of *Helianthemum*. — In the present study, the cpDNA and the concatenated dataset provided strong support for the monophyly of *Helianthemum*, which probably diverged from the clade containing *Cistus*, *Crocانthemum*, *Halimium*, *Hudsonia* and *Tuberaria* during the Miocene (14.10 mya, 95% HPD: 7.07–23.86), and started diversification during the Miocene-Pliocene transition (7.80 mya, 95% HPD: 3.56–14.08) (Fig. 4) coinciding with the Messinian salinity crisis (5.3–6.9 mya; Krijgsman & al., 1999) and the filling of the Mediterranean Sea (2.8–3.4 mya; Suc, 1984). The isolation caused by the disappearance of the land bridges that connected Africa and Europe during the Messinian salinity crisis could have triggered vicariance events and enhanced diversification processes, further diversification taking place mainly in the western part of the Mediterranean Basin where most of its diversity is currently concentrated (e.g., Proctor & Heywood, 1968; Greuter & al., 1984); indeed, only a few very polymorphic, and probably young species, such as *H. oelandicum* or *H. nummularium* (Soubani, 2010), are found throughout the Euro-Asiatic regions (see Fig. 5).

Our phylogenetic reconstruction shows that no *Helianthemum* species occupies an early-diverging, isolated or intermediate position in relation to the rest of the genus, and each species belongs to one of the three main clades retrieved. Several authors (Azevedo & Lorenzo, 1948; Arrigoni, 1971; López-González, 1993) considered *H. squamatum*, with the lowest chromosome number in the genus ($2n = 10$), and *H. caput-felis*, with a unique chromosome number ($2n = 24$) and specific morphological features, to be extant representatives of ancient lineages of the genus. Our analyses revealed a close phylogenetic relationship between *H. squamatum* (a strict gypsophyte almost endemic to the Iberian Peninsula) and *H. syriacum* (a generalist circum-Mediterranean species) plus *H. motae* Sánchez-Gómez & al., together forming the

sister-clade to the rest of the species of sect. *Eriocarpum* and sect. *Pseudomacularia* (see Fig. 3B). Very interestingly, the support for the derived phylogenetic position of *H. squamatum* provides the opportunity for analysing the karyotypic changes (i.e., the drastic reduction in chromosome number) that seem to have occurred in parallel to ecological specialization in this lineage (Levin, 2000; but see Escudero & al., 2014). *Helianthemum caput-felis* (a coastal Tyrrhenian floristic element) has traditionally been included in sect. *Argyrolepis* (= sect. *Polystachium*), along with *H. squamatum* and *H. syriacum* because of their shrubby habit and compound inflorescences (e.g., Janchen, 1925; Proctor & Heywood, 1968) (see Table 1), but our analyses, although they did not resolve the species as monophyletic, robustly confirm its relationship to subg. *Plectolobum*. Interestingly, in the description of the monospecific sect. *Caput-felis*, López-González (1992) emphasised the *Plectolobum*-like ornamentation of the seeds of *H. caput-felis* but, despite suggesting that this species to a certain degree is evolutionarily intermediate between these two subgenera, still assigned this new section to subg. *Helianthemum*. In contrast to this, our study confirms a phylogenetic relationship between *H. caput-felis* and subg. *Plectolobum*.

Clade I. — Clade I consists of subg. *Plectolobum* (= genus *Rhodax*) including all species of sect. *Atlanthemum*, sect. *Macularia* and sect. *Pseudocistus* plus *Caput-felis* (López-González, 1993), whose members extend throughout the Mediterranean and alpine environments of many European and south-west Asian mountain chains (see Fig. 5). In this clade, the unexpected relationship between *H. lunulatum* and *H. pomeridianum* may be revealing a major biogeographical disjunction during, perhaps, the early diversification of subg. *Plectolobum* between the Upper Pliocene and Early Pleistocene (Fig. 4). As the only species of sect. *Macularia*, *H. lunulatum* is a taxonomically and ecologically isolated species restricted to the Ligurian and Maritime Alps (Casazza & al., 2005), and *H. pomeridianum* is a little-known species from the Maghreb (northern Algeria and High Atlas in Morocco) with a rather uncertain taxonomic position (see Table 1) that was initially assigned to sect. *Eriocarpum* in subg. *Helianthemum* (Willkomm, 1856; Grosser, 1903) but later to sect. *Pseudocistus* (Quézel & Santa, 1962). This spectacular split between the south-western Alps and the Atlas Mts. suggests that long-distance dispersal or geographical vicariance represent important speciation forces in early-diverging lineages of this subgenus. Our analyses also

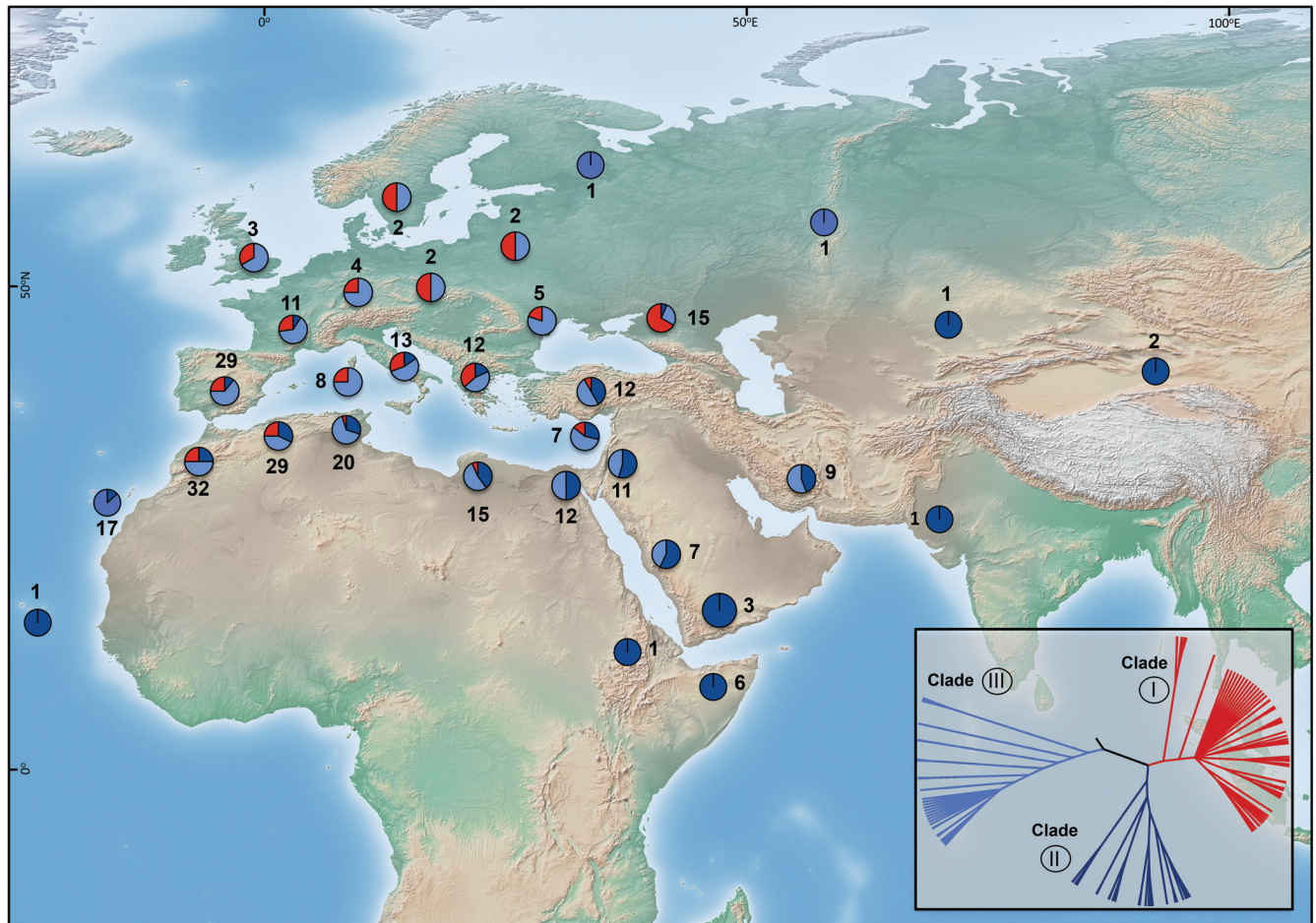


Fig. 5. Distribution of clades I, II and III of *Helianthemum*. Pie charts illustrate the proportion of taxa belonging to each clade of the total number of species in the area. The numbers correspond to the total number of species in the area.

provided support for sect. *Atlantemum* sensu López-González (1993), containing the only therophytic species in this subgenus (*H. sanguineum*; notice that *H. mathezii* is a synonym of *H. pomeridianum*, Aparicio & Albaladejo, 2017) – which rules out the monotypic genus *Atlantemum* (Raynaud, 1987) – and for assigning the seven therophytic species of *Helianthemum* to three different sections (see below). Finally, no evolutionary inferences can be drawn for the taxa in sect. *Pseudocistus* since most species and subspecies of the very polymorphic *H. cinereum*, *H. marifolium* and *H. oelandicum* grouped together without statistical support.

Clade II. — This clade contains the sect. *Argyrolepis*, sect. *Lavandulaceum* and sect. *Pseudomacularia* plus the species included in sect. *Eriocarpum*, all belonging to subg. *Helianthemum* (Fig. 3B), and probably originated in the Late Miocene, Pliocene or Early Pleistocene (4.37 mya, 95% HPD: 1.65–8.57; Fig. 4). This clade mostly includes deserticolous shrub species inhabiting arid and semi-arid environments in Macaronesia, the Mediterranean area, subtropical northern Africa, Anatolia and Central Asia (see Fig. 5). The analysis of the cpDNA+nrDNA concatenated matrix retrieved this clade to consist of two supported sister lineages, one including the species of sect. *Argyrolepis* (*H. squamatum*) and sect. *Lavandulaceum* (*H. syriacum*, *H. motae*), the other containing sect. *Eriocarpum* as a non-monophyletic group due to the inclusion of a minor clade grouping the Irano-Turanian *H. songaricum* (sect. *Pseudomacularia*), *H. antitauricum* and *H. germanicopolitanum* (these latter were ascribed to sect. *Helianthemum* and sect. *Polystachium* respectively; see Table 1). This is a remarkable result that would suggest the need for a taxonomic revision of sect. *Pseudomacularia* taking into account, moreover, the fact that *H. ordosicum* was synonymized with *H. songaricum* in *Flora of China* (Quiner & Gilbert, 2007), but that has, unexpectedly, been retrieved here as a species not most closely related to *H. songaricum*. Indeed, Su & al. (2011, 2017) detected two well-defined genetic groups in western and central China corresponding to *H. songaricum* and *H. ordosicum*, respectively, and our results provide support for this apparent divergence. It is also interesting to note the emergence of two moderately supported clades, one containing the Macaronesian species *H. canariense*, *H. thymiphyllum* and *H. gorgoneum*, the other grouping the morphologically closely similar *H. sicanorum* (one population in southern Sicilia) and the widespread deserticolous shrub *H. kahiricum*. Finally, all non-assigned species (incertae sedis) from Somalia, as expected, turned out to be related to North African and Middle Eastern species (e.g., *H. kahiricum*, *H. lippii*, *H. sancti-antonii* Schweinf. ex Asch. & Schweinf., *H. stipulatum*) from sect. *Eriocarpum*. Gillett (1954) considered the locally endemic *H. somalense* to be a relict species from an ancient wider distribution of *Helianthemum* throughout tropical Africa rather than a Mediterranean-derived floristic element. Therefore, the incorporation of phylogeographical data is necessary for disentangling the diversification pattern of this very interesting group of Mediterranean and subtropical African species (Gillett, 1954), and for the fine-tuning of the relationships between Somalian, Middle Eastern, Macaronesian and Irano-Turanian *Helianthemum* species within sect. *Eriocarpum*.

Clade III. — This clade contains two well-supported sister lineages that represent sect. *Brachypetalum* and sect. *Helianthemum* that also belong to subg. *Helianthemum* (Fig. 3C). This clade probably diversified in parallel to clade II during the Late Miocene, Pliocene or Early Pleistocene (3.76 mya, 95% HPD: 1.39–7.47; Fig. 4) around the Mediterranean Basin. As stated above, all the therophytic species of *Helianthemum* were traditionally assigned to sect. *Brachypetalum* (e.g., Willkomm & Lange, 1880; Grosser, 1903; Proctor & Heywood, 1968; see Table 1). However, López-González (1993) regarded the therophytic habit in *Helianthemum* to be convergent in three different sections in both subgenera: five species in sect. *Brachypetalum*, *H. aegyptiacum* in sect. *Helianthemum*, and *H. sanguineum* in sect. *Atlantemum*. Most of these therophytic species are widespread in the Mediterranean area, which contrasts with a tendency towards a relatively restricted distribution in perennials (cf. Davis, 1965; Proctor & Heywood, 1968; Greuter & al., 1984; López-González, 1993; Thulin, 1993; Tzvelev, 2006), and selfing, through cleistogamy, predominates as a mating systems in these species (Herrera, 1992). This is consistent with Baker's law which states that colonization by self-compatible organisms is more likely to be successful than colonization by self-incompatible organisms due to the ability of the former to produce offspring without pollination agents (Baker, 1959).

Section *Helianthemum* contains more species than any other section in the genus and is also the most geographically and ecologically diverse. These species inhabit Mediterranean and alpine habitats, thriving on limestone, dolomite, marl, gypsum, saline and sandy soils, and are taxonomically complex due to hybridisation plus convergence and phenotypic plasticity (Soubani & al., 2014a, b). Our analyses have retrieved all these species in a large polytomy, but did also retrieve a moderately supported monophyletic lineage, both with the nrDNA (PP = 0.72, BS = 72%) and the concatenated dataset (PP = 0.69, BS = 52%), that contained endemic species from the Canary Islands assigned to sect. *Lavandulaceum* or sect. *Argyrolepis* because of their shrubby habit and ramose inflorescences (Marrero, 1992; Santos-Guerra, 2014) (see also and Tables 1 and 2). As already discussed, we believe that this result, far from reflecting a polyphyletic structure of these sections, indicates the necessity for a taxonomic re-assignment of the species because the shrubby habit and the ramose inflorescence evolved convergently in different sections (López-González, 1992). It is expected that higher-resolution DNA markers will provide stronger support for this monophyletic Canary Islands lineage whose diversification started about 1.28 mya coinciding with the diversification of Canary Islands species of *Cistus* (Guzmán & Vargas, 2010) and other species-rich Macaronesian lineages (Kim & al., 2008) during the Pleistocene, which seems to have taken place over a relatively short period of time.

This unexpected finding reveals that this could be a promising case study for future analysis of the biogeographical processes that have contributed to the biota of this archipelago (Allan & al., 2004; Vargas, 2007; García-Verdugo & al., 2014; Vitales & al., 2014), with up to 15 endemic species of *Helianthemum* (Santos-Guerra, 2014).

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Appendix 1. Taxon name and authority, population number: country (locality), collector's name and collection number (herbarium code) and GenBank accession numbers (in the following order: ITS, *ndhF*, *trnH-psbA* and *trnL-trnF*) included in the molecular analyses. All are newly submitted sequences to GenBank. The n-dash (–) represents not available sequences.

Anisoptera thurifera subsp. *polyandra* (Blume) P.S.Ashton, **E21**: Papua New Guinea (Morobe), *Takeuchi & al.* 16193 (E00310892), KY651254, KY651284, KY651315, KY651346. *Cistus albidus* L., **E14**: Spain (Sevilla, Aznalcázar), *Martín-Hernanz & Rubio s.n.* (SEV286739), KY651255, KY651285, KY651316, KY651347. *Cistus crispus* L., **E15**: Spain (Sevilla, Aznalcázar), *Martín-Hernanz & Rubio s.n.* (SEV286740), KY651256, KY651286, KY651317, KY651348. *Cistus ladanifer* L., **E16**: Spain (Sevilla, Aznalcázar), *Martín-Hernanz & Rubio s.n.* (SEV286741), KY651257, KY651287, KY651318, KY651349. *Cistus libanotis* L., **E23**: Spain (Sevilla, Aznalcázar), *Albaladejo & al. s.n.* (SEV286742), KY651258, KY651288, KY651319, KY651350. *Cistus salvifolius* L., **E17**: Spain (Sevilla, Aznalcázar), *Martín-Hernanz & Rubio s.n.* (SEV286743), KY651259, KY651289, KY651320, KY651351. *Crocantemum bicknellii* Janch., **E216**: U.S.A. (Missouri, Jefferson), *Harris & Harris s.n.* (MO2604040), KX401468, KX498385, KX498510, KX498638. *Crocantemum canadense* Britton, **E204**: U.S.A. (New York, Suffolk Co. Long Island), *Strong 3716* (NY02065469), KX401472, –, KX498514, KX498641. *Crocantemum canadense* Britton, **E205**: U.S.A. (Maryland, Caroline Co. Town Federalsburg), *Longbottom 13827* (NY02065471), KX401473, KX498389, KX498515, KX498642. *Crocantemum georgianum* (Chapm.) Barnhart, **E214**: U.S.A. (Alabama, Roadside of Hwy), *MacDonald 11214* (MO5023665), KX401493, –, KX498533, KX498660. *Crocantemum glomeratum* Janch., **E206**: Costa Rica (San José, Chirripó, valle de los Leones), *Luteyn 15424* (NY309985), KX401497, –, KX498537, KX498663. *Crocantemum pringlei* Janch., **E211**: Nicaragua (Estelí, RN Tisey, Comunidad la Almaciguera), *Rueda & al.* 13098 (MO5610815), KX401550, –, KX498585, KX498705. *Crocantemum rosmarinifolium* Janch., **E207**: U.S.A. (South Carolina, Richland Co. Fort Jackson), *Boyd 4484* (NY02065468), KX401551, –, –, *Crocantemum scoparium* Millsp., **E203**: U.S.A. (California, Riverside Co. Lake Skinner Country Park), *Boyd 4484* (NY02065468), KX401560, –, KX498591, KX498712. *Crocantemum scoparium* Millsp., **E209**: U.S.A. (California, San Diego, lower Otay lake), *Walker 1259* (NY42709), KX401561, –, KX498592, KX498713. *Fumana fontanesii* Clauson ex Pomel, **E29**: Morocco (Agadir, between Oulma and Imouzzer), *Aparicio & Arroyo s.n.* (SEV286744), KY651260, KY651290, –, KY651352. *Fumana laevipes* Spach, **E28**: Morocco (Agadir, between Oulma and Imouzzer), *Aparicio & Arroyo s.n.* (SEV286745), KY651261, KY651291, KY651321, KY651353. *Fumana scoparia* Pomel, **E30**: Italy (Sicily, Gela Torre Manfreda), *Arroyo s.n.* (SEV286746), –, KY651292, KY651322, KY651354. *Fumana thymifolia* Spach, **E13**: Spain (Cádiz, Benamahoma), *Aparicio s.n.* (SEV286747), KX401442, KX498486, KX498486, KX498613. *Halimium calycinum* (L.) K.Koch, **E25**: Spain (Sevilla, Aznalcázar), *Albaladejo & de Vega s.n.* (SEV286748), KY651262, KY651293, KY651323, KY651355. *Halimium halimifolium* (L.) Willk., **E22**: Spain (Sevilla, Aznalcázar), *Albaladejo & al. s.n.* (SEV286749), KY651263, KY651294, KY651324, KY651356. *Halimium lasianthum* (Lam.) Spach, **E27**: Spain (Cádiz, Alcalá de los Gazules, Pileta de la Reina), *Martín-Hernanz s.n.* (SEV286750), KY651264, KY651295, KY651325, KY651357. *Halimium umbellatum* (L.) Spach, **E18**: Spain (Salamanca, between Guijuelo and Valdelacasa), *Campos & al. unknown* (SEV28498), KY651265, –, KY651326, KY651358. *Helianthemum abelardoi* Alcaraz, **38**: Spain (Alicante, Campoamor), *Aparicio & al. s.n.* (SEV286547), KX401448, KX498365, KX498492, KX498619. *Helianthemum aegyptiacum* Mill., **51-1**: Spain (Sevilla, Aznalcollar, Las Barreras), *Aparicio s.n.* (SEV286556), KX401449, KX498366, KX498493, KX498620. *Helianthemum aegyptiacum* Mill., **51-2**: Spain (Sevilla, Aznalcollar, Las Barreras), *Aparicio s.n.* (SEV286556), KX401450, KX498367, KX498494, KX498621. *Helianthemum almeriense* Pau, **40**: Spain (Murcia, Cabo Cope), *Aparicio s.n.* (SEV286550), KX401451, KX498368, –, KX498622. *Helianthemum almeriense* Pau, **42**: Spain (Almería, Antas-Lubrin), *Aparicio s.n.* (SEV286549), KX401452, KX498369, KX498495, KX498623. *Helianthemum alypoides* Losa & Rivas Goday, **45**: Spain (Almería, Río Aguas), *Aparicio s.n.* (SEV286553), KX401453, KX498370, –, KX498624. *Helianthemum alypoides* Losa & Rivas Goday, **46**: Spain (Almería, Río Aguas), *Aparicio s.n.* (SEV286554), KX401454, KX498371, KX498496, KX498625. *Helianthemum angustatum* Pomel, **48**: Spain (Cádiz, Villaluenga del Rosario), *Aparicio & Arroyo s.n.* (SEV286552), KX401455, KX498372, KX498497, KX498626. *Helianthemum angustatum* Pomel, **66**: Spain (Granada, Cúllar, Sierra de Baza, Cortijo del Bordón), *Aparicio & Albaladejo s.n.* (SEV286569), KX401456, KX498373, KX498498, KX498627. *Helianthemum antitauricum* P.H.Davis & Coode, **271-1**: Turkey (Adana, between Arslantaş-Ayvat villages), *Yeşilyurt s.n.* (s.n.) KX401570, KX498471, KX498600, KX498721. *Helianthemum antitauricum*, **271-2**: Turkey (Adana, between Arslantaş-Ayvat villages), *Yeşilyurt s.n.* (s.n.), KX401571, KX498472, KX498601, KX498722. *Helianthemum apenninum* Mill., **169**: Italy (Sicilia, Roca Busambra), *Arroyo & Arroyo s.n.* (SEV286751), KX401457, KX498374, KX498499, KX498628. *Helianthemum apenninum* Mill. subsp. *apenninum*, **30**: France (Cahors, Mt Saint Cyr), *Arroyo & Pérez-Barrales s.n.* (SEV286544), KX401458, KX498375, KX498500, KX498629. *Helianthemum apenninum* subsp. *cavanillesianum* (M.Lainz) G.López, **21**: Spain (Jaén, Cazorla, arenales del Guadalentín), *Aparicio & Albaladejo s.n.* (SEV286541), KX401459, KX498376, KX498501, KX498630. *Helianthemum apenninum* subsp. *estevei* (Peinado & Mart. Parras) G.López, **75**: Spain (Granada, La Zubia, Trevenque), *Aparicio & Albaladejo s.n.* (SEV286573), KX401460, KX498377, KX498502, KX498631. *Helianthemum apenninum* subsp. *stoechadifolium* (Brot.) Samp., **1**: Spain (Cádiz, Grazalema, Sierra del Endrinal), *Aparicio & Albaladejo s.n.* (SEV286512), KX401461, KX498378, KX498503, KX498632. *Helianthemum apenninum* subsp. *suffruticosum* (Boiss.) G.López, **4**: Spain (Málaga, Sierra de las Nieves), *Aparicio & al. s.n.* (SEV286513), KX401462, KX498379, KX498504, KX498633. *Helianthemum apenninum* subsp. *urrielense* (M.Lainz) G.López, **104**: Spain (Cantabria, Picos de Europa), *Albaladejo & de Vega s.n.* (SEV286523), KX401463, KX498380, KX498505, KX498634. *Helianthemum argyreum* Baker, **165**: Yemen (Ras Fartak), *Thulin & al.* 9602 (UPS BOT V-095747), KX401465, KX498382, KX498507, –, *Helianthemum asperum* Lag. ex Dunal, **52**: Spain (Málaga, Sierra de las Nieves), *Aparicio* (SEV286557), KX401466, KX498383, KX498508, KX498636. *Helianthemum asperum* Lag. ex Dunal, **76**: Spain (Granada, La Zubia, Cumbres Verdes), *Aparicio & Albaladejo s.n.* (SEV286576), KX401467, KX498384, KX498509, KX498637. *Helianthemum bramwelliorum* Marrero Rodr., **132**: Spain (Lanzarote, Fuente de Guinate), *Marrero unknown* (MA537010), KX401469, KX498386, KX498511, –, *Helianthemum bystropogophyllum* Svent., **223**: Spain (Gran Canaria, Barranco de Vigaroy), *DNA Bank of the Canarian Flora* (VIAL7777), KX401470, KX498387, KX498512, KX498639. *Helianthemum bystropogophyllum* Svent., **224**: Spain (Gran Canaria, San Nicolás de Tolentino), *DNA Bank of the Canarian Flora* (VIAL7730), KX401471, KX498388, KX498513, KX498640. *Helianthemum canariense* Pers., **110**: Morocco (El-Aïoum,

Appendix 1. Continued.

Beni-Snassen, May Taieb), *Aparicio & al. s.n.* (SEV286528), KX401474, KX498390, KX498516, KX498643. *Helianthemum canariense* Pers., 135: Spain (Fuerteventura, Pájara, La Pared), *Álvarez & al. JC2027* (MA768079), KX401475, KX498391, KX498517, KX498644. *Helianthemum caput-felis* Boiss., 37: Spain (Alicante, Torre de la Horadada), *Aparicio & al. s.n.* (SEV286545), KX401476, KX498392, KX498518, KX498645. *Helianthemum caput-felis* Boiss., 39: Spain (Alicante, Campoamor), *Aparicio & al. s.n.* (SEV286548), KX401477, KX498393, KX498519, KX498646. *Helianthemum caput-felis* Boiss., 335: Italy (Sardinia, Capo Mannu), *Arroyo s.n.* (SEV286752), KY651269, KY651299, KY651330, KY651362. *Helianthemum chihuahuaense* S. Watson, E208: México (Chihuahua, Urique), *Ford 94* (NY1042369), KX401478, –, KX498520, KX498647. *Helianthemum ciliatum* Pers., 147: Tunisia (Kasserine, Fériana, Magil Ben Abbès), *Calvo & al. JC3310* (MA798199), KX401479, KX498394, KX498521, KX498648. *Helianthemum ciliatum* Pers., 217: Tunisia (Kasserine, Fériana, Magil Ben Abbès), *Calvo & al. JC3310* (MA798226), KX401480, KX498395, KX498522, KX498649. *Helianthemum cinereum* Pers. subsp. *cinereum*, 60: Spain (Murcia, Jumilla-Albatera), *Aparicio & Albaladejo s.n.* (SEV286562), KX401481, KX498396, KX498523, KX498650. *Helianthemum cinereum* subsp. *guadicianum* (Font Quer & Rothm.) G.López, 64: Spain (Almería, María, Sierra de María, La Peguera), *Aparicio & Albaladejo s.n.* (SEV286568), KX401482, KX498397, KX498524, KX498651. *Helianthemum cinereum* subsp. *hieronymi* (Sennen) G.López, 62: Spain (Murcia, Alhama de Murcia, Sierra Espuña), *Aparicio & Albaladejo s.n.* (SEV286566), KX401483, KX498398, KX498525, KX498652. *Helianthemum cinereum* subsp. *rotundifolium* (Dunal) Greuter & Burdet, 52: Spain (Málaga, Sierra de las Nieves), *Aparicio s.n.* (SEV286580), KX401485, KX498400, KX498527, KX498654. *Helianthemum cinereum* subsp. *rotundifolium* (Dunal) Greuter & Burdet, 108: Morocco (Berkane, Rislane), *Aparicio & al. s.n.* (SEV286527), KX401484, KX498399, KX498526, KX498653. *Helianthemum citrinum* Ghaz., 198: Oman (Dhofar, above Shabithat), *Miller 6452* (E00454528), KX401486, –, –, –. *Helianthemum confertum* Dunal, 102: Morocco (Essaouira, Ounara), *Aparicio & Arroyo s.n.* (SEV286522), KX401487, KX498401, KX498528, KX498655. *Helianthemum confertum* Dunal, 138: Algeria (Ghardaia, El Golea, El Kouah), *Chevallier unknown* (MA81060–2), KX401488, KX498402, KX498529, KX498656. *Helianthemum crassifolium* Pers., 154: Tunisia (Medine, Zarziz, Slab el Gharbi), *S. Castroviejo and the team of the project CGL2008-02982-C03 unknown* (MA797337), KX401489, KX498403, –, KX498657. *Helianthemum cretaceum* (Rupr.) Juz., 183: Russia (Saratovskaya, Chavlynsk), *Grosser unknown* (LD1630024), KX401490, KX498404, KX498530, KX498658. *Helianthemum cylindrifolium* Verdc., 166: Somalia (Erivago), *Thulin unknown* (UPS4243), KX401491, KX498405, KX498531, –, *Helianthemum ellipticum* Pers., 218: Morocco (Beni-Snassen, Bled al'Adaba), *Bouhmedi & al. unknown* (SEV166793), KX401492, KX498406, KX498532, KX498659. *Helianthemum germanicopolitanum* Borm., 272-1: Turkey (Çankiri, Kalecik-Çankiri road, parting of the İnandık ways), *Yeşilyurt s.n.* (s.n.), KX401494, KX498407, KX498534, KX498661. *Helianthemum germanicopolitanum* Borm., 272-2: Turkey (Çankiri, Kalecik-Çankiri road, parting of the İnandık ways), *Yeşilyurt s.n.* (s.n.), KX401495, KX498408, KX498535, KX498662. *Helianthemum getulum* Pomel, 139: Algeria (Ghardaia, Ghardaia & El-Golâ, Hababza, Oum el Klab), *Chevallier 542* (MA81073), KX401496, KX498409, KX498536, –, *Helianthemum gonzalezferrerii* Marrero Rodr., 222: Spain (Lanzarote, El Bosquecillo), (VIAL 4281), KX401498, KX498410, KX498538, KX498664. *Helianthemum gorgoneum* Webb, 348: Cape Verde (Ilha do Fogo, Chã das Caldeiras), *Martín-Hernanz & al. s.n.* (SEV286753), KY651270, KY651300, KY651331, KY651363. *Helianthemum gorgoneum* Webb, 350: Cape Verde (Santo Antão, Lombo de Figueira), *Martín-Hernanz & al. s.n.* (SEV286754), KY651271, KY651301, KY651332, KY651364. *Helianthemum grosii* Pau & Font Quer, 117: Morocco (Al-Hoceima, 6 km towards Izemmourèn), *Aparicio & al. s.n.* (SEV286533), KX401499, KX498411, KX498539, KX498665. *Helianthemum grosii* Pau & Font Quer, 118: Morocco (Al-Hoceima, Circa Izemmourèn), *Aparicio & al. s.n.* (SEV286534), KX401500, KX498412, KX498666. *Helianthemum guerrae* Sánchez-Gómez, J.S.Carrion & M.A.Carrion, 61-1: Spain (Murcia, Yecla, Sierra del Serral, La Boquera), *Aparicio & Albaladejo s.n.* (SEV286564), KX401501, KX498413, KX498541, KX498667. *Helianthemum guerrae* Sánchez-Gómez, J.S.Carrion & M.A.Carrion, 61-2: Spain (Murcia, Yecla, Sierra del Serral, La Boquera), *Aparicio & Albaladejo s.n.* (SEV286563), KX401502, KX498414, KX498542, KX498668. *Helianthemum helianthemoides* (Desf.) Sennen & Mauricio, 159: Morocco (Timahdate, hacina Col du Zad), *Cirujano & al. R-10242* (MA624971), KX401503, KX498415, KX498543, KX498669. *Helianthemum helianthemoides* (Desf.) Sennen & Mauricio, 164: Morocco (Medium Atlas, Taffert), *Aedo & al. unknown* (MA593297), KX401504, KX498416, KX498544, –, *Helianthemum hirtum* Mill., 19: Spain (Sevilla, Alcalá de Guadaira, Hacienda Los Ángeles), *Aparicio s.n.* (SEV286517), KX401505, KX498417, KX498545, KX498670. *Helianthemum hirtum* Mill., 85: Spain (Málaga, Antequera, hacina Valle de Abdalajís), *Aparicio & Albaladejo s.n.* (SEV286579), KX401507, KX498419, KX498547, KX498672. *Helianthemum hirtum* Mill., 236: Spain (Huelva, Matalascañas), *Aparicio s.n.* (SEV286539), KX401506, KX498418, KX498546, KX498671. *Helianthemum hymettium* Boiss. & Heldr., 180: Greece (Crete, Lasithiou, northern slope of Afendis Karousi), *Ländstrom unknown* (LD1435399), KX401508, KX498420, KX498548, –, *Helianthemum inaguae* Marrero Rodr., Gonz.-Mart. & Gonz.-Art. 225: Spain (Gran Canaria, Inagua), *DNA Bank of the Canarian Flora* (VIAL13308), KX401509, –, KX498549, KX498673. *Helianthemum juliae* Wildpret, 226: Spain (Tenerife, Risco Verde), *DNA Bank of the Canarian Flora* (VIAL2642), KX401510, KX498421, KX498550, KX498674. *Helianthemum kahiricum* Delile, 113: Morocco (Guercif, 15 km towards Taza), *Aparicio & al. s.n.* (SEV286530), KX401511, KX498422, KX498551, KX498675. *Helianthemum kahiricum* Delile, 151: Tunisia (Gabès, Metlaoui, gorjes de Seldja), *S. Castroviejo and the team of the project CGL2008-02982-C03 unknown* (MA795081), KX401512, KX498423, KX498552, KX498676. *Helianthemum kotschyannum* Boiss., 268-1: Turkey (Konya, Aladağ road, near Bademli village), *Yeşilyurt s.n.* (s.n.), KX401513, KX498424, KX498553, KX498677. *Helianthemum kotschyannum* Boiss., 268-2: Turkey (Konya, Aladağ road, near Bademli village), *Yeşilyurt s.n.* (s.n.), KX401514, KX498425, KX498554, KX498678. *Helianthemum ledifolium* (L.) Mill., 19: Spain (Sevilla, Alcalá de Guadaira, Hacienda Los Ángeles), *Aparicio s.n.* (SEV286516), KX401515, KX498426, KX498555, KX498679. *Helianthemum ledifolium* (L.) Mill., 82: Spain (Granada, Alhama de Granada), *Aparicio & Albaladejo s.n.* (SEV286578), KX401516, KX498427, KX498556, KX498680. *Helianthemum lippii* (L.) Dum.Cours., 96: Morocco (Tiznit, Kerdous-Tafrout), *Aparicio & Arroyo s.n.* (SEV286521), KX401518, KX498429, –, KX498682. *Helianthemum lippii* (L.) Dum.Cours., 152: Tunisia (Gabès, Cnenini), *Herrera & al. AH3807* (MA797008), KX401517, KX498428, KX498557, KX498681. *Helianthemum lunulatum* DC., 185: France (Tende, cultivated in Alpine Station Joseph Fourier), *Douzet unknown* (s.n.), KX401519, KX498430, KX498558, –, *Helianthemum lunulatum* DC., 324: Italy (Limone Piemonte, Valle San Giovanni), *Aparicio & al. s.n.* (SEV286756), KY651272, KY651302, KY651333, KY651365. *Helianthemum lunulatum* DC., 329: Italy (Ormea, Colle Caprauna, Monte Armetta), *Aparicio & al. s.n.* (SEV286755), KY651273, KY651303, KY651334, KY651366. *Helianthemum marifolium* subsp. *andalusicum* (Font Quer & Rothm.) G.López, 14: Spain (Cádiz, Grazalema: Las Canteras), *Aparicio s.n.* (SEV286514), KX401520, KX498431, KX498559, KX498683. *Helianthemum marifolium* subsp. *conquense* Borja & Rivas Goday ex G.López, 55: Spain (Cuenca, Huete), *Aparicio & Albaladejo s.n.* (SEV286558), KX401521, KX498432, KX498560, KX498684. *Helianthemum marifolium* subsp. *frigidulum* (Cuatrecasas) G.López, 73: Spain (Jaén, Huelma, Sierra Mágina, Collado de la Cruz), *Aparicio & Albaladejo s.n.* (SEV286572), KX401522, KX498433, KX498561, KX498685. *Helianthemum marifolium* Mill. subsp. *marifolium*, 88: Spain (Málaga, Alhaurín de la Torre, Jarapalo), *Aparicio & Albaladejo s.n.* (SEV286520), KX401523, KX498434, –, KX498686. *Helianthemum marifolium* subsp. *molle* (Cav.) G.López, 254: Spain (Castellón, Eslida, Sierra del Espadán), *Aparicio & al. s.n.* (SEV286540), KX401524, KX498435, KX498562, KX498687. *Helianthemum marifolium* subsp. *organifolium* (Lam.) G.López, 88: Spain (Málaga, Alhaurín de la Torre, Jarapalo), *Aparicio & Albaladejo s.n.* (SEV286519), KX401525, KX498436, KX498563, KX498688. *Helianthemum marifolium* subsp. *organifolium* (Lam.) G.López, 287: Morocco (Nador, Bni Chiker), *Aparicio & al. s.n.* (SEV286757), KY651274, KY651304, KY651335, KY651367. *Helianthemum marminorensis* Alcaraz, Peinado & Mart. Parras, 276: Spain (Murcia, San Pedro del Pinatar, duna de San Pedro), *Aparicio & al. s.n.* (SEV286758), KY651275, KY651305, KY651336, KY651368. *Helianthemum motae* Sánchez-Gómez, J.F.Jiménez & J.B.Vera, 277: Spain (Murcia, Águilas), *Aparicio & al. s.n.* (SEV286759), KY651276, KY651306, KY651337, KY651369. *Helianthemum neopiliferum* Muñoz Garm. & Navarro, 73: Spain (Jaén, Huelma, Sierra Mágina, Collado de la Cruz), *Aparicio & Albaladejo s.n.* (SEV286571), KX401527, KX498438, KX498565, KX498690. *Helianthemum neopiliferum* Muñoz Garm. & Navarro, 125: Spain (Granada, Fornes, La Resinera), *Arroyo & al. s.n.* (SEV286536), KX401526, KX498437, KX498564, KX498689. *Helianthemum nummularium* Mill. subsp. *nummularium*, 171: Italy (Sicilia, Nehodi-Cesaro), *Arroyo & Pérez-Barrales s.n.* (SEV286537), KX401530, KX498441, KX498568, KX498693. *Helianthemum nummularium* subsp. *grandiflorum* (Scop.) Schinz & Thell., 192: Austria (Steiermark, Niedere Tauern, Wölzer Tauern), *Hörandl E8413* (WU030720), KX401528, KX498439, KX498566, KX498691. *Helianthemum nummularium* subsp. *lycaonicum* Coope & Cullen, 269: Turkey (Isparta, Yenice village), *Yeşilyurt s.n.* (s.n.), KX401529, KX498440, KX498692.

Appendix 1. Continued.

KX498692. *Helianthemum nummularium* subsp. *lycaonicum* Coode & Cullen, 270: Turkey (Adana-Aksaray, 14 km from to Ulukışla), *Yeşilyurt s.n.* (s.n.), –, KY651307, KY651338, KY651370. *Helianthemum nummularium* subsp. *obscurum* Holub, 193: Austria (Niederösterreich, Thermenline, Traiskirchen), *Till 110405* (WU060546), KX401531, KX498442, KX498569, –, *Helianthemum nummularium* subsp. *semiglabrum* (Badaró) M. Proctor, 327: Italy (Colle di Nava, Ponte di Nava), *Aparicio & al. s.n.* (SEV286760), KY651277, KY651308, KY651339, KY651371. *Helianthemum obtusifolium* Dunal, 232: Cyprus (Nicosia, road from Mitsero to Kato Moni a Alona), *Aparicio & al. s.n.* (SEV252314), KX401532, KX498443, KX498570, –, *Helianthemum oelandicum* subsp. *alpestre* (Jacq.) Breistr., 184: France (Cervièrès, Col d'Izouard), *Douzet s.n.* (SEV286538), KX401533, –, –, *Helianthemum oelandicum* subsp. *incanum* (Willk.) G. López, 22: Spain (Jaén, Cazorla, Nava de la Correhuela), *Albaladejo s.n.* (SEV286542), KX401534, KX498444, KX498571, KX498694. *Helianthemum oelandicum* subsp. *italicum* (L.) Font Quer & Rothm., 31: Spain (Lleida, Martinet-Lles), *Arroyo & Pérez-Barrales s.n.* (SEV286546), KX401535, KX498445, KX498572, KX498695. *Helianthemum oelandicum* subsp. *pouretii* (Timb.-Lagr.) Greuter & Burdet, 332: France (Bédain, Mont Ventoux), *Aparicio & al. s.n.* (SEV286761), KY651278, KY651309, KY651340, KY651372. *Helianthemum oelandicum* subsp. *rupifragum* (A. Kern.) Breistr., 182: Romania (Alba, Montibus Gilăului, Scărișoara, Belisoara), *Morariu & Danciu unknown* (LUND1629704), KX401536, KX498446, KX498573, KX498696. *Helianthemum oelandicum* subsp. *stevanii* (Rupr. ex Juz. & Pozdeeva) Greuter & Burdet, 190: Ukraine (Crimea, Nikita, monte Martjan), *Beliamina s.n.* (SEV43447), KX401537, –, –, *Helianthemum ordosicum* Zhao, Zong, Zhu & Cao, 127: China (Inner Mongolia, Ordos, Quianlishan), *Su unknown* (s.n.), KX401538, KX498447, KX498574, KX498697. *Helianthemum pannosum* Boiss., 75-1: Spain (Granada, La Zubia, Trevenque), *Aparicio & Albaladejo s.n.* (SEV286574), KX401539, KX498448, KX498575, KX498698. *Helianthemum pannosum* Boiss., 75-2: Spain (Granada, La Zubia, Trevenque), *Aparicio & Albaladejo s.n.* (SEV286575), KX401540, –, KX498576, KX498699. *Helianthemum papillare* Boiss., 63: Spain (Almería, María, Sierra de María, La Peguera), *Aparicio & Albaladejo s.n.* (SEV286567), KX401542, KX498450, –, KX498700. *Helianthemum papillare* Boiss., 106: Morocco (Gouenfuda, towards Jerada), *Aparicio & al. s.n.* (SEV286525), KX401541, KX498449, KX498577, –, *Helianthemum patens* (Hemsl.) Gross., E212: México (San Luis de Potosí, 40 Km towards Santa Teresa), *unknown* (MO4638080), KX401543, –, KX498578, –, *Helianthemum permagaceum* Pomel, 149: Tunisia (Kasserine, Djbel Chambi), *S. Castroviejo and the team of the project CGL2008-02982-C03 unknown* (MA798366), KX401544, KX498451, KX498579, KX498701. *Helianthemum polyanthum* Pers., 117: Morocco (Al-Hoceima, 6 km towards Izemmourén), *Aparicio & al. s.n.* (SEV286532), KX401545, KX498452, KX498580, KX498702. *Helianthemum polygonoides* Peinado, Mart. Parras, Alcaraz & Espuelas, 58: Spain (Albacete, Cordovilla), *Aparicio & Albaladejo s.n.* (SEV286560), KX401546, KX498453, KX498581, KX498703. *Helianthemum polygonoides* Peinado, Mart. Parras, Alcaraz & Espuelas, 59: Spain (Albacete, Saladar de Cordovilla), *Aparicio & Albaladejo s.n.* (SEV286561), KX401547, KX498454, KX498582, –, *Helianthemum pomeridianum* Dunal, 144: Morocco (Marrakech, Taroudant), *Podlech s.n.* (MA472377), KX401548, KX498455, KX498583, KX498704. *Helianthemum pomeridianum* Dunal, 352: Morocco (Taroudant, Sidi Abdellah Oussaid-Alegjane), *Aparicio & Aparicio s.n.* (SEV286762), KY651279, KY651310, KY651341g, KY651373. *Helianthemum raskebdanae* M.A. Alonso, M.B. Crespo, Juan & L. Sáez, 274: Morocco (Nador, Ras-el-Ma), *Aparicio & al. s.n.* (SEV286763), KY651280, KY651311, KY651342, KY651374. *Helianthemum ruficomum* Spreng., 110: Morocco (El-Aïoum, Beni-Snassen, May Taieb), *Aparicio & al. s.n.* (SEV286529), KX401552, KX498456, KX498586, KX498706. *Helianthemum salicifolium* (L.) Mill., 20: Spain (Sevilla, Dos Hermanas, El Baldío), *Aparicio s.n.* (SEV286518), KX401555, KX498459, KX498588, KX498708. *Helianthemum salicifolium* (L.) Mill., 106: Morocco (Gouenfuda, towards Jerada), *Aparicio & al. s.n.* (SEV286526), KX401554, KX498458, KX498587, KX498707. *Helianthemum sancti-antonii* Schweinf. ex Asch. & Schweinf., 194: Jordan (Wadi Rum), *Albert & Watzka 16* (W2010-0000659), KX401556, KX498460, KX498589, –, *Helianthemum sancti-antonii* Schweinf. ex Asch. & Schweinf., 197: Jordan (Ma'an, Rum Eisenbahnstation, Felsen, Geroll), *Frey & Kürschner VO5059* (E00431873), KX401557, KX498461, KX498590, KX498709. *Helianthemum sanguineum* (Lag.) Lag. ex Dunal, 210-1: Spain (Salamanca, Calvarrasa de Arriba, Los Terraplenes), *Rico s.n.* (s.n.), KX401558, KX498462, –, KX498710. *Helianthemum sanguineum* (Lag.) Lag. ex Dunal, 210-2: Spain (Salamanca, Calvarrasa de Arriba, Los Terraplenes), *Rico s.n.* (s.n.), KX401559, KX498463, –, KX498711. *Helianthemum sanguineum* (Lag.) Lag. ex Dunal, 295: Cyprus (Ayia Eirimi), *Aparicio & al. s.n.* (SEV286764), KY651281, KY651312, KY651343, KY651375. *Helianthemum sauvagei* Raynaud., 282-0: Morocco (Agadir, Amerskroud), *Aparicio & al. s.n.* (SEV286765), KY651282, KY651313, KY651344, KY651376. *Helianthemum sicanorum* Brullo, Giusso & Sciandr., 297: Italy (Sicily, Gela Torre Manfria), *Aparicio & al. s.n.* (SEV286766), KY651283, KY651314, KY651345, KY651377. *Helianthemum somalense* Gillett, 167: Somalia (Ceel Afweyn, 43 km to Ceerigaabo), *Thulin 10767* (UPS BOT V-122915), KX401562, KX498464, KX498593, KX498714. *Helianthemum songaricum* Schrenk ex Fisch. & C.A. Mey, 126: China (Xinjiang, Bole city), *Su unknown* (s.n.), KX401563, KX498465, KX498594, KX498715. *Helianthemum songaricum* Schrenk ex Fisch. & C.A. Mey, 175: Kazakhstan (Almaty, Charyn National Park, Top of main gorge), *Rae & al. 16* (E00228039), KX401564, KX498466, KX498595, –, *Helianthemum speciosum* Thulin, 168: Somalia (Al Miskat), *Thulin & al. 10199* (UPS BOT V-104204), KX401565, –, –, *Helianthemum squamatum* Pers., 46: Spain (Almería, Río Aguas), *Aparicio & al. s.n.* (SEV286555), KX401566, KX498467, KX498597, KX498717. *Helianthemum squamatum* Pers., 57: Spain (Albacete, Tobarra-Cordovilla), *Aparicio & Albaladejo s.n.* (SEV286559), KX401567, KX498468, KX498598, KX498718. *Helianthemum stipulatum* C. Chr., 173: Djibouti (Tadjoura, Egeualeita, summit Gouda Mts.), *Lavranos 10508* (E00639602), KX401568, KX498469, –, KX498719. *Helianthemum stipulatum* C. Chr., 200: Iran (Kerman, Rouchoun hills, Khabr-va-Rouchoun protected region), *Parris 75.407* (E00454505), KX401569, KX498470, KX498599, KX498720. *Helianthemum syriacum* (Jacq.) Dum. Cours., 17: Spain (Cádiz, Olvera: Sierra de Lijar), *Aparicio s.n.* (SEV286515), KX401572, KX498473, KX498602, KX498723. *Helianthemum syriacum* (Jacq.) Dum. Cours., 79: Spain (Granada, Padul, Sierra del Manar), *Aparicio & Albaladejo* (SEV286577), KX401573, KX498474, KX498603, KX498724. *Helianthemum teneriffae* Coss., 134: Spain (Tenerife, Güimar), *unknown* (MA620080), KX401574, KX498475, KX498604, –, *Helianthemum tholiforme* J. Ortega & B. Navarro, 228: Spain (Gran Canaria, Faneque), *DNA Bank of the Canarian Flora* (VIAL1360), KX401575, KX498476, –, KX498725. *Helianthemum thymiphyllum* Svent., 227: Spain (Fuerteventura, Vega Río Palma), *DNA Bank of the Canarian Flora* (VIAL11503), KX401576, KX498477, KX498605, KX498726. *Helianthemum vesicarium* Boiss., 195: Jordan (Dana), *unknown* (W2009-0003914), KX401577, KX498478, KX498606, KX498727. *Helianthemum vesicarium* Boiss., 196: Jordan (Wadi Hessa), *unknown* (W2009-0003912), KX401578, KX498479, –, KX498728. *Helianthemum violaceum* Pers., 29: France (Cevennes, St. Etienne de Gorges), *Arroyo & Pérez-Barrales s.n.* (SEV286543), KX401579, KX498480, KX498607, KX498729. *Helianthemum virgatum* (Desf.) Pers., 105: Morocco (Berkane, towards Taforal), *Aparicio & al. s.n.* (SEV286524), KX401464, KX498381, KX498506, KX498635. *Helianthemum virgatum* (Desf.) Pers., 114: Morocco (Aknoul, 18 km towards Taza), *Aparicio & al. s.n.* (SEV286531), KX401580, KX498481, KX498608, KX498730. *Helianthemum viscarium* Boiss. & Reut., 41: Spain (Murcia, Torre del Cabo Cope), *Aparicio & al. s.n.* (SEV286551), KX401583, KX498484, KX498611, KX498733. *Helianthemum viscarium* Boiss. & Reut., 62: Spain (Murcia, Alhama de Murcia, Sierra Espuña), *Aparicio & Albaladejo s.n.* (SEV286565), KX401584, KX498485, KX498612, KX498734. *Helianthemum viscidulum* subsp. *raynaudii* (Ortega Oliv., Romero García & C. Morales) G. López, 70: Spain (Granada, Hueter, Puerto de la Mora), *Aparicio & Albaladejo s.n.* (SEV286570), KX401581, KX498482, KX498609, KX498731. *Helianthemum viscidulum* Boiss. subsp. *viscidulum*, 124: Spain (Granada, Fomes, La Resinera), *Arroyo & al. s.n.* (SEV286535), KX401582, KX498483, KX498610, KX498732. *Hudsonia tomentosa* Nutt., E1: U.S.A. (New York, Suffolk), *Strong 3720* (NY02256776), KX401443, KX498360, KX498487, KX498614. *Hudsonia tomentosa* Nutt., E2: U.S.A. (Maryland, Worcester), *Atha 5000* (NY01087766), KX401444, KX498361, KX498488, KX498615. *Lechea intermedia* Legg. ex Britton & Hollick, E4: U.S.A. (New York, Putnam), *Atha 7869* (NY01132969), KX401445, KX498362, KX498489, KX498616. *Lechea leggettii* Britton & Hollick, E6: U.S.A. (North Carolina, Croatan National Forest), *Atha 9372* (NY01207272), KX401446, KX498363, KX498490, KX498617. *Lechea racemulosa* Lam., E5: U.S.A. (North Carolina, Smoky Mountain NP), *Lendemer 33215* (NY1651749), KX401447, KX498364, KX498491, KX498618. *Tuberaria echioides* (Lam.) Willk., E9: Spain (Sevilla, Dos Hermanas, La Corchuela), *Aparicio & Albaladejo s.n.* (SEV286767), KY651266, KY651296, KY651327, KY651359. *Tuberaria lignosa* (Sweet) Samp., E26: Spain (Cádiz, Alcalá de los Gazules, Pileta de la Reina), *Martin-Hernanz s.n.* (SEV286768), KY651267, KY651297, KY651328, KY651360. *Tuberaria macrosepala* (Coss.) Willk., E8: Spain (Sevilla, Dos Hermanas, La Corchuela), *Aparicio & Albaladejo s.n.* (SEV286769), KY651268, KY651298, KY651329, KY651361.

TAXON

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Electronic Supplement to

**Phylogenetic reconstruction of the genus
Helianthemum (Cistaceae) using plastid and nuclear
DNA-sequences: Systematic and evolutionary
inferences**

**Abelardo Aparicio, Sara Martín-Hernanz, Clara Parejo-Farnés, Juan Arroyo,
Sébastien Lavergne, Emine B. Yeşilyurt, Ming-Li Zhang, Encarnación Rubio &
Rafael G. Albaladejo**

■ APPENDIX S1. FLORISTIC PUBLICATIONS REVIEWED IN THIS STUDY TO ELABORATE THE TAXONOMIC OVERVIEW OF *HELIANTHEMUM*

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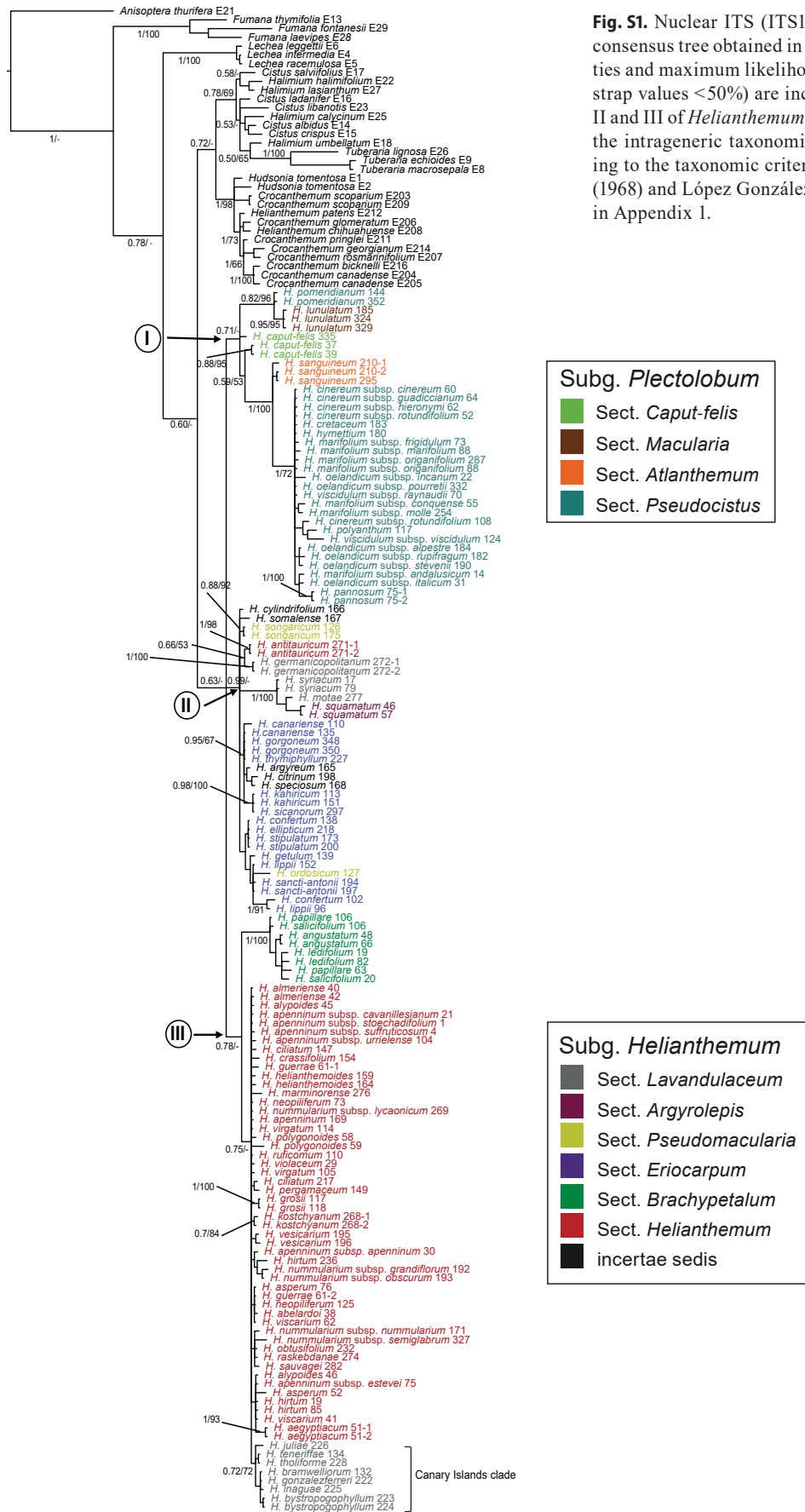


Fig. S1. Nuclear ITS (ITS1+5.8S+ITS2) nrDNA 50% majority-rule consensus tree obtained in the Bayesian analysis. Bayesian probabilities and maximum likelihood bootstrap values (dashes indicate bootstrap values <50%) are included above or below branches. Clades I, II and III of *Helianthemum* are indicated by arrows. Colour illustrates the intragenetic taxonomic assignment of the studied taxa according to the taxonomic criteria in Grosser (1903), Proctor & Heywood (1968) and López González (1993). Identities of accessions are listed in Appendix 1.

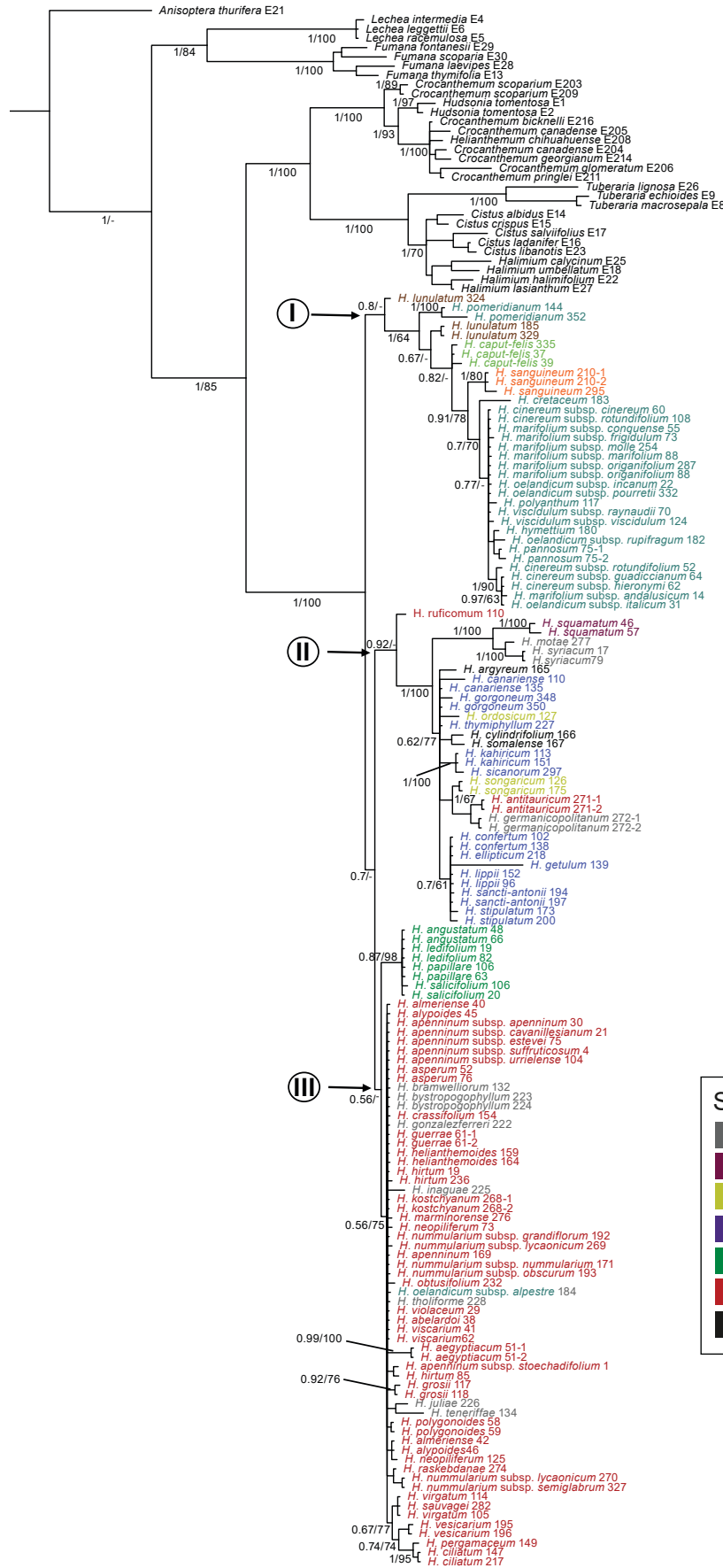


Fig. S2. Concatenated cpDNA (*ndhF*, *psbA-trnH*, *trnL-trnF*) 50% majority-rule consensus tree obtained in the Bayesian analysis. Bayesian posterior probabilities and maximum likelihood bootstrap values (dashes indicate bootstrap values <50%) are included above or below branches. Clades I, II and III of *Helianthemum* are indicated by arrows. Colour illustrates the intrageneric taxonomic assignment of the studied taxa according to the taxonomic criteria in Grosser (1903), Proctor & Heywood (1968) and López González (1993). Identities of accessions are listed in Appendix 1.

Subg. *Plectolobum*

- Sect. *Caput-felis*
- Sect. *Macularia*
- Sect. *Atlanthemum*
- Sect. *Pseudocistus*

Subg. *Helianthemum*

- Sect. *Lavandulaceum*
- Sect. *Argyrolepis*
- Sect. *Pseudomacularia*
- Sect. *Eriocarpum*
- Sect. *Brachypetalum*
- Sect. *Helianthemum*
- incertae sedis

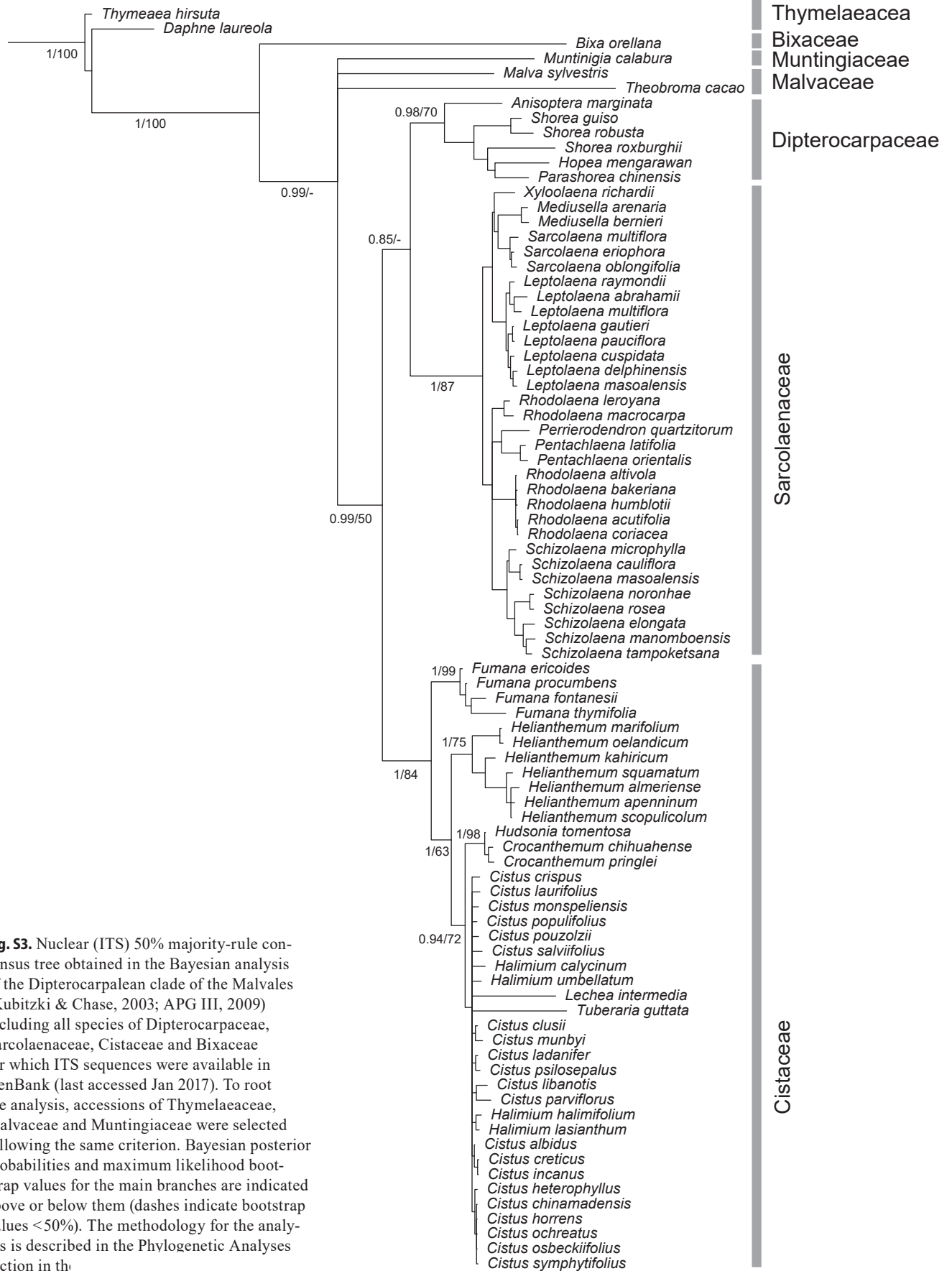


Fig. S3. Nuclear (ITS) 50% majority-rule consensus tree obtained in the Bayesian analysis of the Dipterocarpacean clade of the Malvales (Kubitzki & Chase, 2003; APG III, 2009) including all species of Dipterocarpaceae, Sarcolaenaceae, Cistaceae and Bixaceae for which ITS sequences were available in GenBank (last accessed Jan 2017). To root the analysis, accessions of Thymelaeaceae, Malvaceae and Muntingiaceae were selected following the same criterion. Bayesian posterior probabilities and maximum likelihood bootstrap values for the main branches are indicated above or below them (dashes indicate bootstrap values <50%). The methodology for the analyses is described in the Phylogenetic Analyses section in the



Fig. S4. Plastid *rbcl* 50% majority-rule consensus tree obtained in the Bayesian analysis of the Dipterocarpacean clade of the Malvales (Kubitzki & Chase, 2003; APG III, 2009) including all species of Dipterocarpaceae, Sarcolaenaceae, Cistaceae and Bixaceae for which *rbcl* sequences were available in GenBank (*psbA-trnH* sequences were also included whenever available; last accessed Jan 2017). To root the analysis, accessions of Thymelaeaceae, Malvaceae and Muntingiaceae were selected following the same criterion. Bayesian posterior and maximum likelihood bootstrap values for the main branches are indicated below them (dashes indicate bootstrap values <50%). The methodology for the analyses is described in the Phylogenetic Analyses section in the main text.