

## A phylogenetic hypothesis for *Helianthemum* (Cistaceae) in the Iberian Peninsula

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**Abstract:** Parejo-Farnés, C.; Albaladejo, R. G.; Arroyo, J. & Aparicio, A. 2013. A phylogenetic hypothesis for *Helianthemum* (Cistaceae) in the Iberian Peninsula. *Bot. Complut.* 37: 83-92.

We have sequenced the nuclear DNA internal transcribed spacer (ITS1+5.8S+ITS2) from samples collected in the field belonging to all taxa of *Helianthemum* in the Iberian Peninsula and analysed the data matrix by both Bayesian and Maximum Likelihood approaches. The phylogenetic hypothesis that we present here provides strong support for the monophyly of the genus and for the above-species systematics (sections and subgenera) considered in *Flora iberica*. Nevertheless, most species and subspecies in sections *Helianthemum* and *Pseudocistus* clustered in ample polytomies with poor resolution and branch support. This topology is interpreted to be consequence of frequent events of hybridization and introgression and/or rapid adaptive radiation in the genus *Helianthemum*, the most diversified lineage within the family Cistaceae.

**Key words:** adaptive radiation, Betic-Rifean hot spot, hybridization, Mediterranean flora.

**Resumen:** Parejo-Farnés, C.; Albaladejo, R. G.; Arroyo, J. & Aparicio, A. 2013. Una hipótesis filogenética para el género *Helianthemum* (Cistaceae) en la Península Ibérica. *Bot. Complut.* 37: 83-92.

En este trabajo presentamos una hipótesis filogenética para el género *Helianthemum* en la Península Ibérica. Para ello hemos recolectado en el campo material de todos los taxones y analizado las secuencias del marcador nuclear ITS (internal transcribed spacer) completo (ITS1+5.8S+ITS2) tanto con métodos Bayesianos como de Máxima Verosimilitud. Nuestra hipótesis muestra gran apoyo para la monofilia de género y para los taxones supra-específicos considerados en *Flora iberica* (secciones y subgéneros). En cambio, las especies y subspecies de las secciones más extensas (*Helianthemum* y *Pseudocistus*) se han agrupado en amplias politomías con muy bajo apoyo. Interpretamos que este marcador nuclear tiene baja resolución debido a eventos frecuentes de hibridación e introgresión así como de radiación adaptativa en el género *Helianthemum*, que es el linaje más diversificado de toda la familia Cistaceae.

**Palabras clave:** flora mediterránea, hibridación, hot spot Bético-Rifeño, radiación adaptativa.

### INTRODUCTION

*Helianthemum* Miller is the most diverse genus in the family Cistaceae with about 100 heliophytic species and subspecies of shrubs, subshrubs and annual herbs living from sea level up to circa 3000 meters in a diversity of substrates (limestone, dolomite, marl, gypsum, saline and sand-soils). The genus is distributed from Macaronesia to Central Asia across the Mediterranean floristic region and Europe (Arrington & Kubitzky 2003) with its highest diversity of taxa concentrated in western Mediterranean area (cf. Proctor & Heywood 1968, Greuter *et al.* 1984), particularly in the Betic-Rifean Hot Spot (mountains in south-eastern Spain and eastern Rif in Morocco; see Figs. 1 and 2). The species of *Helianthemum* are often dominant components of disparate woody plant communities.

The high taxonomic and nomenclatural complexity in the genus *Helianthemum* was acknowledged since Linnaeus times (see López-González 1992) and it is outstanding in classical (Dunal 1824, Spach 1836, Willkomm & Lange 1880, Grosser 1903) and recent (Guinea 1954, Proctor & Heywood 1968, Greuter *et al.* 1984, López-González 1992) literature. Most diagnostic features in *Helianthemum* are instable due to micro-evolutionary (i.e., population level) processes and many taxa of hybrid origin are still being described locally (*e. g.* Crespo & Cristóbal 2012). Furthermore, it is also noticeable the diversity of traits inherent in this genus, the species differing in longevity (therophytes to chamaephytes), flower colour (white, pink, yellow, orange) and size, flower biology (cleistogamy and chasmogamy) and breeding and mating systems (autogamy, xenogamy, self-

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compatibility, self-incompatibility) (Arrington & Kubitzky 2003, Rodríguez-Pérez 2005).

The Iberian Peninsula harbours approximately half of the total number of species of *Helianthemum*. In this area, 41 taxa (species and subspecies) have been recognized in the recent-most taxonomical review in *Flora iberica* (López-González 1993) but a few new species have been described since then (*H. scopulicolum*, Sáez et al. 1999; *H. guerrae*, Sánchez et al. 2001; *H. motae*, Sánchez et al. 2011). The taxonomical criteria in *Flora iberica* (López-González 1992) are synthetic and remarkably original in many aspects with regard to other reference floras at different geographical scales (e. g. Guinea 1954, Proctor & Heywood 1968). Based in the morphological diagnostic features usually considered for taxa delimitation in the genus (embryo type, stamen and style height and shape, life-form, inflorescence type, presence and size of stipules, calyx and leaf shape and indumentum, flower colour) and chromosome number, López-González (1992, 1993) considered the genus *Helianthemum* to be integrated by two taxonomic entities at subgenus rank (without ruling out the existence of two separated genera: *Helianthemum* and *Rhodax*, Spach 1836, Markova 1975) and seven Sections: *Argyrolepis* (n = 5), *Lavandulaceum* (n = 10), *Helianthemum* (n = 10), *Brachypetalum* (n = 10) and *Caput-felis* (n = 12) in subgen. *Helianthemum*, and *Pseudocistus* (n = 11) and *Atlantemum* (n = 11) in subgen. *Plectolobum*. From an evolutionary point of view, López-González (1992) considered *H. squamatum* (n = 5) to be a relatively ancient species in the genus without actual relatives and *H. caput-felis* (n = 12) as the only extant representative of other ancient lineage somewhat intermediate between both subgenera. On the other hand, and in contrast to earlier views (e.g. Proctor & Heywood 1968), this author considered the therophytic habit in the genus *Helianthemum* (just six species) to be a convergent trait in three different sections belonging to both subgenera.

In this paper we elaborate the first phylogenetic hypothesis for the genus *Helianthemum* in the Iberian Peninsula based in a consequential field sampling and on ITS sequence variation of nuclear ribosomal DNA of all high taxonomic entities (i.e. two subgenera and seven sections) and most species and subspecies of the genus in the Iberian flora. We aimed to seek concordance among molecular and morphological and karyological classifications (particularly that in López-González 1993) and to detect major lineages of evolution in the most diverse (both taxonomically and biologically) group within the Mediterranean *Cistaceae*. However, we admit that our

hypothesis in this work must be interpreted with caution mainly for two reasons: (1) because a comprehensive phylogenetic hypothesis should rely on an ampler array on nuclear and plastidial DNA markers. And (2) because leaves out the whole sect. *Eriocarpum*, a wealth of species that inhabiting semi-arid regions of N Africa and Middle East is absent from the Iberian Peninsula. The inclusion of sect. *Eriocarpum* sounds critical to comprehensively visualize the evolutionary lines and the above-species systematics of *Helianthemum*, as well as for an in-depth analysis of its phenotypic evolution and diversification.

## MATERIALS AND METHODS

**Study taxa and sampling.** Nomenclature in this study follows the criteria in *Flora iberica* (López-González 1992, 1993). To do the study, during springtime in 2010 and 2011 we collected 2-6 specimens in the field (Table 1) belonging to all species and subspecies of *Helianthemum* in *Flora iberica* (plus additional samples of the newly described species *H. guerrae*) totalling 42 taxa. Vouchers of all samples are available in the herbarium MA. Given that some taxa are endangered and legally protected by law, when necessary we obtained permission to collect samples, a process that always was absolutely non-destructive.

**DNA extraction, PCR amplification and ITS sequencing.** We extracted total genomic DNA from desiccated leaves with the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany) following supplier's instructions. In this study we focused on the nuclear internal transcribed spacer region (ITS1+5.8S+ITS2), which is the most widely used nuclear marker for phylogenetic reconstruction at family level and below (Nieto-Feliner & Roselló 2008). We used the P1A and P4 forward and reverse primers for the amplification of the ITS (Fuertes-Aguilar et al. 1999). We performed PCR in 25 µL reaction volume, containing 16.5 µL of sterile water, 5 µL of polymerase reaction buffer, 1 µL (10 µM) of each primer, 5 U/µL of the MyTaqRed DNA polymerase (Bioline, London, UK), 2 µL of DMSO and 1 µL of template total DNA. PCR conditions were: 35 cycles of denaturation at 95 °C for 15 s, annealing at 51-55 °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 Inc., Ipswich, US) following manufacturer indications. Purified products were directly sequenced in an ABI 3730 (Applied Biosystems, Foster City, CA, US) with the ABI Big Dye terminator kit (Applied Biosystems).

**Cloning.** The ITS is a multicopy DNA region. As a consequence, after direct sequencing some (20) samples displayed ambiguous sequences due to the existence of polymorphic sites. To deal with this issue, we cloned those accessions (9) with at least two polymorphic sites. We obtained amplicons for cloning through amplification with the high

Table 1  
 Localization of the 58 specimens included in the phylogenetic hypothesis in this study. Notice that all taxa (except four subspecies) in *Flora iberica* (López-González 1993) are included plus *H. guerrae*. All localities are from Spain and collection was done during springtime in 2010 and 2011.

Subgen. <i>Helianthemum</i>	Sect. <i>Argyrolepis</i>	<i>H. squamatum</i>	Almería	Río de Aguas	A Aparicio, RG Albaladejo, F García & MA Carrasco
		<i>H. squamatum</i> 1	Albacete	Tobarra, hacia Cordovilla	A Aparicio & RG Albaladejo
	Sect. <i>Lavandulaceum</i>	<i>H. syriacum</i>	Granada	Padul, Sierra del Manar	A Aparicio & RG Albaladejo
		<i>H. syriacum</i> 1	Cádiz	Olvera, Sierra de Lijar	A Aparicio
	Sect. <i>Helianthemum</i>	<i>H. apenninum stoechadifolium</i>	Guadalajara	Somolinos, Sierra de Pela	J Arroyo & R Pérez-Barrales
		<i>H. apenninum estevei</i>	Granada	La Zubia, Trevenque	A Aparicio & RG Albaladejo
		<i>H. apenninum apenninum</i>	Albacete	Tobarra, hacia Cordovilla	A Aparicio & RG Albaladejo
		<i>H. apenninum suffruticosum</i>	Granada	La Zubia, Trevenque	A Aparicio & RG Albaladejo
		<i>H. apenninum covanillesianum</i>	Jaén	Cazorla, arenales del Guadalentín	A Aparicio & RG Albaladejo
		<i>H. asperum</i>	Jaén	Cazorla, Raso del Madrigal	RG Albaladejo
		<i>H. asperum</i> 1	Granada	Cullar, Sierra de Baza, Cortijo del Bordón	A Aparicio & RG Albaladejo
		<i>H. asperum</i> 2	Almería	María, Sierra de María	A Aparicio & RG Albaladejo
		<i>H. neopitiferum</i>	Jaén	Huelma, Sierra Mágina	A Aparicio & RG Albaladejo
		<i>H. neopitiferum</i> 1	Granada	La Zubia, puente de los 7 ojos	A Aparicio & RG Albaladejo
		<i>H. atypoides</i>	Almería	Río de Aguas	A Aparicio, RG Albaladejo, F García & MA Carrasco
		<i>H. atypoides</i> 1	Almería	Río de Aguas	A Aparicio, RG Albaladejo, F García & MA Carrasco
		<i>H. guerrae</i>	Murcia	Yecla, Sierra del Serral, La Boquera	A Aparicio & RG Albaladejo
		<i>H. guerrae</i> 1	Murcia	Yecla, Sierra del Serral, La Boquera	A Aparicio & RG Albaladejo
		<i>H. viscarium</i>	Murcia	Aguilas, torre del Cabo Cope	A Aparicio, RG Albaladejo, F García & MA Carrasco
		<i>H. viscarium</i> 1	Murcia	Alhama de Murcia, Sierra España	A Aparicio & RG Albaladejo
		<i>H. polygonoides</i>	Albacete	Cordovilla	A Aparicio & RG Albaladejo
		<i>H. polygonoides</i> 1	Albacete	Saladar de Cordovilla	A Aparicio & RG Albaladejo
		<i>H. hirtum</i>	Sevilla	Alcalá de Guadaira, Hacienda Los Angeles	A Aparicio
		<i>H. hirtum</i> 1	Málaga	Antequera, hacia Valle de Abdalajís	A Aparicio & RG Albaladejo
		<i>H. almeriense</i>	Almería	Río de Aguas	A Aparicio, RG Albaladejo, F García & MA Carrasco
		<i>H. almeriense</i> 1	Málaga	Pizarra, Sierra del Hacho	A Aparicio & RG Albaladejo
		<i>H. aegyptiacum</i>	Sevilla	Aznatcollar, Las Barreras	A. Aparicio
		<i>H. aegyptiacum</i> 1	Sevilla	Aznatcollar, Las Barreras	A. Aparicio

	<i>H. violaceum</i>	Murcia	Águilas, torre del Cabo Cope	A Aparicio, RG Albaladejo, F García & MA Carrasco
	<i>H. violaceum</i> 1	Granada	Venta del Fraile, Cortijo de los Lagartos	A Aparicio & RG Albaladejo
	<i>H. nummularium</i>	Lérida	El Pont de Suert, hacia Vin de Llevata	J Arroyo & R Pérez-Barrales
Sect. <i>Brachypetalum</i>	<i>H. ledifolium</i>	Sevilla	Alcalá de Guadaíra, Hacienda Los Angeles	A Aparicio
	<i>H. ledifolium</i> 1	Granada	Alhama de Granada, El Navazo	A Aparicio & RG Albaladejo
	<i>H. salicifolium</i>	Sevilla	Dos Hermanas, El Baldío	A Aparicio
	<i>H. salicifolium</i> 1	Granada	La Zúbia, Cumbres Verdes	A Aparicio & RG Albaladejo
	<i>H. angustatum</i>	Cádiz	Villaluenga del Rosario	A Aparicio & J Arroyo
	<i>H. angustatum</i> 1	Granada	Cullar, Sierra de Baza, Cortijo del Bordo	A Aparicio & RG Albaladejo
	<i>H. papillare</i>	Almería	María, Sierra de María, La Peguera	A Aparicio & RG Albaladejo
	<i>H. papillare</i> 1	Almería	María, Sierra de María, La Peguera	A Aparicio & RG Albaladejo
Sect. <i>Caput-felis</i>	<i>H. caput-felis</i>	Alicante	Torre de la Horadada	A Aparicio, RG Albaladejo, F García & MA Carrasco
	<i>H. caput-felis</i> 1	Alicante	Dehesa de Campoamor	A Aparicio, RG Albaladejo, F García & MA Carrasco
Subgen. <i>Plectobolus</i>	<i>H. cinereum rotundifolium</i>	Cádiz	Benamahoma	A Aparicio
Sect. <i>Pseudocistus</i>	<i>H. cinereum cinereum</i>	Alicante	Dehesa de Campoamor	A Aparicio, RG Albaladejo, F García & MA Carrasco
	<i>H. cinereum hieronymi</i>	Murcia	Alhama de Murcia, Sierra Española	A Aparicio & RG Albaladejo
	<i>H. cinereum guadicianum</i>	Almería	María, Sierra de María, La Peguera	A Aparicio & RG Albaladejo
	<i>H. marifolium andalusicum</i>	Cádiz	Grazalema, Las Canteras	A Aparicio
	<i>H. marifolium conguense</i>	Cuenca	Huete	A Aparicio & RG Albaladejo
	<i>H. marifolium frigidulum</i>	Jaén	Huelma, Sierra Mágina	A Aparicio & RG Albaladejo
	<i>H. marifolium origanifolium</i>	Málaga	Alhaurín de la Torre, Jarapalo	A Aparicio & RG Albaladejo
	<i>H. marifolium marifolium</i>	Málaga	Antequera, hacia Valle de Abdalajis	A Aparicio & RG Albaladejo
	<i>H. oelandicum incanum</i>	Guadalajara	Campisábalos, Sierra de Pela	J Arroyo & R Pérez-Barrales
	<i>H. oelandicum italicum</i>	Lérida	Martinet, hacia Lies	J Arroyo & R Pérez-Barrales
	<i>H. viscidulum viscidulum</i>	Granada	Venta del Fraile, Cortijo de los Lagartos	A Aparicio & RG Albaladejo
	<i>H. viscidulum raynaldii</i>	Granada	Hueter, Puerto de la Mora	A Aparicio & RG Albaladejo
	<i>H. pamosum</i>	Granada	La Zúbia, Trevenque	A Aparicio & RG Albaladejo
	<i>H. pamosum</i> 1	Granada	La Zúbia, Trevenque	A Aparicio & RG Albaladejo
Sect. <i>Atlantthemum</i>	<i>H. sanguineum</i>	Salamanca	Calvarrasa de Arriba, Los Terraplenes	E Rico
	<i>H. sanguineum</i> 1	Salamanca	Calvarrasa de Arriba, Los Terraplenes	E Rico

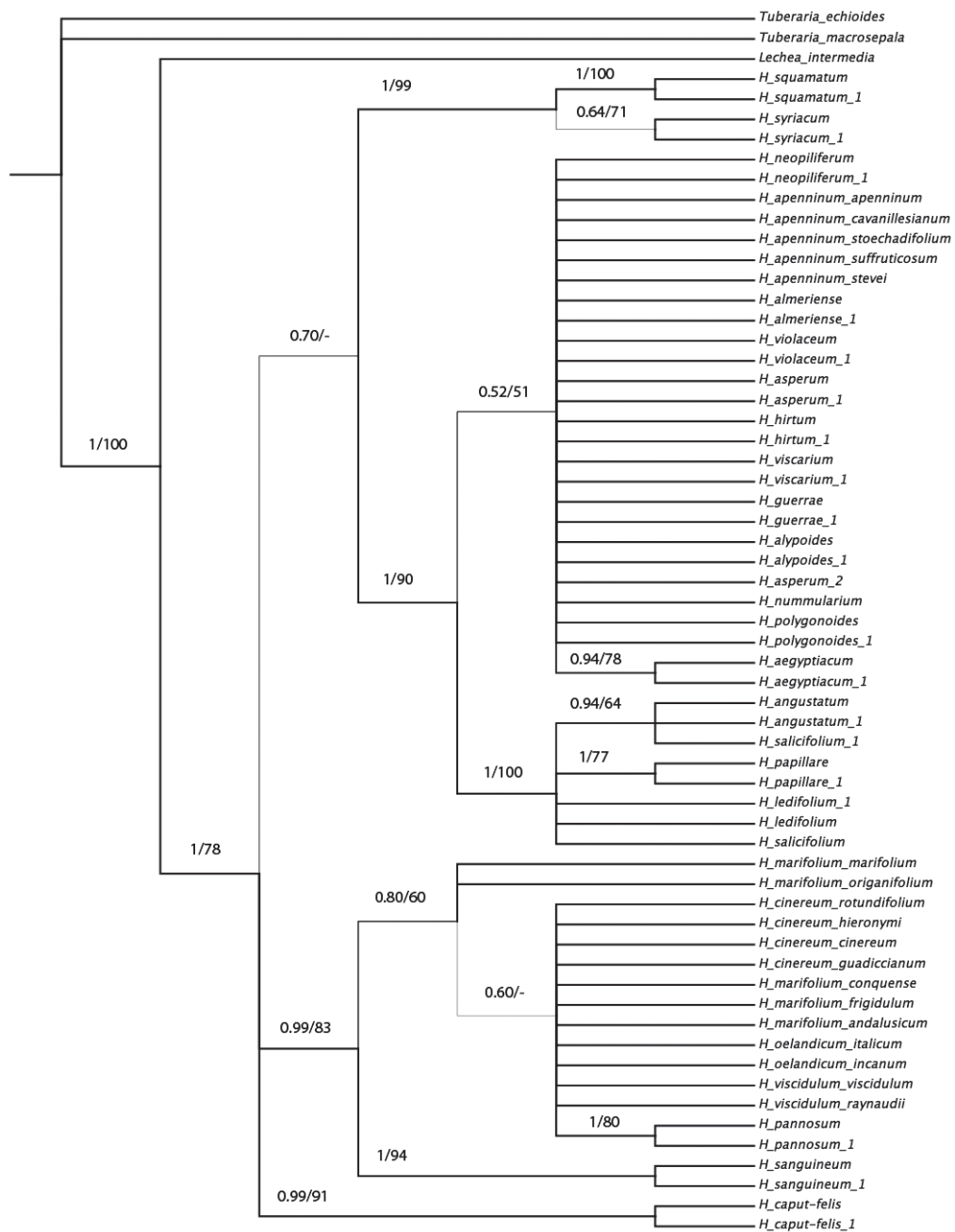


Fig. 3– Phylogenetic hypothesis of *Helianthemum* in the Iberian Peninsula based on ITS sequences and on Bayesian Inference. Numbers indicate posterior probabilities / bootstrap support under Bayesian and Maximum Likelihood analysis (bootstrap support below 50% not shown). Nomenclature follows López-González (1992, 1993).

fidelity Velocity DNA Polymerase (Bioline) and the following cycling conditions: denaturation at 98 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 25 s, followed by a

final extension step at 72 °C for 4 min. Then we cloned the amplicons into the pSpark DNA cloning vector (Canvax Biotech SL, Córdoba, Spain) after T4 ligase (Promega, Madison,

Wis., US) incubation at 22 °C for 1 h. For the transformation process, the ligation reaction was mixed with thawed Z-competent *E. coli* DH5 $\alpha$  strain (Canvax Biotech SL), incubated on ice for 5 min, and seeded onto LB/ampicillin/IPTG/X-Gal plates, prewarmed at 37 °C for 1 h. Finally, we incubated the plates at 37 °C overnight, selected eight different colonies for each cloned sample and then performed the screening by direct PCR of such colonies following amplification, purification and sequencing reactions as above.

**Phylogenetic analyses.** Besides *Helianthemum* taxa, we included three additional samples belonging to *Lechea* and *Tuberaria* as outgroups based on the phylogenetic relationship within the Cistaceae (Guzmán & Vargas 2009). For the ease of representation, in the final ITS matrix we included only one sequence for those samples that were cloned. We constructed a phylogenetic tree with all the cloned sequences with MrBayes 3.1 (Huelsenbeck & Ronquist 2001) with 1000 generations. When several clones from the same accession clustered together in a clade, one representative clone was selected while if some of the clones were variable, we selected the clone with the shortest branch within the clade. We aligned all the ITS sequences with MAFFT (Katoh *et al.* 2005) through the G-INS-i algorithm and then checked them visually for minor corrections. Due to difficult alignment of the ITS2 region we subjected the aligned matrix to Gblocks (Castresana 2000) under the default relaxed stringency level to remove those extremely variable parts from the alignment, which are more prone to homoplasy. We selected the nucleotide substitution model for the ITS region following the AIC values with Modeltest 3.06 (Posada & Crandall 1998). We conducted phylogenetic analyses under both Bayesian inference (BI) and Maximum Likelihood (ML) approaches. For the Bayesian inference we used MrBayes 3.1 (Huelsenbeck & Ronquist 2001) incorporating the potential phylogenetic signal of indels by coding the gaps with the simple method by Simmons & Ochoterena (2000) in SeqState 1.25 (Müller 2005). For that purpose we ran four parallel Markov chains (three hot and one cold) sampling every hundredth tree for a total of  $5 \times 10^6$  generations. For the Maximum Likelihood approach we used PhyML 3.0 (Guindon & Gascuel 2003) with GTR+GAMMA for nucleotide substitution model, tree topology search through SPR moves, and 1000 non-parametric bootstrap replicates for assessing branch support.

## RESULTS

The phylogenetic hypothesis that we present here (Fig. 3) was constructed with 58 ITS sequences from 38 species and subspecies of *Helianthemum* in *Flora iberica* (all except: *H. apenninum* subsp. *cantabricum*, *H. apenninum* subsp. *urriellense*, *H. oelandicum* subsp. *alpestre* and *H. origanifolium* subsp. *molle*) plus *H. guerrae*, representing 90% of the Iberian taxa.

The combined data matrix excluding the outgroups was 777 bp long with a mean G+C content of 65.1%. In

the original data matrix, 144 (18.5%) sites were variable and 100 (12.9%) were parsimony-informative. After passing this matrix through Gblocks the dataset was reduced to 461 bp (59.1% of the original matrix), of which 85 (18.4%) sites were variable and 59 (12.8%) parsimony-informative. The mean number of successfully sequenced clones was 4 per sample (ranging from 2 to 7). The selection of clones per sample was straightforward since cloning resolved all the PS detected in direct sequencing and clones from the same sample tended to cluster together (results not shown).

The phylogenetic relationships recovered through both BI and ML methods were concordant showing the same topology, although with slightly lower branch support in the ML tree. The monophyly of the genus *Helianthemum* was strongly supported with a posterior probability of 1 under BI and 78% bootstrap support in the ML approach (i. e. 1/78%) (Fig. 3). Nevertheless, the tree showed a subsequent polytomy with three large clades at the base of the genus. The first clade was well-supported (0.99/91%) and included only the two accessions of *H. caput-felis* (sect. *Caput-felis*). The second well-supported clade (0.99/83%) clustered all accessions belonging to subgen. *Plectolobum*. Within this clade, the sections *Atlantemum* (*H. sanguineum*) and *Pseudocistus* (*H. marifolium*, *H. cinereum*, *H. oelandicum*, *H. viscidulum* and *H. pannosum*) were supported as monophyletic lineages, the former with a high branch support (1/94%), the latter receiving rather less support in the ML tree (0.80/60%). Sect. *Pseudocistus* was characterized by a large polytomy where the relationships among taxa could not be recovered and only the two accessions of the stenochorous *H. pannosum* clustered together as a strongly differentiated lineage (1/80%).

The third clade comprised the whole subgen. *Helianthemum* whose monophyly was not well supported (0.70/<50%) (stressing in our opinion the necessity of including the sect. *Eriocarpum* in any further phylogenetic hypothesis). Within this subgenus, *H. squamatum* and *H. syriacum* (sections *Argyrolepis* and *Lamvandelaceum*) formed a sister monophyletic lineage to the rest of taxa with a strong statistical support (1/99%). Therophytic species in sect. *Brachypetalum* (*H. ledifolium*, *H. salicifolium*, *H. angustatum* and *H. papillare*) also grouped in a highly supported clade (1/100%). Nevertheless, in our hypothesis the sect. *Helianthemum* was a weakly supported clade (0.52/51%) showing other large polytomy where only the two accessions of the therophytic *H. aegyptiacum* clustered together (0.94/78%).

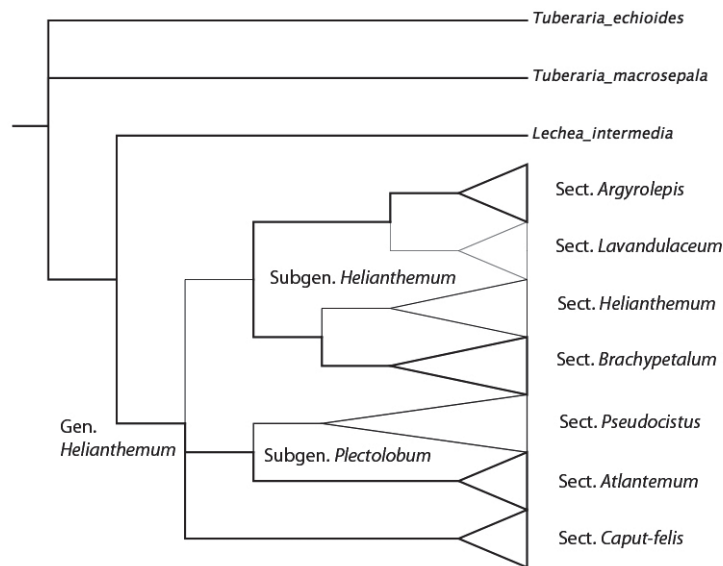


Fig. 4– Phylogenetic hypothesis of *Helianthemum* in the Iberian Peninsula based on ITS sequences and on Bayesian Inference showing clades collapsed above species. Branches support is the same than in Fig. 3. Taxonomy follows López-González (1992, 1993).

## DISCUSSION

Polytomies and low support values in phylogenetic hypothesis are the outcome of low number of molecular polymorphisms at the analysed taxonomic levels. Not unexpectedly, the ITS sequences used here did not provide enough power of resolution at species and subspecies level, the analysis yielding two ample polytomic branches within each subgenus (Fig. 3). This low differentiation of DNA sequences in the studied species and subspecies could be attributable to two non-mutually exclusive micro-evolutionary processes (apart from the idiosyncratic evolution of particular markers). On the one hand, the lability of reproductive barriers that allows frequent events of hybridization and introgression (particularly in subgen. *Helianthemum*) and, on the other, mechanisms of rapid adaptive radiation. Adaptive radiation causes differentiation in phenotypic traits as an adaptation to disparate environments from a recent common ancestor over short periods of time. In *Cistaceae*, Guzmán *et al.* (2009) have documented that the evolution and diversification of the genus *Cistus* was mediated by processes of rapid adaptive radiation from the onset of the Mediterranean climate during the Pleistocene.

Conversely, our molecular phylogenetic hypothesis yielded some interesting results providing support for most of above-species taxa (subgenera and sections, Fig. 4) in López-González (1992, 1993). The monophyly of

*Helianthemum* was strongly supported irrespective of the phylogenetic methods of analysis (both IB and ML methods), but depicted a polytomy with two well-supported clades (subgen. *Plectolobum* and sect. *Caput-felis*) and, interestingly, one low-supported clade corresponding to subgen. *Helianthemum*. We believe that the inclusion of the sect. *Eriocarpum* (which belongs to this subgenus, Grosser 1903) in any further analysis will critically modify the topology of the resulting tree and will provide new insights to disentangle the above-species taxonomy and evolutionary relationships in *Helianthemum*, making our present work, admittedly, preliminary.

It is interesting to underline here that some authors (*e. g.* Markova 1975) have taken under consideration the genus *Rhodax* (Spach 1836) to include all taxa with double-folded embryos, stamens longer than the style and  $n = 11$  chromosomes (*i. e.* the subgen. *Plectolobum*). López-González (1992) does not rule out this possibility emphatically, but our data does not support this view. Nevertheless, this issue remains to deeper biosystematics studies and taxonomist's decision.

*Helianthemum caput-felis* was formerly included in the sect. *Polystachium* together with *H. syriacum* and *H. squamatum* (*e. g.* Proctor & Heywood 1968). Our phylogenetic tree provided support for the sect. *Caput-felis* on its own *sensu* López-González (1992) although did not allow inferring the phylogenetic relationship of this species with regard to any of both subgenera. Further



analysis are required to elucidate the hypothesis of López-González (1992) who considered this species as the unique representative of an ancient and isolated evolutionary line (with  $n = 12$  chromosomes) intermediate between subgen. *Helianthemum* and subgen. *Plectolobum*.

Sections *Argyrolepis* (*H. squamatum*) and *Lavandulaceum* (*H. syriacum*) (López-González 1992) were also supported by our data and were arranged in a well-supported sister clade to sect. *Helianthemum* within subgen. *Helianthemum*. López-González (1992) considered *H. squamatum* as an ancient line of evolution (with the lowest chromosome number in the genus *Helianthemum*,  $n = 5$ ) without close extant relatives. Our data in this paper did not support this view strictly, but, as underlined above, further research is required to assess the actual phylogenetic structure of the subgen. *Helianthemum* (and the genus *Helianthemum* as a whole) by the inclusion of sect. *Eriocarpum* in future phylogenetic hypothesis.

*Helianthemum polygonoides* was described in the subgen. *Plectolobum* (Peinado *et al.* 1987) because all leaves (upper and lower) are exestipulate. However, López-González (1992) based in chromosome number ( $n = 10$ ) and embryo type included this taxon in the subgen. *Helianthemum*. Our molecular data are clear in allocating this very restricted edaphic-endemic species in the broad polytomy that conformed the subgen. *Helianthemum*.

The therophytic habit in plants represents a specialised life-form that has recurrently evolved from perennial life-forms in many seed plant families, particularly in seasonal climates (Ehrendorfer 1970, Pignatti 1979). All six therophytic species of *Helianthemum* have classically conformed the Sect. *Brachypetalum* (e. g. Willkomm & Lange 1880, Grosser 1903, Proctor & Heywood 1968). However, our data, in agreement with López-González (1992), have shown that this relevant trait is convergent in three different lineages: sect. *Brachypetalum* (*H.*

*salicifolium*, *H. angustatum*, *H. ledifolium*, *H. papillare*), sect. *Helianthemum* (*H. aegyptiacum*) and sect. *Atlantemum* (*H. sanguineum*).

In this line, our phylogenetic hypothesis also provided support for the sect. *Atlantemum* within the subgen. *Plectolobum* (López-González 1992, 1993) neglecting the independent monotypic genus *Atlantemum* (*A. sanguineum*) described by Raynaud (1987).

Finally, despite López-González (1992) claimed his own work to be 'provisional', the ITS sequences that we have analysed here mostly converged with morphological and karyological data to provide strong support to his synthesis above species level (Fig. 4). Our data are exhaustive with regard to the Iberian flora because we included all above-species taxa and 90% of the species and subspecies; but this figure felt to about 50% with regard to the Mediterranean area (cf. Greuter *et al.* 1984). Therefore, we stress the necessity of addressing a thorough molecular systematic study in the genus including sections *Eriocarpum*, *Macularia* (one species restricted to Maritime Alps) and *Pseudomacularia* (two Asiatic species) based in an ampler array of nuclear and plastidial DNA sequences. Further microevolutionary and population genetic approaches could enable the study of patterns of species adaptation and diversification, and set the genus *Helianthemum* as a Mediterranean model system suited for testing relevant evolutionary hypotheses.

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