Systematic implications from a robust phylogenetic reconstruction of the genus *Helianthemum* (Cistaceae) based on genotyping-by-sequencing (GBS) data

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Abstract. Molecular systematics requires the establishment of a robust phylogenetic framework including extensive geographical and taxonomie sampling. In this work, we proposed systematic changes in the genus *Helianthemum* based on phylogenetic trees obtained by both maximum likelihood and Bayesian analyses of GBS data. The implications of these phylogenetic results for the systematics of *Helianthemum* entail the establishment of a new subgenus and novel re-ascriptions of sections and species along with some nomenclatural novelties. The following new combinations are proposed: *Helianthemum* subg. *Eriocarpum* (Dunal) Martín-Hernanz, Velayos, Albaladejo & Aparicio; *H. oelandicum* subsp. *conquense* (Borja & Rivas Goday ex G.López) Martín-Hernanz, Velayos, Albaladejo & Aparicio; *H. nummularium* subsp. *cantabricum* (M.Lainz) Martín-Hernanz, Velayos, Albaladejo & Aparicio; *H. nummularium* subsp. *tinetense* (M.Mayor & Fern.Benito) Martín-Hernanz, Velayos, Albaladejo & Aparicio.

Keywords. Cistaceae, molecular systematics, Next Generation Sequencing.


Palabras clave. Cistaceae, secuenciación masiva, sistemática molecular.

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INTRODUCTION

Molecular systematics requires the establishment of a robust phylogenetic framework, including extensive geographical and taxonomic representativeness, well-supported species relationships and high certainty of tree topology. Particularly, for recently diversified lineages, data from Sanger sequencing-based techniques usually provide very low support due to insufficient DNA variability (DeFilippis & Moore 2000). Alternatively, reduced-representation sequencing methods such as genotyping-by-sequencing (GBS; Elshire & al. 2011) have been revealed to be highly resolutive because they allow the discovery of thousands of loci even from non-model species (Fernández-Mazuecos & al. 2018).

Under this perspective, the Palearctic genus *Helianthemum* Mill. (Cistaceae Juss.), whose rapid diversification has been driven by the geo-climatic events that impacted the Mediterranean Basin since the Upper Miocene (see Thompson 2020), is a challenging case of study due to its complex taxonomy and broad geographical distribution.
(Janchen 1925; Quézel & Santa 1962; Proctor & Heywood 1968; Greuter & al. 1984; López-González 1992; Raynaud 1999). To date, two comprehensive molecular phylogenetic reconstructions of the genus Helianthemum have been attempted, one based on the analyses of nrDNA (ITS) plus cpDNA (ndhF, psbA-trnH, trnL-trnF) concatenated Sanger sequences (Aparicio & al. 2017), the other based on GBS data (Martín-Hernanz & al. 2019a). Previous phylogenetic analyses based on Sanger sequencing (Aparicio & al. 2017) provided strong evidence for the generic boundaries in the family Cistaceae as stated by Janchen (1925), i.e., with Crocanthemum Spach restricted to America and Helianthemum to the Old World (also ruling out the segregation of H. subg. Plectolobum Willk. into the separate genus Rhodax Spach). Aparicio & al. (2017) also showed the monophyly of Helianthemum, so defined, and that this genus was integrated by three clades (I, II and III) with no species occupying an early diverging, isolated or intermediate position in relation to the rest of the species. Overall, this study provided support for the above-species classification of the genus, but it was unable to resolve most phylogenetic relationships since most species and subspecies were retrieved in polytomies.

Conversely, the phylogenetic analyses of GBS data (Martín-Hernanz & al. 2019a) (i) provided highly resolved phylogenetic trees with very strong support even at the most external nodes, (ii) robustly confirmed the three clades previously identified by Aparicio & al. (2017), and (iii) showed striking similar topologies among them, each one consisting of one species-rich subclade that corresponded with one of the three largest sections in the genus (H. sects. Eriocarpum Dunal, Pseudocistus Dunal and Helianthemum) plus a few very poorly diversified ones: H. sects. Argyrolepis Spach, Lavandulaceum G.López and Pseudomacularia Grosser in clade I; Caput-felis G.López, Macularia Dunal and Atlantianthemum (Raynaud) G.López, Ortega Oliv. & Romero García in clade II; and Brachypetalum Dunal in clade III. On the one hand, this phylogenetic hypothesis (Martín-Hernanz & al. 2019a) allowed the reconstruction of the biogeographic history of Helianthemum showing that the three largest taxonomic sections represent three evolutionary radiations which synchronously diversified during the Pliocene and Pleistocene, and that clades I, II and III diverged under pre-Mediterranean environmental conditions in northern Africa during the Middle Miocene. Then, clade II expanded throughout arid and semiarid ecosystems in northern Africa, Middle East and central Asia (to a considerably lesser extent to Macaronesia) whereas clades I and III expanded to Mediterranean and temperate regions through much of the Mediterranean Basin, central and northern Europe and the Canary Islands (Martín-Hernanz & al. 2019a; 2021).

On the other hand, the resulting non-monophyletic taxa advised further systematic changes at different taxonomic ranks. For example, at subgenus level, H. subg. Helianthemum as usually considered by taxonomists (i.e., clade II plus clade III) (Spach 1836; Willkomm 1856; Grosser 1903; Janchen 1925; Quézel & Santa 1962; Proctor & Heywood 1968; López-González 1992) was retrieved as a non-monophyletic (paraphyletic) group since clade III (H. sects. Brachypetalum and Helianthemum) and clade I (H. subg. Plectolobum) shared a recent-most common ancestor compared to clade II (see Fig. 1A). At the species level, the most common and taxonomically complex species, such as H. apenninum (L.) Mill., H. cinereum (Cav.) Pers., H. marifolium (L.) Mill., H. nummularium (L.) Mill. or H. oelandicum (L.) Dum. Cours. were also retrieved as non-monophyletic groups. These young species probably impacted by the Pleistocene glacial cycles, whose ample variability is usually described by taxonomists as subspecies arrays or species aggregates (e.g., Janchen 1907; Proctor & Heywood 1968; Greuter & al. 1984). The biological and taxonomic complexity of these species is also reflected in the low statistical support retrieved for their intraspecific relationships as well as in the existence of topological incongruences for some nodes of the phylogenetic trees (see Fig. 4 in Martín-Hernanz & al. 2019a), further revealing that trait convergence, incomplete lineage sorting or hybridization and introgression could be playing an essential role in the differentiation of these lineages (Soubani & al. 2014a, 2014b; Volkova & al. 2016). Overall, these findings emphasize that, beyond the systematic implications, studies at microevolutionary scale are necessary to evaluate diversification dynamics in these complexes and to understand why most species of Helianthemum are endemic or have very restricted distribution ranges (Martín-Hernanz & al. 2019a, 2019b).

In this paper, we update the overall systematic scheme of the genus Helianthemum using a robust molecular phylogenetic framework. To that aim, we considered the intrageneric systematic overview and the set of taxa studied by Aparicio & al. (2017) and Martin-Hernanz & al. (2019a) which targeted a broad taxonomic and geographic representation of the genus including two subgenera, ten sections, 73 species and 25 subspecies (i.e., about 75% of the genus) from the entire geographic distribution range (see Fig. 1 in Aparicio & al. 2017 and Table S2 in Martín-Hernanz & al. 2019a). Considering the overall statistical robustness of the phylogenetic relationships retrieved, particularly those based on GBS data, the subsequent systematic arrangements entail the establishment of a new subgenus, novel re-ascriptions of sections and species, and several nomenclatural novelties. We are aware that numerous species and subspecies relationships are still unknown and that many taxonomic and nomenclatural questions remain unresolved. Addressing these would require a microevolutionary approach, with detailed
population level studies and an increased number of samples per population and taxa.

MATERIAL AND METHODS

The systematic changes proposed here are based on phylogenetic trees obtained by maximum likelihood (RaxML 7.2.8; Stamatakis 2006) and Bayesian (ExaBayes 1.4.1; Aberer & al. 2014) analyses of a GBS assembly, applying the MaxResol configuration and the minimum 15% taxon coverage (Martín-Hernanz & al. 2019a). Briefly, the MaxResol configuration was defined as the parameter set configuration that provided the highest number of supported nodes in the phylogenetic analyses (both Bayesian and Maximum likelihood) as opposed to the MinError configuration, which was designed to minimizing allele and SNP error rates and provided more accurate branch length estimates in the phylogenetic trees (Martín-Hernanz & al. 2019a). Hence, MaxResol configuration retrieves trees suited for molecular systematic inferences whereas MinError configuration yields trees adequate for downstream analyses concerning the evolutionary history of the genus (molecular dating, ancestral area reconstructions or diversification rates). Minimum taxon coverage refers to the minimum number of samples at a given locus required to be retained in the final dataset. Details about the implications of parameter configuration for the phylogenetic reconstructions are in Martín-Hernanz & al. (2019a).

RESULTS AND DISCUSSION

The 50% majority-rule consensus tree resulting from the Bayesian inference analysis of GBS data shows that most nodes received high statistical support with full posterior probability (PP = 1) and bootstrap (BS) values higher than 70% (Fig. 1; see also Fig. S1a in Martín-Hernanz & al. 2019a for bootstrap values). Thus, this tree provides compelling evidence that the genus Helianthemum is integrated by three main lineages (clades I, II and III). Furthermore, it gives very strong support for the non-mono- phyletic status of H. subg. Helianthemum as traditionally considered: i.e., clades II plus III (Spach 1836; Willkomm 1856; Grosser 1903; Janchen 1925; Quézel & Santa 1962; Proctor & Heywood 1968; López-González 1992). Therefore, we are proposing a new subgenus coinciding with clade II, making all three subgenera monophyletic. Thus, H. subg. Eriocarpum (clade II) includes sects. Argyrolepis, Lavandulaceum, Pseudomacularia and Eriocarpum; H. subg. Plectolobum (clade I) contains sects. Caput-felis, Atlanthemum, Macularia and Pseudocistus; and, finally, H. subg. Helianthemum (clade III) is formed by sects. Brachypetalum and Helianthemum. Additional nomenclatural combinations and the re-ascription of taxa to different taxonomic levels are further proposed based on strongly supported phylogenetic relationships.


*Helianthemum* subg. *Eriocarpum* is mostly integrated by fruticose deserticolous plants with the upper leaves alternate inhabiting arid and semiarid ecosystems from the Maghreb to the Middle East, the Horn of Africa and Central Asia. *Helianthemum* subg. *Helianthemum* and *H.* subg. *Pseudocistus* are typical fruticose or suffruticose plants (rarely therophytic) with all leaves opposite inhabiting Mediterranean and Eurosiberian ecosystems from the Mediterranean Basin to northern Europe.


Included taxon


Included taxa


*H. syriacum* (Jacq.) Dum.Cours., Bot. Cult. 3: 129 (1802) [≡ *Cistus syriacus* Jacq., Icon. Pl. Rar. 1: 10 (1784), basion.; *H. lavandulifolium* auct.].


Grosser (1903) described this section on page 62 of the Engler’s Pflanzenreich. Subsequently, on page 92 the author listed the species included without specifying any of them as type of the section. We choose *H. songaricum* as lectotype.

A recent Sanger-based phylogenetic reconstruction which included six out of the seven species of *Helianthemum* endemic from the Horn of Africa (Martin-Hernanz & al. 2021) retrieved the large *H.* sect. *Eriocarpum* as non-monophyletic due to the inclusion in the same clade of the species that conform *H.* sect. *Pseudomacularia*, albeit with low statistical support. This is a remarkable result that would require the re-ascription of *H.* sect. *Pseudomacularia*.
Fig 1. 50% majority-rule consensus tree resulting from Bayesian analyses of Helianthemum GBS data in ExaBayes using the extreme parameter configuration to maximize phylogenetic resolution (MaxResol) under 15% minimum taxon coverage (Martín-Hernanz & al. 2019a). a, Whole phylogenetic tree with the outgroup and the three clades of Helianthemum collapsed for simplicity; b, Subgenus Eriocarpum; c, Subgenus Helianthemum; d, Subgenus Plectolobum. Comb. nov.: newly combined taxa, superscript 1: new taxonomic rank (no new nomenclatural combination required); superscript 2: taxa previously ascribed to a different section or subgenus (see text for details). The original phylogenetic tree was pruned to one tip per taxon following the nomenclature in Anales del Jardín Botánico de Madrid (PP) = 1 and maximum likelihood bootstrap value (BS) > 70%.

Sections

- Brachypetalum
- Helianthemum
- Eriocarpum
- Lavandulaceum
- Lathyrus
- Caput-felis
- Macularia
- Pseudocistus
- Pseudomacularia
- Atlanthemum
- Plectolobum

Colours indicate the intrageneric assignment of taxa. Unless otherwise stated, the support values for all nodes are posterior Bayesian probability (PP) = 1 and maximum likelihood bootstrap value (BS) > 70%.

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Within *H.* sect. *Eriocarpum*, but at present we keep these two sections as separate entities until the confirmation of this result by means of GBS (i.e., highly resolutive) data, when available. Nevertheless, the GBS-based phylogenetic tree here considered (Martin-Hernanz & al. 2019a) confirmed the phylogenetic relationship among the Turkey endemics *H.* antitauricum P.H.Davis & Coode and *H.* germanicopolitanum Bornm. (which were previously ascribed to sects. *Helianthemum* and *Lavandulaceum*, respectively; Aparicio & al. 2017) and the Asiatic species *H.* oridosicum Y.Z.Zhao, Zong.Y.Zhu & R.Cao and *H.* songaricum Schrenk ex Fisch. & C.A.Mey., hence their ascription to *H.* sect. *Pseudomacularia*.

Included taxa


Taxon not included in the phylogenetic analysis

*H.* strickeri Grosser


Dunal (1824) published *H.* sect. *Eriocarpum* in De Candolle’s Prodromus. After the diagnosis of the new section, the author listed the species included, but did not specify a type of the section. For this reason we selected *H.* kahiricum as lectotype.

Included taxa


*H.* confertum Dunal in DC., Prodr. 1: 274 (1824).


Taxa not included in the phylogenetic analysis

*H.* geniorum Maire

*H.* hadedense Thulin

*H.* schwarinfarthii Grosser

*H.* sinuspermiscum Gholamian & F.Ghahrem.

The assignation of the endemic species from the Horn of Africa to this section is based on Aparicio & al. (2017) and Martin-Hernanz & al. (2021).


The GBS-based phylogenetic tree provides strong support for the inclusion of *H.* sect. *Caput-felis* G.López in *H.* subg. *Plectolobum*. López-González (1992) considered this section to be somewhat intermediate among both subgenera but ascribed it to *H.* subg. *Helianthemum* despite emphasizing the *Plectolobum*-like seed morphology of *H.* caput-felis.

Included taxon


Dunal (1824) described this section in De Candolle’s Prodromus. After the diagnosis of the section, that author did not specify the type of the section. We choose *H.* lunulatum as lectotype. *Helianthemum lunulatum* is
a species restricted to high-altitude ecosystems in the Maritime Alps and the only species usually ascribed to *H. sect. Macularia* (e.g., Gasser 1903; Janchen 1925; Proctor & Heywood 1968). However, the analyses retrieved an unexpected sister-relationship among this species and *H. pomeridianum* Dunal (formerly ascribed either to *H. sect. Eriocarpum* or to *H. sect. Chamaecistus* Willk. in different subgenera; Aparicio & al. 2017), a species restricted to northern Algeria and the Moroccan Atlas Mountains range. This relationship reveals a major intercontinental disjunction happened during the early diversification of *H. subg. Plectolobum* in the Upper Pliocene (Aparicio & Albaladejo 2017).

Included taxa

*H. lunulatum* (All.) DC. in Lam. & DC., Fl. Franç., ed. 3, 4: 816 (1805) [= *Cistus lunulatus* All., Auct. Fl. Pedem.: 30 (1789), basion.].


Taxon not included in the phylogenetic analysis

*H. petiolatum* Thibaud ex Pers.


Similarly, the phylogenetic reconstructions considered in this paper provide strong support for the inclusion of *H. sanguineum* (Lag.) Lag. in the monotypic *H. sect. Atlanthemum* as proposed by López-González (1992), ruling out the consideration of the separate genus *Atlanthemum* Raynaud (Raynaud 1987).

Included taxon

*H. sanguineum* (Lag.) Lag. ex Dunal in DC., Prodr. 1: 273 (1824).


Some complex species in this section as currently defined have been retrieved non-monophyletic. The GBS data provide support for the consideration of *H. frigidulum* Cuatrec. and *H. raynaudii* Ortega Oliv., Romero García & C. Morales at species level, as originally described, i.e., not subordinated either to *H. marifolium* or to *H. viscidulum* Boiss., respectively. Conversely, the consideration of *H. marifolium* and *H. origanifolium* (Lam.) Pers. as different species (e.g., Guinea 1954; Proctor & Heywood 1968; Greuter & al. 1984; Crespo & al. 2016) resulted firmly unsupported. *Helianthemum oelandicum* is an extremely polymorphic complex usually considered as a species’ aggregate (Janchen 1907; Yuzepchuk 1974; Tzvelev 2006; Soubani 2010); very interestingly, to this complex is to be ascribed a xerophile specialist plant endemic to central Spain which was subordinated to *H. marifolium* (syn. *H. marifolium* subsp. *conquense* Borja & Rivas Goday ex G.López) albeit its morphological relationship with *H. oelandicum* was originally suspected (López-González 1992). For a comprehensive insight of the polymorphic species *H. cinereum, H. marifolium* and *H. polyanthum* (Desf.) Pers., it is a requisite to take into account the ample variability that exists at both sides of the strait of Gibraltar.

Included taxa

*H. cinereum* (Cav.) Pers., Syn. Pl. 2: 76 (1806) subsp. *cinereum*


*H. marifolium* (L.) Mill., Gard. Dict. ed. 8, n.° 24 (1768) subsp. *marifolium*


*H. oelandicum* (L.) Dum.Cours., Bot. Cult. 3: 129 (1802) subsp. *oelandicum*


Taxon not included in the phylogenetic analysis

H. assadii F. Ghahrem. & Gholamian

2. Sect. Helianthemum

The complex species H. apenninum and H. nummularium, as currently delimited, have been retrieved as non-monophyletic making necessary some nomenclatural combinations and taxonomic re-assignments. Future studies are required to evaluate the variability of H. apenninum and to ascertain its relationships with H. croceum (Desf.) Pers. (see López-González 1992) and H. neopiliferum Muñoz Garm. & C. Navarro. On the other hand, a huge ecological and morphological variation is inherent to H. nummularium (Proctor & Heywood 1968; Volkova & al. 2016) and the results that we analyse in this paper increase the complexity of this taxon. For example, we have unexpectedly found that the sample identified in the phylogenetic tree as H. apenninum subsp. stoechadifolium 236, which represents a few populations restricted to maritime sandy soils around the Doñana National Park in the coast of southern Spain (see Table S2 in Martín-Hernanz & al. 2019a and Fig. 1), clustered within the clade of H. nummularium. Although these plants can be readily identified as H. apenninum subsp. stoechadifolium (Brot.) Samp., the facts that this taxon is not cited for this geographic area by López-González (1993) in Flora iberica and the retrieved phylogenetic relationship, stress that a detailed study is necessary to ascertain the real taxonomic identity of these populations. Similarly, the stenochorous H. apenninum subsp. urielense (M.Lainz) G.López and H. apenninum subsp. cantabricum (M.Lainz) G.López were retrieved within the clade of H. nummularium together with H. morisianum Bertol., an endemic taxon from Sar-

Also, in this section a cluster of species whose relationships are not fully supported is retrieved [H. guerrae Sánchez-Gómez, J.S.Carrión & M.A.Carrión, H. viscidulum Boiss. & Reut., H. violaceum (Cav.) Pers., H. asperum Lag. ex Dunal, H. marinoreense Alcaraz, Peinado & Mart.Parras and H. fontqueri Sennen plus H. hirtum (L.) Mill. and H. scopulicola L.Sáez, Rosselló & Alomar]. Indeed, this is an extremely complex group of species almost restricted to eastern and southern Spain where phenotypic plasticity and hybridization is very common (e.g., Mateo Sanz 2012; Pérez Da Costa & Mateo Sanz 2012). Again, the study of this complex of species would require a population level approach with an enlarged number of samples per population and species. This last consideration can be extended to


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Taxa not included in the phylogenetic analysis

H. baschkirorum (Juz. ex Kupat.) Juz.

H. bushii (Palib.) Juz. & Pozdeeva

H. ciscaucasicum Juz. & Pozdeeva

H. cretaceum Juz. ex Dobrocz.

H. croticolav Klokov & Dobrocz.

H. crotophilum Klokov & Dobrocz.

H. canum auct.

H. hynettium Boiss. & Heldr.

H. marmoreum Stevan., Matevski & Kit Tan

H. oelandicum subsp. rupifragum (A.Kern.) Breistr.

H. pinegense Juz.

H. zheguliense Juz. ex Tzvelev

Helianthemum subg. Helianthemum


Lectotype: H. ledifolium (L.) Mill.

Included taxa


the lineage integrated by species mostly from south-eastern Spain and northern Maghreb such as *H. almeriensense* Pau, *H. alpyoides* Losa & Rivas Goday, *H. virgatum* (Desf.) Pers., *H. pergammaceum* Pomel, *H. ciliatum* (Desf.) Pers. and *H. vesicarium* Boiss. where hybridization is also common and the species limit result many times unclear (Quézel & Santa, 1962; Raynaud 1999). Finally, the analysed GBS data firmly support the existence of the ‘Canary Island clade’, a monophyletic lineage endemic to the Canary Islands explosively diversified during the Pleistocene (Albaladejo & al. 2021) whose species were previously ascribed to *H*. sects. *Argyrolepis* or *Lavandulaceum* (Aparicio & al. 2017).

Included taxa


*H. aguloi* Marrero Rodr. & R.Mesa, Candollea 58: 151 (2003). [The inclusion is based on Albaladejo & al. (2021)].


*H. asperum* Lag. ex Dunal, DC., Prodr. 1: 283 (1824).


*H. braunsonnetii* Dunal in DC., Prodr. 1: 279 (1824).


*H. fontqueri* Sennen [= *H. abelardoi* Alcaraz, Flora Montiber. 60: 140 (2015)].


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H. violaceum Pomel (Cav.) Pers., Syn. Pl. 2: 78 (1806)


