

## Phylogenetic relationships in *Helichrysum* (Compositae, Gnaphalieae) and related genera: incongruence between nuclear and plastid phylogenies, biogeographic and morphological patterns, and implications for generic delimitation

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### Abstract

The *Helichrysum-Anaphalis-Pseudognaphalium* (HAP) clade is a major component of the tribe Gnaphalieae (Compositae) and includes the genera *Helichrysum*, *Anaphalis*, *Achyrocline* and *Pseudognaphalium*. Allopolyploid origins for at least two clades within the HAP clade have previously been suggested, one involving the genus *Anaphalis* and the Mediterranean-Asian *Helichrysum* species, and a second one involving part of *Pseudognaphalium*. In the present paper, with the use of two nuclear ribosomal and two plastid DNA markers and an extensive sampling of the HAP clade, further evidence relevant to the origin, composition and closest relatives of these clades is provided, and additional cases of incongruence are discussed. The superposition of distribution areas on the phylogeny suggests that the HAP clade originated in the Cape region of southern Africa and subsequently dispersed to and diversified in the Afrotropical regions of east southern Africa, mainly the Drakensberg, before spreading northward and giving rise to several lineages in afrotropical and afroalpine areas of central tropical Africa and in Madagascar. Allopolyploidy may have preceded the dispersal and diversification of the HAP lineage out of Africa to the Mediterranean area, and to the Americas and Asia. Finally, discussion on the distribution of several morphological characters in the phylogeny and their taxonomic relevance is also provided, with views on the need for a new generic delimitation.

**Key words** *Achyrocline*; allopolyploidy; *Anaphalis*; ETS; hybridisation; *Helichrysum*; *Humeocline*; ITS; *ndhF*; paraphyly; *Pseudognaphalium*; reticulate evolution; *rpl32-trnL* intergenic spacer

**Running head** Phylogenetic incongruence in *Helichrysum*

**Supplementary Material** Appendix 1 and Table S1 (all in the Electronic Supplement) are available under “Supplementary Data” of the online version of this article (<http://www.ingentaconnect.com/content/iapt/tax>).

## INTRODUCTION

The use of multiple DNA regions in phylogenetic reconstructions often provides better resolved phylogenies than the use of only one marker, nuclear or plastid (e.g. Barres & al., 2013). However, conflicting (incongruent) phylogenetic signals are sometimes recovered from different genes (or genomes, e.g. between nuclear and plastid loci; Pelser & al., 2010). Combining incongruent data partitions into a single data matrix ignores the potential that incongruence results from different loci having different evolutionary histories, while separate analysis of incongruent data partitions may provide insight into the evolution of a group (e.g. Kim & Donoghue, 2008). Incongruence between different loci is often interpreted as the result of independent sorting (or retention) of ancestral polymorphisms at different loci or as the result of hybridisation (Pelser & al., 2010 and references therein).

Hybridisation between related species is known to be common in the evolution of plants. In some cases the hybrids produced can back-cross to the parental species, and occasionally allow gene flow between them (Marhold & al., 2002; Smitsen & al., 2007; Conesa & al., 2010). In other cases the hybrids produced give rise to novel lineages as a new species of hybrid origin. Homoploid hybrid speciation has been documented to occur in plants (Gross & Rieseberg, 2005; Howarth & Baum, 2005; Fjellheim & al., 2009), although hybrid speciation has most commonly been studied in cases in which allopolyploidy is involved (Perný & al., 2005; Ma & al., 2010). The importance of allopolyploidy in adaptive radiations in plants has been extensively documented (Grant, 1981; Barrier & al., 1999; Soltis & Soltis, 2000; Joly & al., 2009). Polyploid plants have been suggested to have broader ecological tolerances and lower rates of inbreeding depression (Soltis & Soltis, 2000). Several studies have also shown that polyploids could be particularly advantageous in long-distance dispersal and establishment on oceanic islands (Barrier & al., 1999; Doyle & al., 2000, 2002).

The tribe Gnaphalieae (Compositae) are an example of a group in which adding additional markers to phylogenetic analyses has not lead to convergence on a well resolved and supported phylogeny (Bayer & al., 2000, 2002; Bergh & Linder, 2009; Ward & al., 2009; Galbany-Casals & al., 2010; Smitsen & al., 2011). Incongruence between plastid DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) based phylogenies contributes to uncertainty in the estimation of phylogenetic relationships among Gnaphalieae species, although other challenges, such as uneven rates of nucleotide substitution among lineages and rapid radiation of the extant clades, have also played a role. Low copy nuclear gene phylogenies suggest that multiple clades within Gnaphalieae have allopolyploid origins (Smitsen & al., 2011).

Despite their limitations, phylogenetic studies of Gnaphalieae consistently recovered a basal divergence between a relatively small southern African “*Relhania* clade” (sensu Bergh & Linder, 2009; Ward & al., 2009; Smitsen & al., 2011) or Relhaniinae s.str. (sensu Bayer & al., 2000) and most of the genera in the tribe. Within the main lineage of the tribe there is consistent evidence for a grade of southern African taxa at the base of the group and then a crown radiation including a mixture of African and non-African taxa (Bergh & Linder, 2009; Ward & al., 2009; Galbany-Casals & al., 2010; Smitsen & al., 2011). A large portion of the genus *Helichrysum* Mill. along with at least parts of the genera *Achyrocline* (Less.) DC., *Anaphalis* DC. and *Pseudognaphalium* Kirp. (HAP clade sensu Smitsen & al., 2011), has been consistently recovered as a clade within the crown radiation (Galbany-Casals & al., 2009a; Ward & al., 2009; Galbany-Casals & al., 2010; Smitsen & al., 2011). Its monophyly is

well supported and *Langebergia* Anderb. and *Petalacte* D. Don seem to be the most closely related taxa (Bayer & al., 2000; Bergh & Linder, 2009; Ward & al., 2009).

Within the HAP clade, multiple instances of hybridisation have been reported. In the genus *Helichrysum* an important evolutionary role for historical hybridisation has been suggested as an explanation for the low resolution found in previous nrDNA phylogenies accompanied by a geographical, rather than taxonomic, grouping of the specimens (Galbany-Casals & al., 2009a) and the presence of trans-specific plastid DNA polymorphisms (Galbany-Casals & al., 2011). Several levels of ploidy are known to occur in *Anaphalis*, *Helichrysum* and *Pseudognaphalium* (Ward & al., 2009) and allopolyploid origins for at least two clades within the HAP clade have been suggested on the basis of low copy nuclear DNA sequences, one constituted by *Anaphalis margaritacea* (L.) Benth. & Hook. f. and the Mediterranean-Asian *Helichrysum* species, and a second one constituted by part of *Pseudognaphalium* (Smitsen & al., 2011). Contemporary wild interspecific hybridisation has also been demonstrated with the use of morphological and molecular characters in Mediterranean *Helichrysum* (Galbany-Casals & al., 2012).

A recent nrDNA based phylogeny focused on *Anaphalis*, but also including some *Pseudognaphalium* representatives confirmed the position of all members sampled of these two genera within the HAP clade (Nie & al., 2013). In this work, the Eurasian clade of *Helichrysum* was shown as the sister clade to *Anaphalis*, and the South African *Helichrysum populifolium* DC. was sister to *Pseudognaphalium*, although the sampling of *Helichrysum* and *Pseudognaphalium* was sparse. *Pseudognaphalium* was recovered as monophyletic whereas monophyly of *Anaphalis* received low support. Sampling of *Achyrocline* in phylogenetic studies has been very limited, and its monophyly, phylogenetic relationships among its species, and its closest relatives within the HAP clade have not been investigated. Traditional generic treatments of *Helichrysum* and related genera have been quite artificial. As a result of morphological and molecular work, numerous extraneous elements have been transferred from *Helichrysum* to other genera such as *Castroviejoa* Galbany, L. Sáez & Benedí, *Dolichothrix* Hilliard & B. L. Burtt, *Ozothamnus* R. Br., *Plecostachys* Hilliard & B. L. Burtt, *Syncarpha* DC. and *Vellereophyton* Hilliard & B. L. Burtt, among others, or are still awaiting placement elsewhere (Hilliard & Burtt, 1981; Bayer & al., 2000; Galbany-Casals & al., 2004, 2009a; Bergh & Linder, 2009; Smitsen & al., 2011; see Ward & al., 2009 for a review). The current generic definition of *Helichrysum* is the one provided by Hilliard & Burtt (1981) based on the type species, the Mediterranean *Helichrysum orientale* (L.) Gaertn., and most of the African species. The genus is characterised by capitula generally with hermaphroditic florets outnumbering the pistillate ones; involucre bracts with a divided stereome, i.e., that the basal coriaceous part of the bracts have a transparent patch; receptacle generally smooth, honeycombed or fimbriiferous; and pappus usually uniseriate. Nevertheless, in some species the pistillate florets outnumber the hermaphroditic ones; some other species lack a pappus or have a biseriate one. Moreover, several species have a paleate receptacle, or an undivided stereome. All these anomalous species have been kept within the genus (Hilliard & Burtt, 1981; Hilliard, 1983; Anderberg, 1991), though not without reservation.

The other members of the HAP clade, *Achyrocline*, *Anaphalis* and *Pseudognaphalium* all have the stereome divided – except *Pseudognaphalium oligandrum* (DC.) Hilliard & B. L. Burtt – the receptacle epaleate and the pappus uniseriate. *Anaphalis* is mainly recognised because it is subdioecious, i.e. some capitula are predominantly pistillate, with a few central, functionally staminate florets, and some others are predominantly staminate, with a few external pistillate florets (Anderberg, 1991; Nesom, 2006a). This character probably explains why its affinities with *Helichrysum* were not suspected before molecular data were available, although some *Anaphalis* species do resemble some Asian *Helichrysum* in their gross

morphology. *Pseudognaphalium* is mainly distinguished from *Helichrysum* by having capitula in which the pistillate florets always outnumber the staminate ones, although this character is also found in some species of *Helichrysum* (Hilliard & Burtt, 1981). *Achyrocline* is also characterised by having capitula in which the pistillate florets outnumber the hermaphroditic ones, although in this genus capitula are reported to be much smaller than in *Pseudognaphalium*, having less than 20 florets (Anderberg, 1991). Hilliard & Burtt (1981) noted that some *Helichrysum* species also have these characters and that *Achyrocline* does not differ enough morphologically to be separated from *Helichrysum*. However, they refrained from including the former in the latter. Anderberg (1991) in his cladistic analysis based on morphology also found that the two genera were closely related.

The biogeography of the HAP clade is also a topic of major interest. *Helichrysum* occurs in a variety of habitats in many ecoregions of the world. It seems to have had an initial diversification in southern Africa followed by several independent northward migrations up the African continent, one giving rise to a Macaronesian-Mediterranean-Asian clade, and probably several independent colonisations establishing several lineages in afroalpine and afroalpine areas of tropical Africa, as well as an additional Macaronesian lineage (Galbany-Casals & al., 2009a). One of the major interests in phytogeography is investigating the origin, diversification and dispersal or migration of plant groups that show a great diversity in southern Africa (e.g., Linder, 2003; Galley & al., 2007; McKenzie & Barker, 2008; Devos & al., 2010). Often, part of the lineages highly diversified in the Cape region of southern Africa also occurs in the Afrotropical region, and two hypotheses have been postulated for this distribution pattern: (1) an origin in tropical Africa and migration through the Afrotropical region southwards into the Cape (Levyns, 1964; McGuire & Kron, 2005); and (2) an origin in the Cape and migration northwards into tropical Africa (Linder, 1994), often through the Drakensberg range (Galley & al., 2007). *Helichrysum* seems to fit the second pattern, although the limited sampling of the genus in previous phylogenies (Galbany-Casals & al., 2009a) made any conclusion tentative. Finally, although 115 *Helichrysum* species occur in Madagascar, none of them has ever been included in any molecular phylogeny, so their origin and the number of times of colonisation of this island by the lineage is unknown.

*Anaphalis* has 110 species and is widely distributed in Asia, with only two species in the New World: *A. margaritacea*, which grows in Asia and North America (Anderberg, 1991), and *Anaphalis chilensis* Reiche, which is endemic to the South American Andes (Nie & al., 2013). *Pseudognaphalium* contains about 90 species, which are distributed throughout Africa, America and Eurasia, although one species, *Pseudognaphalium luteoalbum* (L.) Hilliard & B. L. Burtt, is also considered indigenous in Australia and New Zealand (Anderberg, 1991). *Achyrocline* has about 32 species and has a disjunct distribution between Africa, Madagascar and South America (Hilliard & Burtt, 1981; Anderberg, 1991). The fact that *Achyrocline*, *Anaphalis* and *Pseudognaphalium* are now known to be nested within *Helichrysum* (Galbany-Casals & al., 2009a; Ward & al., 2009; Galbany-Casals & al., 2010; Smitsen & al., 2011) expands the current geographic distribution of the lineage throughout the world, and opens new phytogeographic questions, such as how many times it has dispersed from Africa to Asia and the Americas, and the role of allopolyploidy in the dispersal and diversification of new lineages out of Africa.

Despite the limitations of cpDNA and nrDNA markers in recovering phylogenetic relationships within Gnaphalieae, and the incongruities detected between them (Ward & al., 2009; Smitsen & al., 2011), they are still useful to detect some clades, especially those affected by allopolyploidization, and incongruities may provide insight into ancient hybridization events (Galbany-Casals & al., 2010; Smitsen & al., 2011). Also, nrDNA markers have shown a notable correlation with morphology and/or biogeography (Galbany-Casals & al., 2009a), and they could help in identifying morphological characters to be used

in future generic delimitation and infrageneric classification. For these reasons, we provide molecular phylogenies of the HAP clade based on nrDNA and cpDNA markers, and consider these results in the context of information on chromosome numbers, morphology and biogeography, with the following aims: (1) to expand the sampling of *Helichrysum* and provide a general phylogeny for this genus; (2) to test the monophyly of *Achyrocline*, *Anaphalis* and *Pseudognaphalium*, and examine their relationships to clades within *Helichrysum*; (3) to examine the congruence between nrDNA and cpDNA phylogenies in the light of hypothesised allopolyploidy within the HAP clade; and (4) to provide evidence on the biogeographic history of the HAP clade and the role of allopolyploidy in its dispersal out of Africa.

## MATERIALS AND METHODS

**Taxon sampling.** – Sequences used in this work include both new sequences and previously published sequences. Voucher details for all samples are shown in Appendix 1 along with European Nucleotide Archive accession numbers. We have included 153 *Helichrysum* specimens representing 145 different species (24-29% of the genus), which include: 17 representatives of the previously identified Macaronesian-Mediterranean-Asian clade (Galbany-Casals & al., 2009a); four specimens belonging to the other three endemic species from Macaronesia (*Helichrysum alucense* García-Cas., S. Scholz & E. Hernández, *Helichrysum monogynum* B. L. Burtt & Sunding and *Helichrysum nicolai* N. Kilian, Galbany & Oberpr.) which constituted an independent Macaronesian clade in previous studies (Galbany-Casals & al., 2009a); 23 specimens from eastern Tropical Africa and the Arabian peninsula, representing wide morphological, biogeographical and ecological variation; 91 specimens from Southern Africa, representing 28 of the 30 informal infrageneric groups recognised by Hilliard (1983); and 18 specimens from Madagascar, representing six of the 11 informal infrageneric groups recognised by Humbert (1962). We also included three *Achyrocline* species (9% of the genus), 10 *Anaphalis* specimens representing 7 different species (8% of the genus) and 12 *Pseudognaphalium* species (13% of the genus), plus the monotypic Malagasy genus *Humeocline* Anderb., which had never been included in any previous phylogeny. *Langebergia canescens* (DC.) Anderb. and *Petalacte coronata* (L.) D. Don have also been included, since these species appear as sister to the HAP clade (Bayer & al., 2000; Bergh & Linder, 2009; Ward & al., 2009) or closely related to the South African *Helichrysum dasyanthum* (Willd.) Sweet, which is placed outside the HAP clade (Bayer & al., 2000; Galbany-Casals & al., 2004; Ward & al., 2009). Finally, a selection of 17 species of other Gnaphalieae genera has been included based on previous phylogenetic results at the tribal level (Ward & al., 2009; Galbany-Casals & al., 2010; Smissen & al., 2011). These include *Helichrysum lanceolatum* (Buchanan) Kirk from New Zealand, which is already known to be relatively distantly related to the HAP clade (Galbany-Casals & al., 2004; Ward & al., 2009; Smissen & al., 2011), and is also misplaced in *Ozothamnus* (e.g., Anderberg, 1991; see Ward, 2009). In total, we have included in the analyses 199 ITS sequences, of which 108 are new; 199 ETS sequences, of which 156 are new; 178 *rpl32-trnL* sequences, of which 160 are new; and 178 *ndhF* sequences, of which 153 are new. The cpDNA sequences could not be obtained from several specimens (see Appendix for details).

**DNA extraction.** – Total genomic DNA was extracted following the CTAB method of Doyle & Dickson (1987) as modified by Cullings (1992) and Tel-Zur *et al.* (1999) from silica-gel-dried leaves collected in the field or from herbarium material. In some cases NucleoSpin® Plant (Macherey-Nagel GmbH & Co. KG, Düren, Germany) or DNeasy extraction kits (Qiagen Inc., Hilden, Germany) were used, following the manufacturers' instructions.

**nrDNA amplification strategies.** – The ITS DNA region (ITS1, 5.8S ribosomal gene and ITS2) was amplified using the 17SE forward and the 26SE reverse primers (Sun & al., 1994). The profile used for amplification was as described in Galbany-Casals & al. (2004). The ETS DNA region was amplified using the forward primer ETS1f (Linder & al., 2000) and reverse primer 18S-ETS (Markos & Baldwin, 2001). In some cases, Ast-1 and Ast-2 were also used as internal primers (Markos & Baldwin, 2001). The profile used for amplification was as described in Galbany-Casals & al. (2009a).

**cpDNA amplification strategies.** – The *rpl32-trnL* intergenic spacer was amplified using the forward primer rpl32F and reverse primer trnL(UAG) (Shaw & al., 2007). The profile used for amplification was as described in Galbany-Casals & al. (2010). The *ndhF* gene was amplified using the forward primer 3'F (Eldenas & al., 1999) and reverse primer +607 (Kim & Jansen, 1995). Two internal primers were also used: 1783R and 1626F (Barres & al., 2013). The profile used for amplification was as described in Galbany-Casals & al. (2012).

**Purification of PCR products and sequencing.** – Double-stranded PCR products were purified with either QIAquick® Purification Kit (Qiagen Inc., Hilden, Germany), DNA Clean & Concentrator-5 (Zymo Research, Orange, CA, USA) columns, or with ExoSAP-IT (USB Corp., Cleveland, OH, USA). Direct sequencing of the amplified DNA segments was performed with a Big Dye® Terminator v3.1 kit (Applied Biosystems, Foster City, CA, USA), following the protocol recommended by the manufacturer. Nucleotide sequencing was carried out at the “Serveis Científic-Tècnics” of the University of Barcelona on an ABI PRISM 3700 DNA analyzer (Applied Biosystems) or at the DNA Sequencing Core, CGRC/ICBR of the University of Florida on a ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

**Alignments.** – Nucleotide sequences were edited using Chromas 2.0 (Technelysium Pty. Ltd., Tewantin, Australia), Bioedit 7.0.1 (Hall, 1999) and Mega 3.1 (Kumar & al., 2004), and aligned visually or with the program ClustalX 2.0.10 (Thompson & al., 1997) with subsequent visual inspection and manual correction. In the ETS, *rpl32-trnL* intergenic spacer and *ndhF* datasets, ambiguous regions in alignments were detected and excluded by the use of Gblocks 0.91 (Castresana, 2000; Talavera & Castresana, 2007). This software allows the automatic, objective and repeatable detection and elimination from the dataset of those parts that cannot be unambiguously aligned. It was used with relaxed conditions in order to preserve as much information as possible: “Minimum Number Of Sequences For A Conserved Position” and “Minimum Number Of Sequences For A Flank Position” were half the number of sequences, “Minimum Number Of Contiguous Nonconserved Positions” was 5, “Maximum Number Of Contiguous Nonconserved Positions” was 10, “Minimum Length of a Block” was 5, and “Allowed Gap Positions” was “With Half”. Final aligned length analysed for each region is shown in Table 1, with indication of the percentage of the original datasets that it represents after the exclusion of ambiguously aligned regions. Data matrices are available on request from the corresponding author.

**Analyses.** – The evolutionary relationships of *Helichrysum* and related genera were examined analysing cpDNA and nrDNA separately, given the existence of several well supported incongruent patterns in previous phylogenies (Smitsen & al., 2011). The two cpDNA loci were analysed together, and *Athrixia phyllicoides* DC., *Leysera gnaphalodes* (L.) L. and *Relhania pungens* L'Hérit., three members of the *Relhania* clade, were coded as outgroup taxa in agreement with previous phylogenies (Bayer & al., 2000; Bergh & Linder, 2009; Ward & al., 2009). The nrDNA sequences were analysed at two levels using two different datasets. The tribe nrDNA dataset comprised all species included in this study and used the same three members of the *Relhania* clade as outgroup taxa. This dataset was composed of the ITS region and a conserved 3' portion of the ETS, which corresponds to the

fragment amplified by the Ast-1 and 18S-ETS primers (Markos & Baldwin, 2001). The second nrDNA dataset –the HAP clade dataset– included taxa selected from results of the tribe nrDNA dataset analyses. It comprised all the species included in the HAP clade and used *H. dasyanthum* and *Syncarpha mucronata* (P. J. Bergius) B. Nord. as outgroup taxa. This dataset was composed of the ITS region and a longer portion of the ETS, which corresponds to the fragment amplified by the ETS1f (Linder & al., 2000) and 18S-ETS (Markos & Baldwin, 2001) primers, and provided better results in resolving phylogenetic relationships within the HAP clade due to the possibility of aligning a longer portion, and therefore analysing a larger number of characters from the ETS region.

Bayesian inference (BI) of phylogeny was conducted using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The best-available model of molecular evolution required for Bayesian estimations of phylogeny was selected for each marker using the Akaike Information Criteria (AIC; Akaike, 1973) as implemented in the software jModelTest 0.0.1 (Guindon & Gascuel, 2003; Posada, 2008). The best fitting model for each marker was used in each case for all analyses (see Table 1), and partitions were defined when necessary in combined analyses. For each dataset, two simultaneous and independent analyses of four Metropolis-coupled Markov chains were run for five million generations (see Table 1), starting from different random trees, and saving one out of every 200 generations. For all analyses, the variance of split frequencies was <0.01, which indicated convergence of chains. After checking the convergence diagnostic Potential Scale Reduction Factor (PSRF) in MrBayes, and the LnL values, the first 25% of the trees of each analysis were discarded (burn-in), which amply ensured the exclusion of trees that might have been sampled prior to the convergence of the Markov chains. A 50% majority-rule consensus tree was computed with MrBayes with the remaining trees. Posterior probability support (PP) was considered to be significant for nodes with  $PP \geq 0.95$ . Bootstrap analyses (Felsenstein, 1985) were performed with PAUP\* v.4.0b10 (Swofford, 2002) with 1000 replicates, random taxon addition with 20 replicates, and no branch swapping. Bootstrap support (BS) values are shown for nodes with  $BS \geq 70\%$ .

**Mapping of distribution areas, chromosome numbers and morphological characters.** – Geographic area, ploidy level and some morphological characters relevant for generic delimitation were mapped for each species of the HAP clade on the nrDNA phylogenetic tree. Distribution areas of all species included were compiled from Reiche (1903), Kirpicznikov (1959), Humbert (1962), Davis & Kupicha (1975), Hilliard (1983), Anderberg (1991), Mesfin Tadesse & Rielly (1995), Wood (1997), Bentjee (2002), Nesom (2006a, b) and Galbany-Casals & al. (2006, and unpublished data). From this information, 14 geographic areas were defined (Fig. 1), in an attempt to reflect the main distribution patterns observed for the sampled species of the HAP clade. Mediterranean, Macaronesian and Irano-Turanian Regions approximately correspond to those described by Takhtajan (1986). For Africa, some of the geographic areas defined approximately correspond to phytogeographical regions shown in Burgoyne & al. (2005), although the Afromontane and Afroalpine Region has been divided into three regions: Afroalpine Region of Central East Africa, Tropical Africa Afromontane Region and Southern Africa Afromontane and Afroalpine Region, and Madagascar has been considered a unique region. Chromosome numbers were retrieved from the “Index to chromosome numbers in Compositae” website ([http://www.lib.kobe-u.ac.jp/infolib/meta\\_pub/CsvDefault.exe](http://www.lib.kobe-u.ac.jp/infolib/meta_pub/CsvDefault.exe)), Nesom (2006a, b), Galbany-Casals & al. (2009b) and Meng & al. (2010), and are compiled in Table S1. Relevant morphological characters for generic delimitation of members of the HAP clade were selected based on Hilliard & Burt (1981), Hilliard (1983) and Anderberg (1991) and those are number of florets per capitulum, capitula sex ratio, type of receptacle and type of pappus. Character states for each species were compiled from Humbert (1962), Hilliard (1983), Mesfin Tadesse & Rielly (1995),

Bentjee (2002), Galbany-Casals & al. (2006, and unpublished data), Nesom (2006a, b), Kilian & al. (2010) and personal observations of the specimens sequenced.

## RESULTS

**The HAP clade in the tribe, placement and composition.** – The HAP clade was placed in two different positions in the nrDNA and cpDNA analyses. In the nuclear tree (Fig. 2A), the HAP clade (PP = 0.99, BS = 100%) is sister to the remaining taxa from the crown radiation. In the cpDNA tree (Fig. 2B), it is nested within the crown radiation (PP = 1, BS = 98%), within a clade that also contains the Australasian taxa (AUS clade) and the South African *P. coronata* and *H. dasyanthum* (PP = 1, BS = 79%).

In both cpDNA and nrDNA analyses the HAP clade was composed of all *Achyrocline*, *Anaphalis* and *Pseudognaphalium* species sampled, the monotypic *Humeocline*, and all *Helichrysum* species sampled except for *H. dasyanthum* and *H. lanceolatum*, which were already known to be outside the HAP clade (Bayer & al., 2000; Galbany-Casals & al., 2004; Bergh & al., 2009; Ward & al., 2009; Smissen & al., 2011).

**Phylogenetic relationships within the HAP clade.** – The nrDNA tree of the HAP clade (Fig. 3A) and the geographic areas mapped on it (Fig. 4) show a basal grade, mainly constituted by species from SW southern Africa (Figs. 3A and 4, clades P and Q and *Helichrysum lambertianum* DC.), followed by subsequent supported clades (Figs. 3A and 4, clades K, L, M, N and O, and *Helichrysum isolepis* H. Bol.) that comprised species from the tropical and southern Africa Afromontane and Afroalpine regions, Madagascar and South Arabian peninsula. After those, a main clade (Fig. 3A, marked with an asterisk) contained clades A to J and several isolated species in polytomy. All members of *Achyrocline*, *Anaphalis*, *Humeocline* and *Pseudognaphalium* were placed within this clade. The three members of *Achyrocline* sampled were nested in clade D3 (PP = 1, BS = 94%), together with the African *Helichrysum epapposum* H. Bol. and *Helichrysum odoratissimum* (L.) Sweet (Figs. 3A and 4). The genus *Anaphalis* (Figs. 3A and 4, clades B2 + B3) was recovered as monophyletic with moderate support (PP = 0.99, BS = 76%) and sister to clade B1, composed by all the Mediterranean, Asian and some of the Macaronesian *Helichrysum* species (PP = 1, BS = 100%). Finally, all sampled members of *Pseudognaphalium* were placed in clade A (Fig. 3A) and constituted two main clades sister to each other without support: A3 (PP = 1, BS = 100%) contained the African *P. oligandrum* at the base of all North American species (PP = 1, BS = 86%), and A4 was constituted by *P. luteoalbum* and the two South American species *Pseudognaphalium gayanum* (J. Rémy) Anderb. and *Pseudognaphalium landbeckii* Phil. (PP = 1, BS = 100%). In clade E (Fig. 3A), an additional Macaronesian clade was recovered, constituted by *H. monogynum*, *H. alucense* and *H. nicolai*, and sister to the African and Arabian *Helichrysum glumaceum* DC.

Mapping morphological character states onto the nrDNA tree showed that *Achyrocline*, *Anaphalis* and *Pseudognaphalium* have almost unique combinations of morphological features as regards the number of florets per capitulum and sex ratio (Fig. 4). Species with fimbriiferous receptacles, species with paleate receptacles and species with reduced pappi did not constitute monophyletic groups, but were distributed in several clades. In contrast, species with biseriate pappi constituted a monophyletic group.

All *Achyrocline*, *Anaphalis* and *Pseudognaphalium* for which chromosome numbers are available ( $x = 7$ , Fig. 4 and Table S1) as well as the Mediterranean, Asian and Macaronesian *Helichrysum* are at least tetraploid ( $2n = 28$ ) – except for *P. luteoalbum* that is reported as diploid or tetraploid and *P. beneolens* that is reported as diploid (cf. Nesom, 2006b). Several diploid *Helichrysum* species were closely related to these clades.



The cpDNA tree showed less resolution than that obtained from the nrDNA markers, and the tree topology differed considerably (Figs. 3A and 3B). The only well-supported clades (i.e., PP  $\geq$  0.95 and/or BS  $\geq$  75%) that were congruent as regards their composition, i.e. they contained exactly the same members in both the nrDNA and cpDNA trees, were clades A5, A6, B1, B1.1, B2, D2, and the clades containing *Helichrysum ephelos* Hilliard with *Helichrysum glomeratum* Klatt, and *Helichrysum crispum* (L.) D. Don with *Helichrysum indicum* (L.) Grierson, although their position or closest relatives differed. Clades D5, F, N and O were also recovered in the cpDNA tree, but did not contain all the species that were contained by the equivalent clade in the nrDNA tree. Many species presented an incongruent position between the nrDNA and the cpDNA trees, and this is reported in detail in Table 2.

The details of the incongruities between the nrDNA and the cpDNA trees are difficult to summarise, given the different levels of resolution and the different composition of clades in both trees. However, the main differences between the tree topologies are that members of clade A (PP = 0.99) in the nrDNA tree (Fig. 3A) appear scattered in clades 1, 2, 3, 4, 5 and 6 in the cpDNA tree (Fig. 3B), and one species, *Helichrysum transmontanum* Hilliard, is not included in any of the supported clades. Similarly, members of clade B in the nrDNA tree (Fig. 3A) appear scattered in clades 2 and 4 in the cpDNA tree (Fig. 3B).

## DISCUSSION

**Incongruities between cpDNA and nrDNA, and allopolyploidy as a potential force for dispersal and diversification in the HAP clade.** – Incongruence between cpDNA and nrDNA markers has been previously reported for several genera of tribe Gnaphalieae (Galbany-Casals & al., 2010; Smissen & Breitwieser, 2008; Smissen & al., 2004, 2011) and has largely been interpreted in those studies as evidence of past or present hybridisation events rather than sorting or retention of ancestral polymorphisms. For some particular clades, and with the additional evidence from low copy markers and chromosome data, an allopolyploid origin has been hypothesised (Smissen & al., 2011). The data presented here are generally consistent with an allopolyploid origin of some clades within the HAP clade, although the scenarios presented by Smissen & al. (2011) are insufficient to explain the phylogenetic incongruence observed with our increased taxa sampling.

One case involves *Pseudognaphalium*. In our nrDNA tree (Fig. 3A) the sampled members of this genus are distributed in two clades, with a non-supported sister relationship to each other: clade A3 comprises *P. oligandrum*, an African species of unknown chromosome number, sister to all sampled species from North America; and clade A4 comprises the subcosmopolitan *P. luteoalbum* sister to the two South American species sampled. In the cpDNA (Fig. 3B) all species from the Americas form a distinct clade nested in a larger clade which also includes the Macaronesian *H. nicolai*, the subcosmopolitan *P. luteoalbum*, and the African *Helichrysum psilolepis* Harv. and *Helichrysum basalticum* Hilliard. *Pseudognaphalium oligandrum* is placed in a different clade (clade 4) together with some African species of *Helichrysum* and two *Anaphalis* species. Based on very limited taxon sampling, Smissen & al. (2011) suggested an allopolyploid origin for part of *Pseudognaphalium* with parents from within diploid *Helichrysum*. Considering the additional taxon sampling for nrDNA and cpDNA reported here, along with that reported by Nie & al. (2013), it seems likely that more complex scenarios are required to explain the distribution of sequences among these species. Sequencing of the low copy number nuclear DNA regions used by Smissen & al. (2011) for more species of *Pseudognaphalium* and associated species of *Helichrysum* (particularly, but not limited to clade A) may help unravel this history.

Another group of putative allopolyploid origin is clade B from the nrDNA tree (Fig. 3A) which includes all Mediterranean and Asian *Helichrysum* species as well as some from Macaronesia (clade B1), and all *Anaphalis* species sampled (clades B2 and B3). Clade B1 had been identified in previous work (Galbany-Casals & al., 2004) and relationships within this group were described by Galbany-Casals & al. (2009a). In their molecular phylogeny of *Anaphalis* based on nrDNA markers, Nie & al. (2013) also obtained the same relationships among *Anaphalis* and the Macaronesian-Mediterranean-Asian *Helichrysum* clade, although in their analysis the monophyly of *Anaphalis* was not statistically supported. The two clades we recovered within *Anaphalis* (Fig. 3A, B2 and B3) agree with two clades shown by Nie & al. (2013) and called by them the “non decurrent” and the “decurrent” clades.

The species of *Helichrysum* from nrDNA clade B1 and those of *Anaphalis* from nrDNA clade B2 (Fig. 3A) occur in cpDNA clade 2 (Fig. 3B), along with several other *Helichrysum* species from the distantly related clade N in the nrDNA tree. *Anaphalis* species from nrDNA clade B3 (Fig. 3A) appear in cpDNA clade 4 (Fig. 3B), along with several species of *Helichrysum* from multiple nrDNA clades. Smissen & al. (2011) hypothesised that *Anaphalis* and the Mediterranean-Asian *Helichrysum* had a common allopolyploid origin with possible parental species shared (Smissen & al., 2011) on the basis of low copy number nuclear gene trees. However, *Anaphalis* was represented in their study only by a single species, *A. margaritacea*. The existence of two *Anaphalis* clades in the nrDNA analyses in the present work, and the incongruent position of one of them in the cpDNA analyses, suggest that this genus could have had two or more independent allopolyploid origins or at least evolved from a polymorphic polyploid ancestral complex. Although the exact number of allopolyploidy events that would have led to clade B1 and *Anaphalis* is not certain with the present data, some hypotheses can be presented about possible parental species involved. The close relationship of *Helichrysum arwae* J. R. I. Wood, *Helichrysum citrispinum* Del. and *Helichrysum gofense* Cufod., from tropical Africa and Yemen, with members of clades B1 and B2 in the cpDNA tree (Fig. 3B, clade N) could implicate them as possible species (or descendants of species) involved in the origin of clade B members. Other species involved in the hypothesised allopolyploidy events could have been *Helichrysum splendidum* (Thunb.) Less., placed in clade C in the nrDNA tree (Fig. 3A) and sister to clade A + B although without statistical support, and a member of clade 4 together with part of *Anaphalis* in the cpDNA tree (Fig. 3B), or some species of clade A1, which are also related to clade B in the nrDNA (Fig. 3A) –although without statistical support– and are members of clade 4 with part of *Anaphalis* in the cpDNA tree (Fig. 3B). All species of *Anaphalis* and clade B1 for which the chromosome number is known are at least tetraploid, and some of them are hexaploid or octoploid (Fig. 4 and Table S1), whereas *H. splendidum* is a diploid as are some members of clade A1 (Fig. 4 and Table S1). Unfortunately, chromosome numbers of *H. arwae*, *H. citrispinum* and *H. gofense* are unknown. Given the current distribution area of these species, the *Anaphalis* and B1 lineages might have originated in east tropical Africa. The ancestors of clade B1 could have dispersed and diversified in the Macaronesian and the Mediterranean areas, and later dispersed to Asia through the Irano-Turanian Region up to the Pamir and Tien Shan Central Asian mountains, as was described in detail in Galbany-Casals & al. (2009a), while possibly one or two additional new lineages, constituting *Anaphalis*, would have dispersed to Asia independently. The present distribution area of *Anaphalis* in Asia seems to indicate that the colonisation of Asia would have followed a migration route from east Africa, it would have radiated in the Himalayan range, east Asia, and would later have reached North America, where only *A. margaritacea* is currently present, across the Bering Strait. Similar migration routes through Asia and America have been proposed for *Datisca* L. (Datisceae: Liston & al., 1992), the tribe Betoideae (Amaranthaceae: Hohmann & al., 2006) and *Plectocephalus* D. Don (Compositae, Cardueae: Susanna & al., 2011).

In our nrDNA tree the Cape Verde endemic *H. nicolai* is part of a Macaronesian clade with *H. alucense* and *H. monogynum* from the Canary islands, and this clade is in turn sister to *H. glumaceum* (Figs. 3A and 4, part of clade E2). However, in our cpDNA tree *H. nicolai* is related to *P. luteoalbum*, *H. psilolepis* and *H. basalticum* (Fig. 3B). Although the chromosome number of *H. nicolai* is unknown, this incongruence could suggest a possible hybrid origin for this species. The facts that it shares with *P. luteoalbum* a higher number of pistillate than hermaphroditic florets in the capitula (Fig. 4), and that it is closely related to this species in the cpDNA tree, could point at this species as a possible parent. It is also interesting that they both grow together in the only known locality of *H. nicolai* (M. Galbany-Casals, pers. obs.). The fact that *H. monogynum* is reported to be tetraploid or hexaploid (Fig. 4 and Table S1) could imply either that this species, and possibly also *H. alucense*, could have also been originated by allopolyploidy, or alternatively that *H. nicolai* would be of homoploid hybrid origin, although the present data are not conclusive.

In the present work, many other cases of incongruence have been detected between nrDNA and cpDNA trees (Figs. 3A and 3B and Table 2). However, for most of them chromosome numbers, their closest relatives and other relevant information are missing. Therefore, any explanation of the incongruities would be speculative and the possibility that they result from retention or sorting of ancestral polymorphisms cannot be excluded.

Finally, in the nrDNA tree *Achyrocline satureioides* (Lam.) DC. and *Achyrocline alata* DC., both endemic to South America, constitute a highly supported clade, which is part of a larger clade that includes *Achyrocline stenoptera* (DC.) Hilliard & B. L. Burtt from the Arabian Peninsula, tropical and southern Africa, and the African *H. odoratissimum* and *H. epapposum* (Figs. 3A and 4, clade D3). *Achyrocline satureioides* is reported to be tetraploid (Fig. 4 and Table S1), and the closely related *H. odoratissimum* is reported to have different chromosome numbers, from diploid to hexaploid ( $2n = 42$ ). Although evidence is scarce at the moment, these data could also be interpreted as suggesting a possible polyploid origin of *Achyrocline* in Africa, and a subsequent dispersal to the New World. However, given that *Achyrocline* and its closest relatives do not have an incongruent position in the cpDNA tree, there is no evidence of allopolyploidy in this case. It would be interesting to see if all the South American species have a common origin, especially after Hilliard & Burtt's (1981) observations on the diversity of habit and leaf form among the species of *Achyrocline*, and the similarity of several African-South American species pairs in relation to these characters. For example, the African *A. stenoptera* was said to be morphologically similar to the South American *A. alata* (Hilliard & Burtt, 1981) and they are both placed in clade D3 (Fig. 4). The absence of the genus in Asia might suggest a long-distance dispersal event from Africa to South America, where most of the species of the genus are found. However, any hypothesis would be speculative at the moment, especially taking into consideration Anderberg's (1991) comments on the morphological similarities of some South American *Achyrocline* and *Pseudognaphalium* species, suggesting a possible close relationship among them. The addition of other American *Pseudognaphalium* and *Achyrocline* species in future work could provide alternative hypotheses for the colonisation of the New World by both genera.

**Biogeographic patterns.**— The superposition of distribution areas on the phylogeny (Fig. 4) suggests that the HAP clade could have originated in the Cape region of southern Africa and then dispersed to the Afromontane regions of east southern Africa, mainly the Drakensberg, where it would have diversified considerably. From east southern Africa, several independent lineages then spread northwards, and gave rise to the species of the afromontane and afroalpine areas of central tropical Africa. This pattern parallels that found for *Disa* Bergius (Orchidaceae) and tribe Iridae of Iridaceae (Galley & al., 2007). Other groups such as Restionaceae, *Pentaschistis* (Nees) Spach (Poaceae), and *Euryops* (Cass.) Cass. (Compositae) have also been found to have a Cape origin followed by northward

migration to central Africa's montane areas, although with absence of local diversification in the Drakensberg (Galley & al., 2007; Devos & al., 2010). *Helichrysum* has also dispersed to Madagascar, an island which seems to have been colonised by the genus at least five times independently (Fig. 4). Our present data suggest that several lineages would have originated in eastern tropical Africa and would have dispersed out of Africa and diversified elsewhere. The Mediterranean region would have been colonised once by members of clade B1.1 and B1.2; the Macaronesian region would have been colonised at least twice by members of clades B1.3 and E; the Asian continent would have been colonised two or three times by members of clades B1.1, B2 and B3; and the New World would have been colonised at least two or three times by members of clades A3, A4 and D3. If our hypotheses of allopolyploid origins for all these clades are proven, this would imply that allopolyploidy would have preceded most of the dispersal events and further diversification of the HAP clade out of Africa. Several studies have previously suggested that polyploids could be particularly advantageous in long-distance dispersal and establishment on oceanic islands (Barrier & al., 1999; Doyle & al., 2000, 2002). Although it is clear that *Achyrocline*, *Anaphalis* and *Pseudognaphalium* are nested within the currently broadly defined *Helichrysum*, further work, including more comprehensive sampling of at least *Achyrocline* and *Pseudognaphalium* is needed to better understand the complexity of their origin, dispersal and diversification.

**Morphological patterns and generic delimitation.**— The fact that *Achyrocline*, *Anaphalis* and *Pseudognaphalium* are nested within *Helichrysum*, therefore rendering it paraphyletic, presents the classic dilemma of phylogenetic systematics: *Helichrysum* can be split so as to render it, and the genera nested within it, reciprocally monophyletic, or the genera can be lumped to avoid paraphyly. Transferring all species of *Achyrocline*, *Anaphalis* and *Pseudognaphalium* to *Helichrysum* would certainly overcome the paraphyly of the current classification. However, this would involve numerous new nomenclatural combinations and would add about 230 species to the genus, making it even larger and less wieldy. Application of this lumping approach across the whole tribe might ultimately render its species classified in very few large genera, reversing the trend of recent decades towards the splitting of large heterogenous genera (see Ward & al., 2009).

Alternatively, splitting *Helichrysum* into demonstrably monophyletic genera based on current understanding of phylogenetic relationships would require the introduction of numerous small (or monotypic) genera often lacking clear morphological synapomorphies. However, for those content to accept some paraphyletic genera (e.g. Hörandl & Stuessy, 2010) if only as an interim measure (e.g. Entwistle & Weston, 2005), opportunities remain to reclassify so as to better reflect phylogenetic relationships than the *status quo* does. As also discussed by Smitsen & al. (2011) for the case of Gnaphalieae, since reticulate relationships among genera and species cannot be represented by a dichotomizing tree, in such cases, paraphyletic groups might be considered a necessary expedience. Leaving *Helichrysum* paraphyletic and retaining all or some of the other traditionally recognised genera might be justified by the hypotheses that *Achyrocline*, *Anaphalis* and *Pseudognaphalium* have allopolyploid origins, by their distinguishable morphology, and by remaining uncertainties as to phylogenetic relationships, both in the HAP clade and in the tribe at large. However, this raises other questions. For example, the circumscription of *Pseudognaphalium* and *Anaphalis* is debatable since their monophyly as currently recognised is not certain. Furthermore, it is not clear if *P. luteoalbum* and *P. oligandrum* are part of a distinct clade corresponding with *Pseudognaphalium*, or part of a group within *Helichrysum* that provided one parent of an allopolyploid *Pseudognaphalium*. If so, these species should be recognised in *Helichrysum*, while the remainder of *Pseudognaphalium* is retained at least in the short term.

Moreover, maintaining *Achyrocline*, *Anaphalis* and *Pseudognaphalium* because of their hypothesised hybrid origins would also favour the description of new genera to

recognise other putative allopolyploid clades within *Helichrysum*. This would have significant nomenclatural implications. The type species of *Helichrysum* is *H. orientale*, a Mediterranean species in a clade for which we have hypothesised an allopolyploid origin. Recognising all allopolyploid clades as different genera would force either the proposal of a different type for *Helichrysum* (presumably a diploid African species) or the transfer of most species of *Helichrysum* to another genus or genera. Splitting *Helichrysum* into a number of genera would be consistent with other taxonomic progress in the tribe (see Ward & al., 2009), but given the current lack of phylogenetic resolution among many of the species, much further work would be required before new genera could be proposed as monophyletic.

Given the uncertainty and complexity of phylogenetic relationships within the HAP clade suggested by DNA sequence data, morphological characters need to be given particular attention. Hilliard & Burt (1981) discussed anomalies shown by several species of African *Helichrysum* in their stereome, sex ratio, receptacle structure, and pappus morphology, and those are discussed here on the basis of the nrDNA tree (Fig. 4) and in relation to the other genera involved.

Division of the stereome seems to be a very constant character within the HAP clade. Among all species recovered within this clade, the only one with an undivided stereome is *P. oligandrum*, while all other species have a fenestrated stereome (Hilliard, 1983; Anderberg, 1991). Hilliard & Burt (1981) commented on some aberrant *Helichrysum* with undivided stereomes, which have in common several other morphological features, i.e. similar habit and capitulum morphology. Among those, *H. dasyanthum* is the only species represented in the present work and is excluded from the HAP clade (Figs. 2A and 2B). In the nrDNA tree (Fig. 2A) this species is placed in a clade along with *P. coronata* and *L. canescens*, a clade already obtained by Bayer & al. (2000) with cpDNA markers, although these authors also recovered *Anaxeton* Gaertn., *Syncarpha*, *Anderbergia* B. Nord. and *Helichrysum cylindriflorum* (L.) Hilliard & B. L. Burt in the same clade. All these taxa have been reported to have an undivided stereome as well (Hilliard, 1983; Anderberg, 1991), except for *H. cylindriflorum*, for which there is no mention about the stereome (Hilliard, 1983), and *Syncarpha*, that is variable for this character (Anderberg, 1991). With the present data this feature seems to be diagnostic for the HAP clade, although future studies should consider other *Helichrysum* species with undivided stereomes, together with a more comprehensive sampling of *Anaxeton* and *Syncarpha*.

Characters related to capitulum sex ratio show interesting patterns in the phylogenies. *Anaphalis* is readily recognised by an exclusive feature within the HAP clade, which is subdioecy. This seems to support the possible monophyly of the genus, in agreement with the nrDNA tree (Fig. 3A), but not supported by the cpDNA tree, as discussed above (Fig. 3B).

Another diagnostic character for generic delimitation in the HAP clade is capitulum sex ratio in combination with the number of florets per capitulum. Capitula with pistillate florets outnumbering hermaphroditic florets are diagnostic of *Achyrocline* and *Pseudognaphalium*, and these two genera are distinguished from each other by the number of florets per capitula: while *Achyrocline* is said to have less than 20 florets per capitulum, *Pseudognaphalium* has larger capitula with more numerous florets. Although numerous *Helichrysum* species have few-flowered capitula with less than 20 florets, and others have capitula with more pistillate than hermaphroditic florets, these two character states are scattered across several clades in the nrDNA tree and not correlated (Fig. 4). In our analyses, *A. satureioides* and *A. alata* constitute a highly supported clade, which is part of a larger clade (Fig. 4, D3) that includes *A. stenoptera*. Thus, although these results do not provide support for the monophyly of *Achyrocline*, they are consistent with it. Also, these three species are grouped with high support with some members of *Helichrysum* that have several morphological characters in common with them. Both *H. epapposum* and *H. odoratissimum*

have very small and narrowly cylindrical capitula, which are densely congested in cymose clusters which are secondarily arranged in corymbose synflorescences. Hilliard & Burt (1981) noted the similarity between *H. odoratissimum* and *A. stenoptera*. In *H. odoratissimum* the hermaphroditic florets outnumber pistillate florets, and *H. epapposum* has homogamous capitula, whereas other *Helichrysum* species of clade D have capitula with pistillate florets outnumbering hermaphroditic florets, for example *Helichrysum forskahlii* (J. F. Gmel.) Hilliard & B. L. Burt, *Helichrysum interjacens* Hilliard and *Helichrysum polycladum* Klatt. However, none of these species have both character states diagnostic of *Achyrocline*.

The *Pseudognaphalium* clade shows another almost exclusive combination of character states, big capitula with more than 40 florets with pistillate florets outnumbering hermaphroditic florets. With our present sampling, only *H. basalticum*, placed also within clade A and close to *Pseudognaphalium*, has this character state combination. The circumscription of *Pseudognaphalium* remains debatable, in particular whether *P. luteoalbum* should be included in *Pseudognaphalium* or in *Helichrysum* (e.g., Galbany-Casals & al., 2004), or should be treated as *Laphangium* (Hilliard & B. L. Burt) Tzvelev (Greuter, 2005--2007). Hilliard & Burt (1981) placed this species in *Pseudognaphalium* subgenus *Laphangium* recognising it as distinct from most other members of the genus in having cymose rather than corymbose synflorescences, and urceolate rather than campanulate capitula. However, the same authors recognised that these features are found elsewhere in *Helichrysum*.

The presence of fimbriiferous receptacles seems to be a defining character shared by most members of the clades where the character appears (i.e., D, E1 and J, Fig. 4). Although some species of these clades have receptacles flat or honeycombed, and fimbrials also appear in other species scattered across other clades, our results suggest that more complete and detailed analyses could show this character as diagnostic of some infrageneric groups, possibly correlated with other morphological characters that need to be explored. In contrast, our results are not conclusive as regards the presence of paleae in the receptacle. *Helichrysum paleatum* Hilliard, *Helichrysum platypterum* DC. and *Helichrysum argyrophyllum* DC. have paleate receptacles and, although they are not grouped together in the nrDNA tree (Fig. 3A), the tree does not contradict a possible common origin of this feature due to lack of resolution. *Helichrysum platypterum* and *H. argyrophyllum* are both in clade 3 in the cpDNA tree (Fig. 3B), while *H. paleatum* was not included.

Pappus morphology is variable in *Helichrysum*. Most species have a uniseriate pappus with scabrid, barbellate or subplumose setae (Hilliard & Burt, 1981) but several species have a reduced pappus, composed of a small number of short bristles, while others have no pappus at all. The fact that these species appear scattered across several clades in the nrDNA tree (Fig. 4), and that they are sometimes closely related to other species with a normally developed pappus, suggests that this structure could have been lost independently several times. One of the species without pappus is *Humeocline madagascariensis* (Humbert) Anderb. This monotypic genus was described by Anderberg (1991) based on *Humea madagascariensis* Humbert. Despite the close relationship to species with capitula grouped in corymbose synflorescences and with fimbriiferous receptacle, this species has a particular morphology: the synflorescence has a generally pyramidal shape, with capitula arranged in axillary and terminal spiciform glomerules, and the receptacle is very narrow and naked. Another member of the same clade is *Helichrysum manopappoides* Humbert, which has in common with *Humeocline* homogamous capitula and an anomalous pappus.

Three of the sampled species in the present study have a biseriata pappus and constitute a highly supported clade (Fig. 4, part of clade P). *Helichrysum spiralepis* Hilliard & B. L. Burt and *H. litorale* H. Bol. were members of *Leontonyx* Cass., characterized by a biseriata pappus, but that genus was synonymised with *Helichrysum* by Hilliard & Burt

(1981). *Helichrysum zwartbergense* H. Bol., although it has never been included in *Leontonyx*, also has a biseriata pappus and is closely related to members of the old genus *Leontonyx* in the phylogeny. These three species have also in common that their capitula are composed of four series of involucre bracts, soon caducous, the two outer series being shorter and webbed in a dense tomentum together with the surrounding leaves, whereas the two inner series equal the florets in length, and the three species have an unusual chromosome number in the HAP clade,  $2n = 8$ . Future studies including other morphologically very similar species that have a uniseriate pappus (cf. Hilliard, 1983) would be necessary to show the value of this character in the establishment of generic or infrageneric groups.

**Conclusions.**— Although some trends are detected in the distribution of morphological traits in the phylogeny, *Helichrysum* shows much more morphological variation than any of the segregated hypothesised allopolyploid genera, and the diagnostic characters of these genera are not always exclusive of them, but also present in some species of *Helichrysum*. The information currently available is still incomplete as regards species and markers sampled, and chromosome number. *Helichrysum* is only represented by about one fourth of its species; and *Achyrocline* and *Pseudognaphalium* are also underrepresented. Although the HAP clade is well defined and highly supported, the complexity of phylogenetic relationships and morphological variation among its species implies that a decision on generic delimitation would be premature with the present data. However, current evidence suggests the following incremental steps toward a satisfactory generic delimitation of the group: 1. Accept a paraphyletic *Helichrysum* but transfer the species outside the HAP clade – the New Zealand species (Ward & al., in progress) and some South African species – to other, possibly new genera; 2. Retain *Achyrocline*, *Anaphalis* and *Pseudognaphalium* pending possible further splitting of each when it is demonstrated that they have had multiple origins; and 3. Segregate the other allopolyploid clades from *Helichrysum* once more evidence is provided about their origin and circumscription. This includes the Mediterranean-Asian clade and possibly others, and could involve the proposal of a new type for the genus. Finally, splitting the remaining diploid *Helichrysum* into numerous smaller genera would probably be necessary to arrange the morphological diversity involved once this has been explored more deeply.

Although nrDNA regions have proved useful in delimiting some clades, they present problems in phylogenetic reconstructions when allopolyploidy is involved. When multiple divergent copies of the nrDNA repeat are present in the genome, concerted evolution could result in the exclusive presence of one type, or the emergence of a recombinant form, or the coexistence of multiple copies (Álvarez & Wendel, 2003). Also, levels of variation in cpDNA are low and relationships are not well resolved, but the addition of more cpDNA markers has been shown to be of limited value (Smitsen & al., 2011). In addition, lineage sorting could be influencing the results we present here, which up to now have been mostly interpreted or discussed as resulting from hybridisation. Available data on low copy DNA markers (Smitsen & al., 2011) showed more structure in the phylogenies obtained. Future phylogenetic work on the HAP clade might use low copy DNA markers in addition to nrDNA and cpDNA markers for a better sample of the whole lineage, to provide more solid hypotheses about their origins and phylogenetic relationships.

## ACKNOWLEDGMENTS

We thank the curators of all herbaria and staff of Botanical Gardens that provided material, and all colleagues who helped in field trips or provided their own material from field collections; they are all cited in the Appendix. D. Gutiérrez, E. Hinojosa and P. Carnicero-Campmany provided technical assistance; J. Jardim, A. Marticorena, J. Ready and Mesfin

Tadesse and S. Freire helped with some species identification. Two anonymous reviewers provided helpful and constructive criticism of the manuscript. Finally, the National Science Foundation (0301824/IRFP), CSIRO, Plant Industry (Australia), the Spanish government (CGL2007-60781/BOS, CGL2009-13322-C03-03/BOS, CGL2010-18631/BOS) and the Catalan government ('Ajuts a grups consolidats' 2009/SGR/00439) have partly financed this work.

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**Table 1.** Characteristics of data matrices and substitution models applied in the Bayesian analyses.

DNA region	cpDNA (tribe)	nrDNA (tribe)	nrDNA (HAP clade)
Number of taxa	178	199	179
Number of characters included after removing ambiguously alignable regions	1062 (43% of the complete aligned dataset)	1089 (100% of the complete aligned dataset)	1676 (78% of the complete aligned dataset)
Parsimony informative characters	184	535	918
Number of MCMC generations	5 million	5 million	5 million
Substitution model	GTR+G (Yang, 1996) for the <i>ndhF</i> region; GTR+I+G (Gu & al., 1995) for the <i>rpl32-trnL</i> intergenic spacer	GTR+I+G (Gu & al., 1995)	GTR+I+G (Gu & al., 1995)

**Table 2.** Taxa with well-supported (BS = 75% and/or PP = 0.95) incongruent positions.

Species	ITS/ETS trees	Plastid trees
<i>Helichrysum transmontanum</i>	Member of clade A1 (PP = 1)	Not placed in any of the supported clades of the HAP clade, excluded from clade A1
<i>Pseudognaphalium oligandrum</i>	Member of clade A3 (PP = 1, BS = 100)	Constitutes clade 4 (PP = 1) together with most members of clade A1, clade B3, <i>Helichrysum trilineatum</i> and <i>H. splendidum</i>
<i>Pseudognaphalium luteoalbum</i>	Member of clade A4 (PP = 1, BS = 100) together with <i>Pseudognaphalium landbeckii</i> and <i>P. gayanum</i>	Not included in clade 3 (PP = 1) that groups most members of clades A3 and A4
<i>Helichrysum populifolium</i>	Member of clade A (PP = 1)	Member of clade 1 (PP = 0.99), that does not include any species of clade A
<i>Helichrysum italicum</i> subsp. <i>italicum</i> and subsp. <i>microphyllum</i>	Member of clade B1.2 (PP = 1, BS = 98) within clade B1	Within clade 2 (PP = 1), nested with members of clade B1.3 (PP = 0.98)
<i>Helichrysum splendidum</i>	Nested with <i>Helichrysum montanum</i> (PP = 1, BS = 100) in clade C together with <i>H. marginatum</i> (PP = 0.96, BS = 80)	Constitutes clade 4 (PP = 1) together with most members of clade A1, clade B3, <i>Helichrysum trilineatum</i> and <i>Pseudognaphalium oligandrum</i>
<i>Helichrysum melanacme</i>	Nested with <i>Helichrysum interjacens</i> , <i>H. polycladum</i> and <i>H. simillinum</i> (PP = 1, BS = 100) within clade D4	Nested with <i>Helichrysum griseolanatum</i> (PP = 1) in clade 1 (PP = 0.99)
<i>Helichrysum petiolare</i>	Constitutes clade D5 with two specimens of <i>Helichrysum patulum</i> (PP = 1, BS = 100)	Within clade 1 (PP = 0.99), but not nested with <i>Helichrysum patulum</i>
<i>Helichrysum albobrunneum</i>	Nested in clade D6 (PP = 1, BS = 100)	Nested in clade 3 (PP = 1), that does not include any species of clade D6
<i>Helichrysum felinum</i>	Nested in clade D7 (PP = 1, BS = 100)	Nested in clade 3 (PP = 1), that does not include any species of clade D7
<i>Helichrysum globosum</i>	Nested in clade E1 (PP = 0.99, BS = 72)	Nested in clade 3 (PP = 1), that does not include any species of clade E1
<i>Helichrysum nicolai</i>	Nested in clade E2 (PP = 1, BS = 100)	Within clade 3 (PP = 1), nested in a clade (PP = 1, BS = 76) that includes most members of clade A3, members of clade A4, <i>Helichrysum psilolepis</i> and <i>H. basalticum</i>
<i>Helichrysum maranguense</i>	Nested with <i>Helichrysum arwae</i> (BS = 80) within clade N (PP = 1, BS = 93)	Nested in clade 1 (PP = 0.99), that does not include any species of clade N
<i>Helichrysum galpinii</i>	Member of clade O (PP = 1, BS = 84)	Constitutes clade 6 (PP = 1) with clade A6 and <i>Helichrysum stuhlmannii</i>
<i>Helichrysum dasycephalum</i> and <i>H. callicomum</i>	Nested together (PP = 1, BS = 100) and constitute clade O (PP = 1, BS = 84) with <i>Helichrysum galpinii</i>	Within clade 1 (PP = 0.99), both species constitute a clade without support and this is nested with <i>Helichrysum glomeratum</i> and <i>H. ephelos</i> (PP = 1)
<i>Helichrysum crispum</i> and <i>H. indicum</i>	Nested together (PP = 1, BS = 100) and constitute clade Q (PP = 1, BS = 100) with <i>Helichrysum hamulosum</i> and <i>H. retortum</i>	Nested together (PP = 0.98) and constitute clade 7 (PP = 0.95) with members of clade P

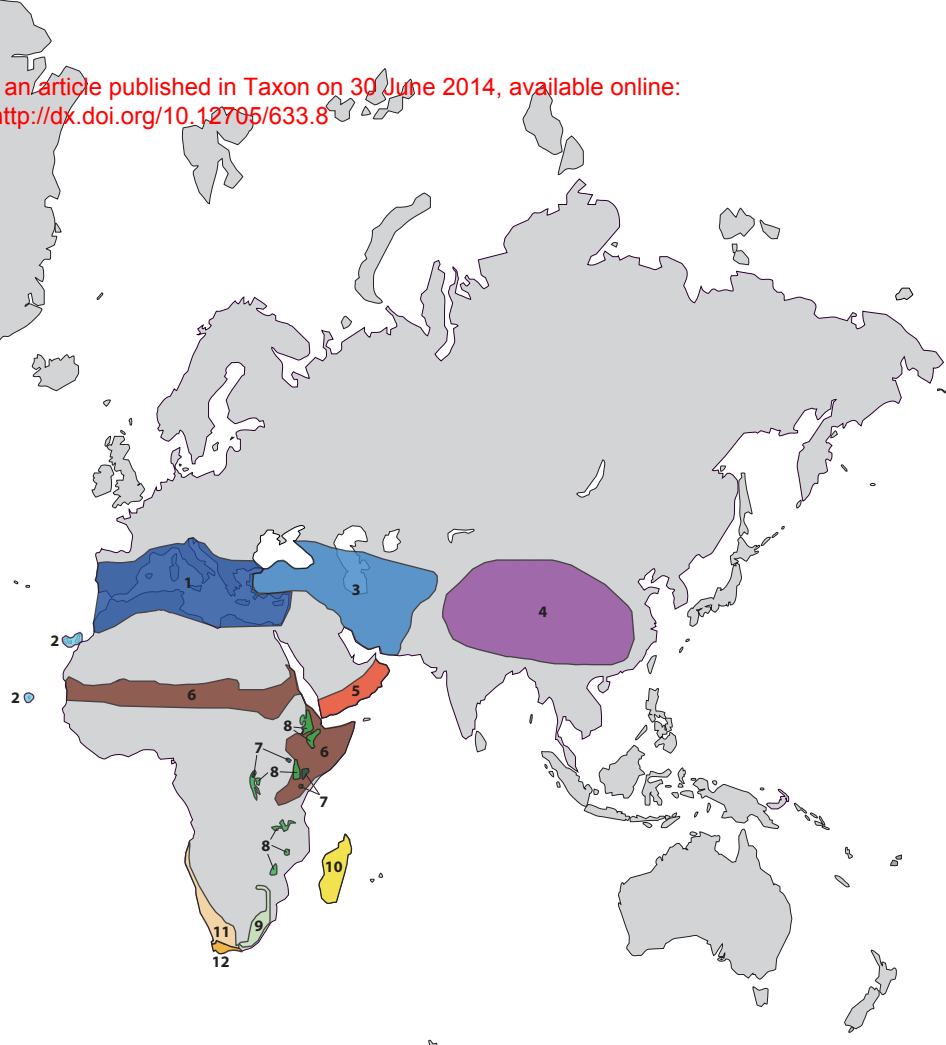
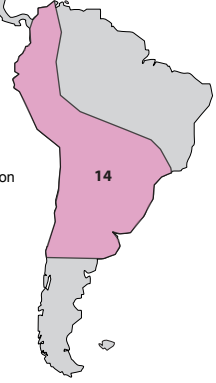
**Figure captions:**

**Fig. 1.** Geographical areas defined to map the distribution of the sampled species onto the phylogenetic tree.

**Fig. 2. A.** Phylograms obtained from the Bayesian analysis at the tribal level. Only  $\geq 0.95$  Bayesian posterior probabilities and  $> 70\%$  bootstrap values are shown above branches. AUS: Australasian; HAP: *Helichrysum-Anaphalis-Pseudognaphalium*. Bold: *Helichrysum* species and the HAP clade. **A.** ITS and ETS nrDNA regions. Grey: species not included in the cpDNA analysis. **B.** *rpl32-trnL* and *ndhF* cpDNA regions.

**Fig. 3.** Phylograms obtained from the Bayesian analyses of the HAP clade. Only  $\geq 0.95$  Bayesian posterior probabilities and  $> 70\%$  bootstrap values are shown. *H.*: *Helichrysum*. Genera other than *Helichrysum* are highlighted in coloured boxes. Names of species with an incongruent position between the nrDNA and cpDNA trees are highlighted in coloured letters. **A.** ITS and ETS nrDNA regions. Supported clades discussed in the text are labelled with capital letters. **B.** *rpl32-trnL* and *ndhF* cpDNA regions. Supported clades discussed in the text are labelled with numbers.

**Fig. 4.** Rectangular cladogram obtained from the Bayesian analysis of ITS and ETS nrDNA regions of the HAP clade. Only  $\geq 0.95$  Bayesian posterior probabilities and  $> 70\%$  bootstrap values are shown. Supported clades labelled with capital letters are the same as in Fig. 3A. Information on distribution areas, morphological characters and ploidy levels for the available species has been mapped. For distribution areas, black represents subcosmopolitan. For morphological characters, black represents unknown. For chromosome numbers, the base number is 7, otherwise it is indicated.

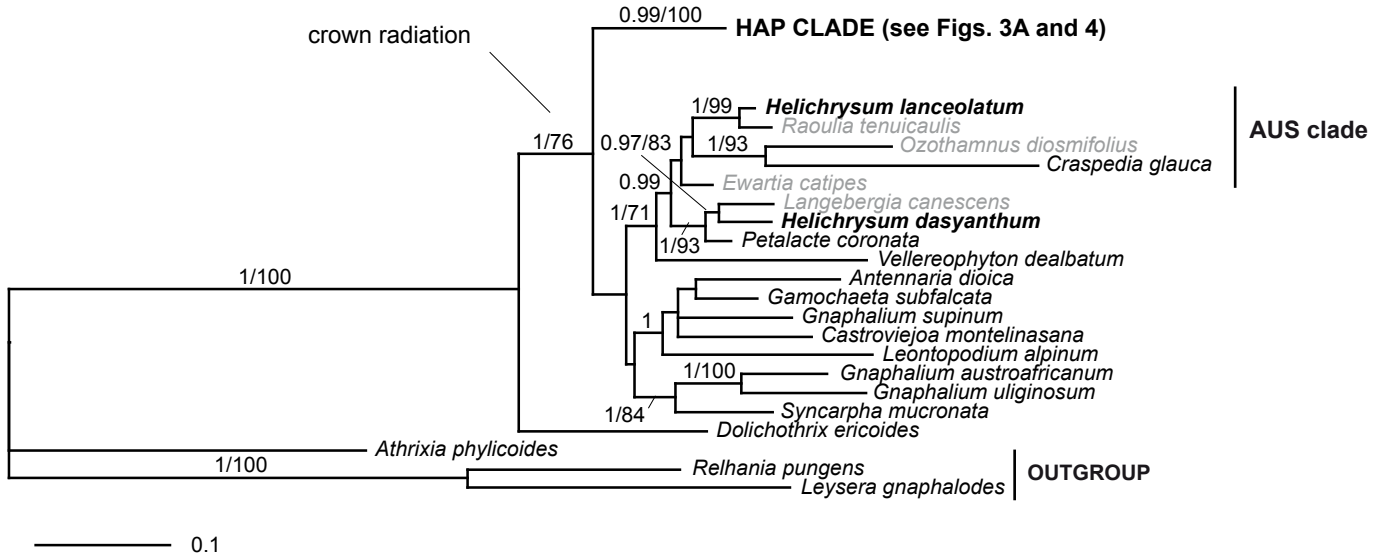


- 1. Mediterranean Region
- 2. Macaronesian Region
- 3. Irano-Turanian Region
- 4. Eastern and Southern Asia
- 5. South Arabian peninsula
- 6. Sahel and Somalia-Masai Regions
- 7. Afroalpine region of Central East Africa
- 8. Tropical Africa Afromontane Region
- 9. Southern Africa Afromontane and Afroalpine Region
- 10. Madagascar
- 11. Inner SW southern Africa (mainly Karoo)
- 12. Coastal SW southern Africa (Cape Region)
- 13. N America
- 14. S America

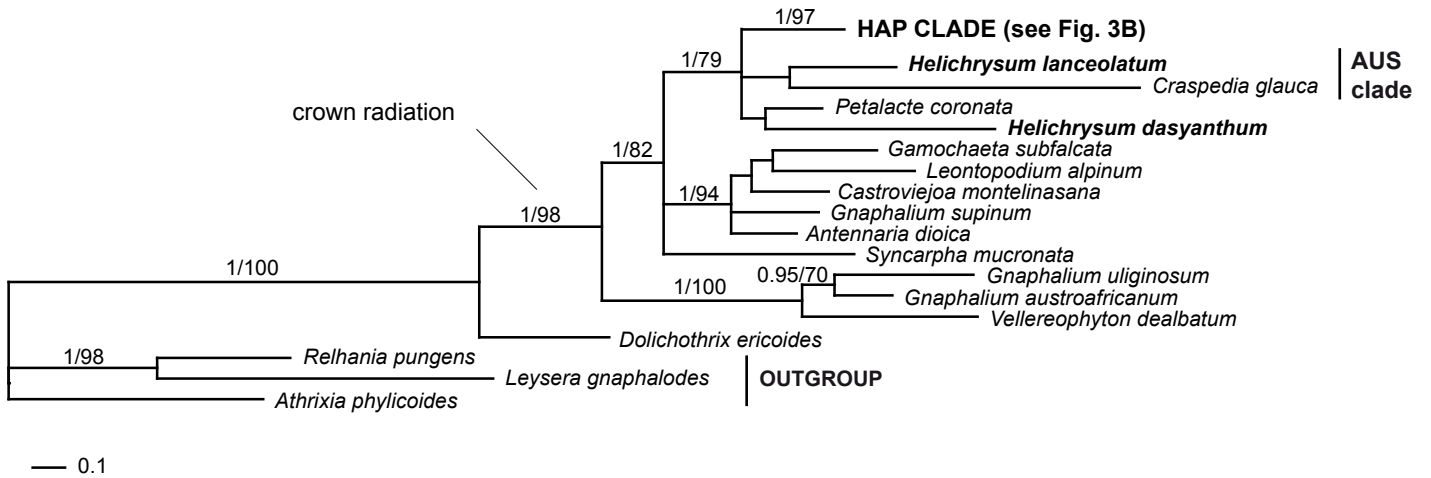
1500 km



## A. nrDNA



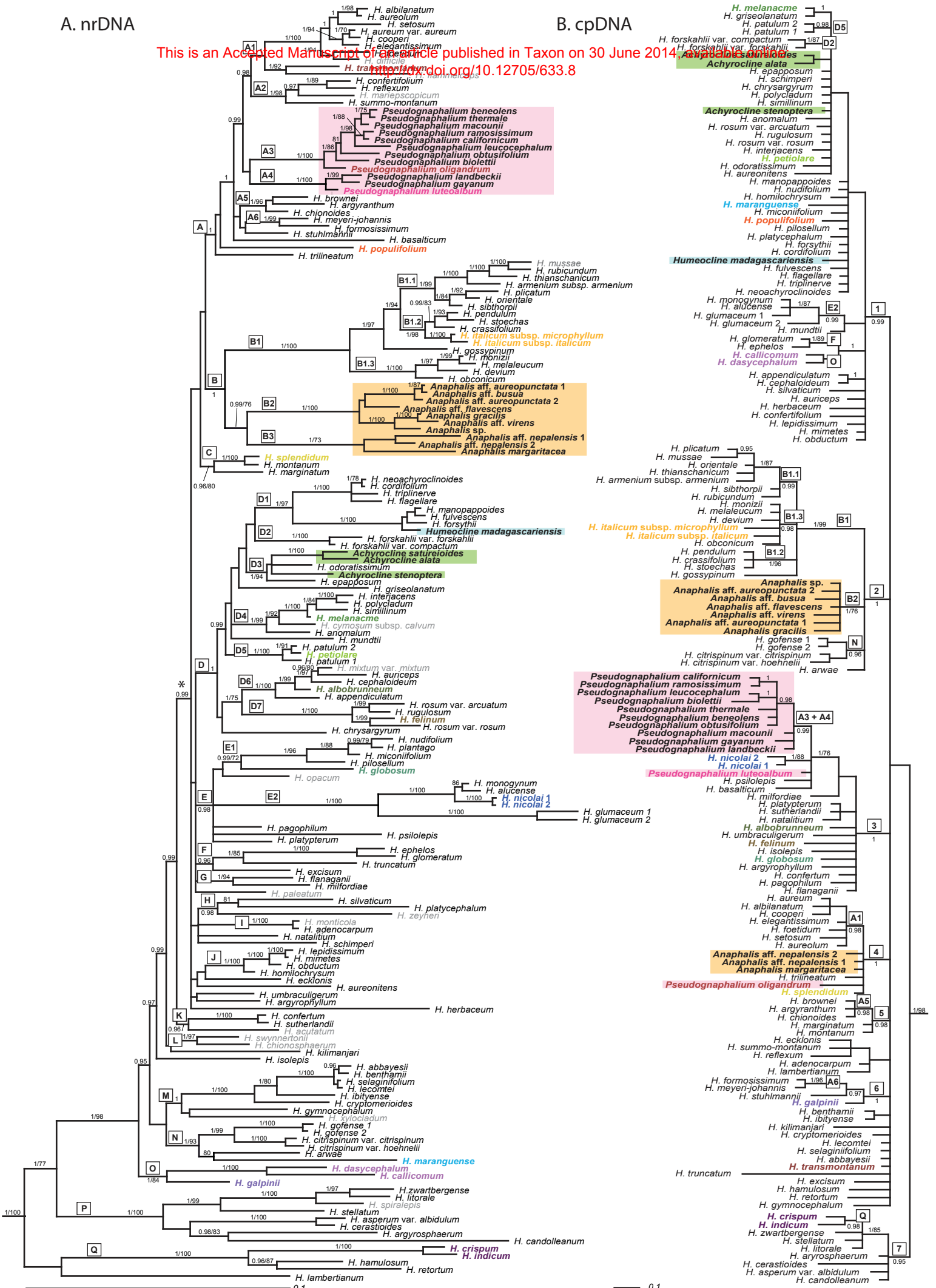
## B. cpDNA



A. nrDNA

B. cpDNA

This is an Accepted Manuscript of an article published in Taxon on 30 June 2014. <https://doi.org/10.12705/633.8>



**Geographic areas**

- 1. Mediterranean region
- 2. Macaronesian region
- 3. Irano-turanian region
- 4. Eastern and Southern Asia
- 5. South Arabian peninsula
- 6. Sahel and Somalia-Masai regions
- 7. Afroalpine region of Central East Africa
- 8. Tropical Africa Afromontane region
- 9. Southern Africa Afromontane and Afroalpine region
- 10. Madagascar
- 11. Inner SW southern Africa (mainly Karoo)
- 12. Coastal SW southern Africa (Cape region)
- 13. N America
- 14. S America

**Morphological characters**

**Number of florets per capitulum**

- ▲ > 150
- ▲ 40-150
- ▲ 15-40
- ▲ 8-20
- ▲ < 8

**Capitula sex ratio**

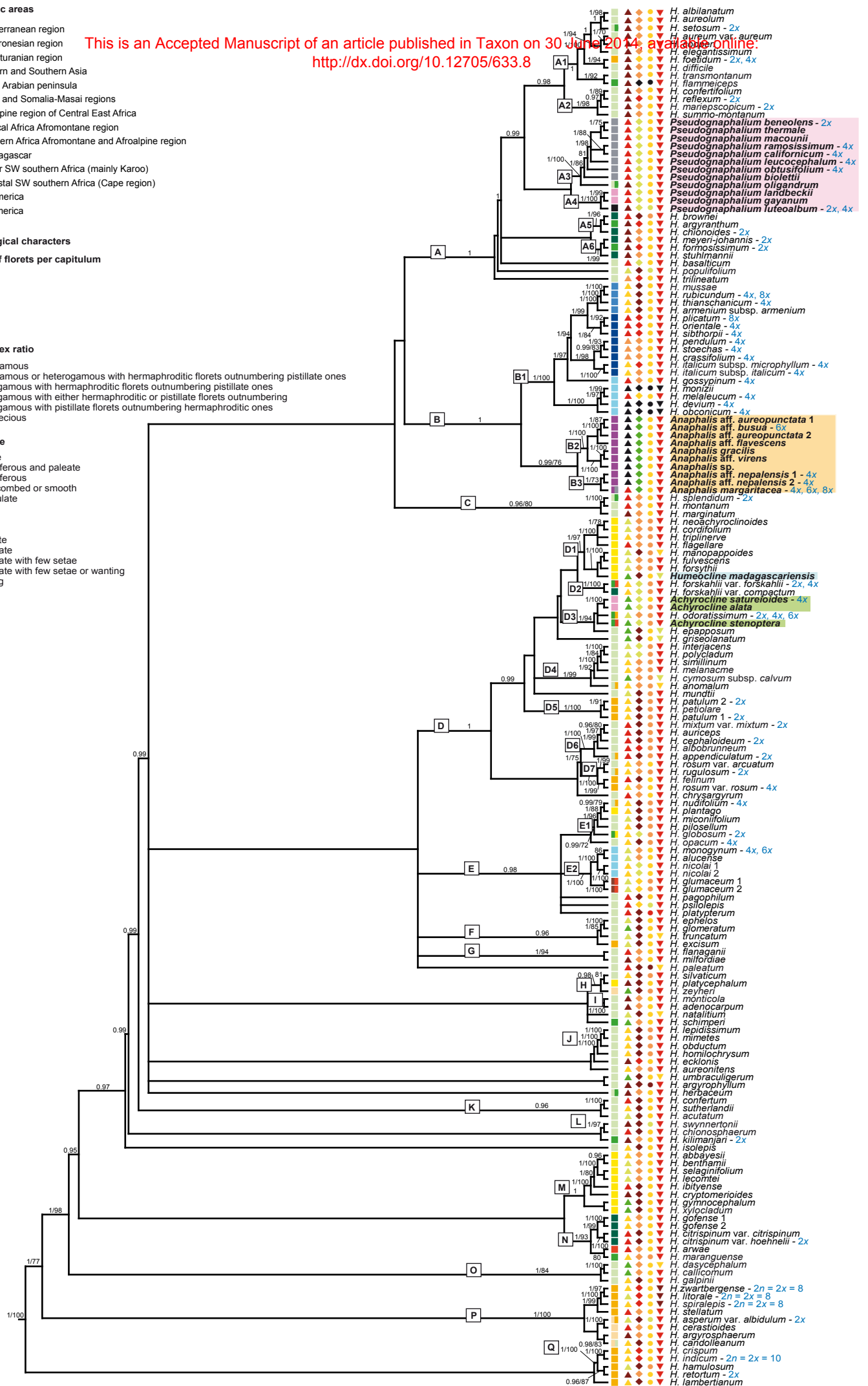
- ◆ homogamous
- ◆ homogamous or heterogamous with hermaphroditic florets outnumbering pistillate ones
- ◆ heterogamous with hermaphroditic florets outnumbering pistillate ones
- ◆ heterogamous with either hermaphroditic or pistillate florets outnumbering
- ◆ heterogamous with pistillate florets outnumbering hermaphroditic ones
- ◆ subdioecious

**Receptacle**

- paleate
- fimbriiferous and paleate
- fimbriiferous
- honeycombed or smooth
- tuberculate

**Pappus**

- ▼ biseriata
- ▼ uniseriata
- ▼ uniseriata with few setae
- ▼ uniseriata with few setae or wanting
- ▼ wanting



- H. albilanatum*
- H. aureolum*
- H. setosum* - 2x
- H. aurum* var. *aurum*
- H. elegantissimum*
- H. foetidum* - 2x, 4x
- H. officinale*
- H. transmontanum*
- H. flammeiceps*
- H. confertifolium*
- H. reflexum* - 2x
- H. mariescopium* - 2x
- H. summo-montanum*
- Pseudognaphalium beneolens* - 2x
- Pseudognaphalium thermale*
- Pseudognaphalium macounii*
- Pseudognaphalium ramosissimum* - 4x
- Pseudognaphalium californicum* - 4x
- Pseudognaphalium leucocephalum* - 4x
- Pseudognaphalium obtusifolium* - 4x
- Pseudognaphalium biolettii*
- Pseudognaphalium oligandrum*
- Pseudognaphalium landbeckii*
- Pseudognaphalium gayanum*
- Pseudognaphalium luteoalbum* - 2x, 4x
- H. brownii*
- H. argyranthum*
- H. chionoides* - 2x
- H. meyeri-johannis* - 2x
- H. formosissimum* - 2x
- H. stuhlmannii*
- H. basalticum*
- H. populifolium*
- H. trilineatum*
- H. mussae*
- H. rubicundum* - 4x, 8x
- H. thianschanicum* - 4x
- H. armenium* subsp. *armenium*
- H. plicatum* - 8x
- H. orientale* - 4x
- H. stibthorpii* - 4x
- H. pendulum* - 4x
- H. stoechas* - 4x
- H. crassifolium* - 4x
- H. italicum* subsp. *microphyllum* - 4x
- H. italicum* subsp. *italicum* - 4x
- H. gossypinum* - 4x
- H. monzii*
- H. meileucum* - 4x
- H. devium* - 4x
- H. obconicum* - 4x
- Anaphalis* aff. *aureopunctata* 1
- Anaphalis* aff. *bursus* - 6x
- Anaphalis* aff. *aureopunctata* 2
- Anaphalis* aff. *flavescens*
- Anaphalis gracilis*
- Anaphalis* aff. *virens*
- Anaphalis* sp.
- Anaphalis* aff. *nepalensis* 1 - 4x
- Anaphalis* aff. *nepalensis* 2 - 4x
- Anaphalis margaritacea* - 4x, 6x, 8x
- H. splendidum* - 2x
- H. montanum*
- H. marginatum*
- H. neochryoclinoides*
- H. cordifolium*
- H. triplinerve*
- H. flagellare*
- H. chinopappoides*
- H. fulvescens*
- H. forsythii*
- Humeocline madagascariensis*
- H. forskahlii* var. *forskahlii* - 2x, 4x
- H. forskahlii* var. *compactum*
- Achyrocline satureioides* - 4x
- Achyrocline alata*
- H. odoratissimum* - 2x, 4x, 6x
- Achyrocline stenoptera*
- H. epapposum*
- H. griseolanatum*
- H. interjacens*
- H. polydium*
- H. simillimum*
- H. melanacme*
- H. cymosum* subsp. *calvum*
- H. anomalum*
- H. mundtii*
- H. patulum* 2 - 2x
- H. petiolare*
- H. patulum* 1 - 2x
- H. mixtum* var. *mixtum* - 2x
- H. auiceps*
- H. cephaloideum* - 2x
- H. albidum*
- H. appendiculatum* - 2x
- H. rosum* var. *arcuatum*
- H. rugulosum* - 2x
- H. feinum*
- H. rosum* var. *rosum* - 4x
- H. chrysargyrum*
- H. nudifolium* - 4x
- H. plantago*
- H. miconiifolium*
- H. pilosellum*
- H. globosum* - 4x
- H. opacum* - 4x
- H. monogynum* - 4x, 6x
- H. alucense*
- H. nicolai* 1
- H. nicolai* 2
- H. glumaceum* 1
- H. glumaceum* 2
- H. pagophilum*
- H. psilolepis*
- H. platypterum*
- H. ephelos*
- H. glomeratum*
- H. truncatum*
- H. excisum*
- H. fianaganii*
- H. milfordiae*
- H. paleatum*
- H. silvaticum*
- H. platycephalum*
- H. zeyheri*
- H. monticola*
- H. adenocarpum*
- H. natalitium*
- H. schimperi*
- H. leptisissimum*
- H. mimetes*
- H. obductum*
- H. homiochrysum*
- H. ecklonis*
- H. aureonitens*
- H. umbraculigerum*
- H. argyrophyllum*
- H. herbaceum*
- H. confertum*
- H. sutherlandii*
- H. acutatum*
- H. swynnertonii*
- H. chionosphaerum*
- H. kilimanjari* - 2x
- H. isolepis*
- H. abbayesii*
- H. benthamii*
- H. selaginifolium*
- H. lecoimae*
- H. ibityense*
- H. cryptomerioides*
- H. gymnocephalum*
- H. xylocladum*
- H. gofense*
- H. gofense* 2
- H. citrispinum* var. *citrispinum*
- H. citrispinum* var. *hoehnelii* - 2x
- H. arwae*
- H. maranguense*
- H. dasycephalum*
- H. callicomum*
- H. galpini*
- H. zwanbergense* - 2n = 2x = 8
- H. littorale* - 2n = 2x = 8
- H. spiralepis* - 2n = 2x = 8
- H. stellatum*
- H. asperum* var. *albidulum* - 2x
- H. crassifolium*
- H. argyrosphaerum*
- H. cardolleianum*
- H. crispum*
- H. indicum* - 2n = 2x = 10
- H. hamulosum*
- H. retortum* - 2x
- H. lambertianum*

**Appendix 1.** Species included in the molecular analyses with voucher information and European Nucleotide Archive accession numbers (ITS, ETS, *rpl32-trnL* intergenic spacer, *ndhF* gene). Newly generated sequences are marked with an asterisk (\*) and a dash (--) indicates missing sequences.

*Achyrocline alata* DC.; Brasil, Rio Grande do Sul, São Francisco de Paula, *Grazziotin 8941 & al.* (W 1995-4937); \*HG797714, \*HG797978, \*HG798135, \*HG797824. *Achyrocline satureioides* (Lam.) DC.; Argentina, Entre Ríos, Departamento Colón, *D. G. Gutiérrez 658* (LP); \*HG797713, \*HG797976, \*HG798133, \*HG797822. *Achyrocline stenoptera* (DC.) Hilliard & B. L. Burt; Tanzania, Olmoti, *Galbany & Arrabal s. n.* (BC 867819); FJ211449 and FJ211507, \*HG797977, \*HG798134, \*HG797823. *Anaphalis aff. aureopunctata* Lingelsh. & Borza (1); China, Sichuan Province, *Boufford 35751 & al.* (HUH); \*HG797715, \*HG797979, \*HG798136, \*HG797825. *Anaphalis aff. aureopunctata* Lingelsh. & Borza (2); China, Sichuan Province, *Boufford 32622 & al.* (HUH); \*HG797716, \*HG797980, \*HG798137, \*HG797826. *Anaphalis aff. busua* DC.; China, Sichuan Province, *Boufford 37045 & al.* (HUH); \*HG797717, \*HG797981, \*HG798138, \*HG797827. *Anaphalis aff. flavescens* Hand.-Mazz.; China, Xizang (Tibet) Province, *Boufford 31246 & al.* (HUH); \*HG797718, \*HG797982, \*HG798139, \*HG797828. *Anaphalis gracilis* Hand.-Mazz.; China, Sichuan Province, *Boufford 34675 & al.* (HUH); \*HG797719, \*HG797983, \*HG798140, \*HG797829. *Anaphalis margaritacea* (L.) Benth. & Hook. f.; Canada, West, *J. M. Blanco & E. Blanco s. n.* (BC); FN645827, FN645632, FN649352, HM445660. *Anaphalis aff. nepalensis* (Spreng.) Hand.-Mazz. (1); China, Sichuan Province, *Boufford 36783 & al.* (HUH); \*HG797720, \*HG797984, \*HG798141, \*HG797830. *Anaphalis aff. nepalensis* (Spreng.) Hand.-Mazz. (2); China, Xizang (Tibet) Province, *Boufford 31830 & al.* (HUH); \*HG797721, \*HG797985, \*HG798142, \*HG797831. *Anaphalis aff. virens* C. C. Chang; China, Xizang (Tibet) Province, *Boufford 31568 & al.* (HUH); \*HG797722, \*HG797986, \*HG798143, \*HG797832. *Anaphalis sp.*; China, Sichuan Province, *Boufford 32750 & al.* (HUH); \*HG797723, \*HG797987, \*HG798144, \*HG797833. *Antennaria dioica* (L.) Gaertn.; Spain, Huesca, *Santos-Vicente & al. MS 428* (SALA); FN645833, FN645610, FN649336, HM445686. *Athrixia phyllicoides* DC.; South Africa, Eastern Cape Province, *Romo 14395 & al.* (BC); FN645816, FN645634, FN649330, HM445664. *Castroviejoa montelinasana* (Schmid) Galbany, L. Sáez & Benedí; Italy, Sardinia, Monte Línas, *Galbany & Sáez s. n.* (BCN 4644); AY445229, FN645559, FN649341, HM445687. *Craspedia glauca* Spreng.; Australia, Tasmania, Eaglehawk Neck, *Ford & Purves 21/03* (CHR 565520); EF187655, EF187629, --, --. *Dolichotheix ericoides* (Lam.) Hilliard & B. L. Burt; South Africa, Western Cape Province, *Romo 14514 & al.* (BC); FN645828, FN645622, FN649332, HM445665. *Ewartia catipes* Beauverd; Australia, Tasmania, Ben Lomond, *Ward 94098/9* (CANU 37226); U95290, FJ404694, --, --. *Gamochoeta subfalcata* (Cabrera) Cabrera; Spain, Girona, *Galbany & al. s. n.* (BCN); FN645834, FN645557, FN649338, HM445688. *Gnaphalium austroafricanum* Hilliard; South Africa, Kwazulu-Natal Province, *Romo 14365 & al.* (BC); FN645830, FN645630, FN649353, HM445679. *Gnaphalium supinum* L.; Andorra, Port Creussans, *Galbany & Lluent s. n.* (BCN 6121); AY445230, FN645558, FN649354, HM445683. *Gnaphalium uliginosum* L.; Armenia, Shirak Province, *Vitek & al. s. n.* (BCN 39933); FN645823, FN645624, FN649359, HM445680. *Helichrysum abbayesii* Humbert; Madagascar, Fianarantsoa Province, Maharumona road, *Bayer MAD-04026 & al.* (CANB 660363); \*HG797724, \*HG797988, \*HG798145, \*HG797834. *Helichrysum acutatum* DC.; South Africa, Mpumalanga Province, *Romo 14596 & al.* (BC 867799); FJ211457 and FJ211515, \*HG797989, --, --. *Helichrysum adenocarpum* DC.; South Africa, Mpumalanga Province, *Romo 14611 & al.* (BC 867814); \*HG797725, \*HG797990, \*HG798146, \*HG797835. *Helichrysum albilanatum* Hilliard; South Africa, Mpumalanga

Province, *Romo 14603 & al.* (BC 867806); \*HG797726, \*HG797991, \*HG798147, \*HG797836. *Helichrysum albobrunneum* S. Moore; Lesotho, ex Roy. Bot. Gard. Kew (BCN 6097); AY445215, \*HG797992, \*HG798148, \*HG797837. *Helichrysum alucense* García-Cas., S. Scholz & E. Hernández; Spain, Canary Islands, La Gomera, *García Casanova s. n.* (TFC 36682); AY445223, \*HG797993, \*HG798149, \*HG797838. *Helichrysum anomalum* Less.; South Africa, Eastern Cape Province, *Romo 14463 & al.* (BC 867691); \*HG797727, \*HG797994, \*HG798150, \*HG797839. *Helichrysum appendiculatum* (L. f.) Less.; South Africa, Kwazulu-Natal Province, *Romo 14362 & al.* (BC 867635); \*HG797728, \*HG797995, \*HG798151, \*HG797840. *Helichrysum argyranthum* O. Hoffm.; Tanzania, Olmoti, *Galbany & Arrabal s. n.* (BC 867822); FJ211465 and FJ211523, \*HG797996, \*HG798152, \*HG797841. *Helichrysum argyrophyllum* DC.; South Africa, Eastern Cape Province, *Romo 14472 & al.* (BC 867699); \*HG797729, \*HG797997, \*HG798153, \*HG797842. *Helichrysum argyrosphaerum* DC.; South Africa, Free State Province, *Koekemoer 3532* (BC); \*HG797730, \*HG797998, \*HG798154, \*HG797843. *Helichrysum armenium* DC. **subsp. armenium**; Turkey, Adiyaman, *Susanna 2346 & al.* (BCN 6127); AY445208, FJ211577, \*HG798155, \*HG797844. *Helichrysum arwae* J. R. I. Wood; Yemen, ex Roy. Bot. Gard. Kew (BCN 6103); AY445219, \*HG797999, \*HG798156, \*HG797845. *Helichrysum asperum* (Thunb.) Hilliard & B. L. Burtt **var. albidulum** (DC.) Hilliard; South Africa, Western Cape Province, *Romo 14526 & al.* (BC 867744); FJ211470 and FJ211528, \*HG798000, \*HG798157, \*HG797846. *Helichrysum aureolum* Hilliard; South Africa, Mpumalanga Province, *Koekemoer 3494* (BC); \*HG797731, \*HG798001, \*HG798158, \*HG797847. *Helichrysum aureonitens* Sch. Bip.; South Africa, Mpumalanga Province, *Romo 14579 & al.* (BC 867786); \*HG797732, \*HG798002, \*HG798159, \*HG797848. *Helichrysum aureum* (Houtt.) Merrill **var. aureum**; South Africa, Eastern Cape Province, *Romo 14414 & al.* (BC 867667); \*HG797733, \*HG798003, \*HG798160, \*HG797849. *Helichrysum auriceps* Hilliard; South Africa, Kwazulu-Natal Province, *Romo 14371 & al.* (BC 867644); \*HG797734, \*HG798004, \*HG798161, \*HG797850. *Helichrysum basalticum* Hilliard; Lesotho, ex Roy. Bot. Gard. Kew (BCN 6095); AY445211, \*HG798005, \*HG798162, \*HG797851. *Helichrysum benthamii* R. Vig. & Humbert; Madagascar, Fianarantsoa Province, Massif de Itremo, *Bayer MAD-04031 & al.* (CANB 660368); \*HG797735, \*HG798006, \*HG798163, \*HG797852. *Helichrysum brownei* S. Moore; Kenya, Mount Kenya, *Galbany & Arrabal s. n.* (BC 867843); FJ211464 and FJ211522, \*HG798007, \*HG798164, \*HG797853. *Helichrysum callicomum* Harv.; South Africa, Free State Province, *Romo 14342 & al.* (BC 867623); FJ211473 and FJ211531, \*HG798008, \*HG798165, \*HG797854. *Helichrysum candolleianum* Buek; Mozambique, Gaza Province, *Burrows 8560* (Buffelskloof herb.); \*HG797736, \*HG798009, \*HG798166, \*HG797855. *Helichrysum cephaloideum* DC.; South Africa, Mpumalanga Province, *Romo 14578 & al.* (BC 867785); \*HG797737, \*HG798010, \*HG798167, \*HG797856. *Helichrysum cerastioides* DC.; South Africa, Mpumalanga Province, *Burrows 8508* (Buffelskloof herb.); \*HG797738, \*HG798011, \*HG798168, \*HG797857. *Helichrysum chionoides* Philipson; Kenya, Mount Kenya, *Galbany & Arrabal s. n.* (BC 867840); FJ211463 and FJ211521, \*HG798012, \*HG798169, \*HG797858. *Helichrysum chionosphaerum* DC.; South Africa, Eastern Cape Province, *Romo 14450 & al.* (BC 867682); FJ211461 and FJ211519, \*HG798013, --, --. *Helichrysum chrysargyrum* Moeser; South Africa, Mpumalanga Province, *McMurtry 8871* (Buffelskloof herb.); \*HG797739, \*HG798014, \*HG798170, \*HG797859. *Helichrysum citrispinum* Del. **var. citrispinum**; Ethiopia, Mount Choke, *Aldasoro 9952 & Alarcón* (BC); \*HG797740, \*HG798015, \*HG798171, \*HG797860. *Helichrysum citrispinum* Del. **var. hoehnelii** (Schweinf.) Hedberg; Ethiopia, Bale Mountains plateau, *Aldasoro 10378 & Alarcón* (BC); \*HG797741, \*HG798016, \*HG798172, \*HG797861. *Helichrysum confertifolium* Klatt; South Africa, Mpumalanga Province, *Romo 14599 & al.*

(BC 867802); \*HG797742, \*HG798017, \*HG798173, \*HG797862. *Helichrysum confertum* N. E. Br.; Ex Roy. Bot. Gard. Kew (BCN 6096); AY445214, \*HG798018, \*HG798174, \*HG797863. *Helichrysum cooperi* Harv.; South Africa, Free State Province, *Romo 14345 & al.* (BC 867626); \*HG797743, \*HG798019, \*HG798175, \*HG797864. *Helichrysum cordifolium* DC.; Madagascar, Antananarivo Province, *Bayer MAD-04003 & al.* (CANB 660340); \*HG797744, \*HG798020, \*HG798176, \*HG797865. *Helichrysum crassifolium* (L.) D. Don; Spain, Balearic Islands, Majorca, *Galbany & Sáez s. n.* (BCN 6117); AY445190, FJ211540, \*HG798177, \*HG797866. *Helichrysum cryptomerioides* Baker; Madagascar, Fianarantsoa Province, Massif de Itremo, *Bayer MAD-04045 & al.* (CANB 660382); \*HG797745, \*HG798021, \*HG798178, \*HG797867. *Helichrysum crispum* (L.) D. Don; South Africa, Western Cape Province, *Romo 14532 & al.* (BC 867748); \*HG797746, \*HG798022, \*HG798179, \*HG797868. *Helichrysum cymosum* (L.) D. Don **subsp. calvum** Hilliard; South Africa, Eastern Cape Province, *Romo 14410 & al.* (BC 867663); \*HG797748, \*HG798024, --, --. *Helichrysum dasyanthum* (Willd.) Sweet; Ex J. Bot. Mar i Murtra Blanes (BCN 6107); AY445226, HM450869, \*HG798181, HM445678. *Helichrysum dasycephalum* O. Hoffm.; South Africa, Eastern Cape Province, *Romo 14411 & al.* (BC 867664); \*HG797749, \*HG798025, \*HG798182, \*HG797870. *Helichrysum devium* J. Y. Johnson; Portugal, Madeira island, *Jardim s. n.* (MADJ); FJ211441 and FJ211499, FJ211579, \*HG798183, \*HG797871. *Helichrysum difficile* Hilliard; South Africa, Mpumalanga Province, *Romo 14588B & al.* (BC 867793); \*HG797750, \*HG798026, --, --. *Helichrysum ecklonis* Sond.; South Africa, Eastern Cape Province, *Romo 14485 & al.* (BC 867712); \*HG797751, \*HG798027, \*HG798184, \*HG797872. *Helichrysum elegantissimum* DC.; South Africa, Eastern Cape Province, *Romo 14435 & al.* (BC 867676); \*HG797752, \*HG798028, \*HG798185, \*HG797873. *Helichrysum epapposum* H. Bol.; South Africa, Mpumalanga Province, *Romo 14581 & al.* (BC 867788); FJ211459 and FJ211517, \*HG798029, \*HG798186, \*HG797874. *Helichrysum ephelos* Hilliard; South Africa, Mpumalanga Province, *Romo 14601 & al.* (BC 867804); \*HG797753, \*HG798030, \*HG798187, \*HG797875. *Helichrysum excisum* (Thunb.) Less.; South Africa, Western Cape Province, *Koekemoer 3433* (BC); \*HG797754, \*HG798031, \*HG798188, \*HG797876. *Helichrysum felinum* Less.; South Africa, Eastern Cape Province, *Romo 14469 & al.* (BC 867696); \*HG797755, \*HG798032, \*HG798189, \*HG797877. *Helichrysum flagellare* Bak; Madagascar, Antananarivo Province, *Bayer MAD04004 & al.* (CANB 660341); \*HG797756, \*HG798033, \*HG798190, \*HG797878. *Helichrysum flammeiceps* Brenan; Malawi, Nyika National Park, *Koekemoer 1854* (BC); \*HG797757, \*HG798034, --, --. *Helichrysum flanaganii* H. Bol.; South Africa, Eastern Cape Province, *Romo 14424 & al.* (BC 867673); \*HG797758, \*HG798035, \*HG798191, \*HG797879. *Helichrysum foetidum* (L.) Moench; Ex Dresden Bot. Gard. (BCN 8219); AY445221, \*HG798036, \*HG798192, HM445667. *Helichrysum formosissimum* (Sch. Bip.) A. Rich.; Kenya, Mount Kenya, *Galbany & Arrabal s. n.* (BC 867832); FJ211446 and FJ211504, \*HG798037, \*HG798193, \*HG797880. *Helichrysum forskahlii* (J. F. Gmel.) Hilliard & B. L. Burtt **var. compactum** (Vatke) Mesfin; Kenya, Mount Kenya, *Galbany & Arrabal s. n.* (BC 867830); \*HG797759, \*HG798038, \*HG798194, \*HG797881. *Helichrysum forskahlii* (J. F. Gmel.) Hilliard & B. L. Burtt **var. forskahlii**; Tanzania, Empaakai, *Galbany & Arrabal s. n.* (BC 867823); FJ211447 and FJ211505, \*HG798039, \*HG798195, \*HG797882. *Helichrysum forsythii* Humbert; Madagascar, Fianarantsoa Province, 30 km from Ivato, *Bayer MAD-04021 & al.* (CANB 660358); \*HG797760, \*HG798040, \*HG798196, \*HG797883. *Helichrysum fulvescens* DC.; Madagascar, Antananarivo Province, *Bayer MAD04001 & al.* (CANB 660338); \*HG797761, \*HG798041, \*HG798197, \*HG797884. *Helichrysum galpinii* N. E. Br.; South Africa, Mpumalanga Province, *Romo 14569 & al.* (BC 867776); \*HG797762, \*HG798042, \*HG798198, \*HG797885. *Helichrysum globosum* A. Rich.; Kenya, Mount Kenya, *Galbany*

& *Arrabal s. n.* (BC 867838); FJ211454 and FJ211512, \*HG798043, \*HG798199, \*HG797886. *Helichrysum glomeratum* Klatt; South Africa, Kwazulu-Natal Province, *Romo 14390 & al.* (BC 867657); FJ211474 and FJ211532, \*HG798044, \*HG798200, \*HG797887. *Helichrysum glumaceum* DC. (1); Oman, Musadam, *McLeish 3727* (E 121502); FJ211469 and FJ211527, \*HG798045, \*HG798201, \*HG797888. *Helichrysum glumaceum* DC. (2); Ethiopia, SW of Negele, *Aldasoro 10262 & Alarcón* (BC); \*HG797763, \*HG798046, \*HG798202, \*HG797889. *Helichrysum gofense* Cufod. (1); Ethiopia, Bale Mountains plateau, *Aldasoro 10336 & Alarcón* (BC); \*HG797764, \*HG798047, \*HG798203, \*HG797890. *Helichrysum gofense* Cufod. (2); Ethiopia, Bale Mountains plateau, *Aldasoro 10376 & Alarcón* (BC); \*HG797765, \*HG798048, \*HG798204, \*HG797891. *Helichrysum gossypinum* Sch. Bip.; Spain, Canary Islands, Lanzarote, *Galbany & Arrabal s. n.* (BCN 25226); FJ211440 and FJ211498, FJ211578, \*HG798205, \*HG797892. *Helichrysum griseolanatum* Hilliard; South Africa, Eastern Cape Province, *Romo 14413 & al.* (BC 867666); FJ211476 and FJ211534, \*HG798049, \*HG798206, \*HG797893. *Helichrysum gymnocephalum* (DC.) Humbert; Madagascar, Antananarivo Province, Mt. Ibity, *Bayer MAD-04053 & al.* (CANB 660390); \*HG797766, \*HG798050, \*HG798207, \*HG797894. *Helichrysum hamulosum* [E. Mey. ex] DC.; South Africa, Western Cape Province, *Romo 14540 & al.* (BC 867751); \*HG797767, \*HG798051, \*HG798208, \*HG797895. *Helichrysum herbaceum* (Andr.) Sweet; South Africa, Kwazulu-Natal Province, *Romo 14369 & al.* (BC 867642); \*HG797768, \*HG798052, \*HG798209, \*HG797896. *Helichrysum homilochrysum* S. Moore; South Africa, Mpumalanga Province, *Burrows 7748* (Buffelskloof herb.); \*HG797769, \*HG798053, \*HG798210, \*HG797897. *Helichrysum ibityense* Humbert; Madagascar, Fianarantsoa Province, Ambatosira, *Bayer MAD-04052 & al.* (CANB 660389); \*HG797770, \*HG798054, \*HG798211, \*HG797898. *Helichrysum indicum* (L.) Grierson; South Africa, Western Cape Province, *Romo 14547 & al.* (BC 867758); \*HG797771, \*HG798055, \*HG798212, \*HG797899. *Helichrysum interjacens* Hilliard; South Africa, Mpumalanga Province, *Romo 14570 & al.* (BC 867777); \*HG797772, \*HG798056, \*HG798213, \*HG797900. *Helichrysum isolepis* H. Bol.; South Africa, Eastern Cape Province, *Romo 14477 & al.* (BC 867704); \*HG797773, \*HG798057, \*HG798214, \*HG797901. *Helichrysum italicum* (Roth) G. Don **subsp. italicum**; Bosnia-Herzegovina, Herzegovina, *Redžić & al. s. n.* (BCN 20756); FJ211422 and FJ211480, FJ211548, \*HG798215, \*HG797902. *Helichrysum italicum* (Roth) G. Don **subsp. microphyllum** (Willd.) Nyman; Spain, Balearic Islands, Majorca, *Galbany & Sáez s. n.* (BCN 6115); AY445195, FJ211546, \*HG798216, \*HG797903. *Helichrysum kilimanjari* Oliv.; Kenya, Mount Kenya, *Galbany & Arrabal s. n.* (BC 867836); FJ211453 and FJ211511, \*HG798058, \*HG798217, \*HG797904. *Helichrysum lambertianum* DC.; South Africa, Western Cape Province, *Romo 14556 & al.* (BC 867767); FJ211472 and FJ211530, \*HG798059, \*HG798218, \*HG797905. *Helichrysum lanceolatum* (Buchanan) Kirk; New Zealand, Nelson, Pelorus Valley, *Glenny 8969* (CHR 574730); HM244720, HM450855, \*HG798219, HM445692. *Helichrysum lecomtei* R. Vig. & Humbert; Madagascar, Fianarantsoa Province, Analamay, *Bayer MAD-04016 & al.* (CANB 660353); \*HG797774, \*HG798060, \*HG798220, \*HG797906. *Helichrysum lepidissimum* S. Moore; South Africa, Mpumalanga Province, *Burrows s. n.* (Buffelskloof herb.); \*HG797775, \*HG798061, \*HG798221, \*HG797907. *Helichrysum litorale* H. Bol.; South Africa, Eastern Cape Province, *Romo 14500 & al.* (BC 867722); HM244706, \*HG798062, \*HG798222, HM445669. *Helichrysum manopappoides* Humbert; Madagascar, Fianarantsoa Province, *Bayer MAD-04023 & al.* (CANB 660360); \*HG797776, \*HG798063, \*HG798223, \*HG797908. *Helichrysum maranguense* O. Hoffm.; Tanzania, Empaakai, *Galbany & Arrabal s. n.* (BC 867825); FJ211452 and FJ211510, \*HG798064, \*HG798224, \*HG797909. *Helichrysum marginatum* DC.; South Africa, Eastern Cape Province, *Romo 14434 & al.* (BC 867675); FJ211460 and

FJ211518, \*HG798065, \*HG798225, \*HG797910. *Helichrysum mariepsopicum* Hilliard; South Africa, Mpumalanga Province, *Romo 14592 & al.* (BC 867795); \*HG797777, \*HG798066, --, --. *Helichrysum melaleucum* Rchb. ex Holl; Portugal, Madeira island, *Jardim s. n.* (MADJ); FJ211443 and FJ211501, FJ211581, \*HG798226, \*HG797911. *Helichrysum melanacme* DC.; South Africa, Free State Province, *Romo 14334 & al.* (BC 867616); \*HG797778, \*HG798067, \*HG798227, \*HG797912. *Helichrysum meyeri-johannis* Engl.; Kenya, ex Roy. Bot. Gard. Kew (BCN 6104); AY445216, \*HG798068, \*HG798228, \*HG797913. *Helichrysum miconiifolium* DC.; South Africa, Free State Province, *Romo 14348 & al.* (BC 867629); \*HG797779, \*HG798069, \*HG798229, \*HG797914. *Helichrysum milfordiae* Killick; Lesotho, ex Roy. Bot. Gard. Kew (BCN 6101); AY445212, FJ211537, \*HG798230, \*HG797915. *Helichrysum mimetes* S. Moore; South Africa, Mpumalanga Province, *Romo 14610 & al.* (BC 867813); FJ211462 and FJ211520, \*HG798070, \*HG798231, \*HG797916. *Helichrysum mixtum* (O. Kuntze) Moeser var. *mixtum*; South Africa, Kwazulu-Natal Province, *Romo 14374 & al.* (BC 867647); \*HG797780, \*HG798071, --, --. *Helichrysum monizii* Lowe; Portugal, Madeira island, *Jardim s. n.* (MADJ); FJ211444 and FJ211502, FJ211582, \*HG798232, \*HG797917. *Helichrysum monogynum* B. L. Burtt & Sunding; Spain, Canary Islands, Lanzarote, *Galbany & Arrabal s. n.* (BCN 25227); FJ211468 and FJ211526, \*HG798072, \*HG798233, \*HG797918. *Helichrysum montanum* DC.; South Africa, Kwazulu-Natal Province, *Romo 14392 & al.* (BC 867659); \*HG797781, \*HG798073, \*HG798234, \*HG797919. *Helichrysum monticola* Hilliard; South Africa, Mpumalanga Province, *Romo 14591 & al.* (BC 867794); \*HG797782, \*HG798074, --, --. *Helichrysum mundtii* Harv.; South Africa, Kwazulu-Natal Province, *Romo 14368 & al.* (BC 867641); \*HG797783, \*HG798075, \*HG798235, \*HG797920. *Helichrysum mussae* Nevski; Tadjikistan, Zeravshchan Mts., *Filatov & al. 81* (LE); FJ211426 and FJ211484, FJ211555, --, --. *Helichrysum natalitium* DC.; South Africa, Kwazulu-Natal Province, *Burrows 8431* (Buffelskloof herb.); \*HG797784, \*HG798076, \*HG798236, \*HG797921. *Helichrysum neoachyroclinoides* Humbert; Madagascar, Antananarivo Province, Mt. Ibity, Bayer *MAD-04067 & al.* (CANB 660404); \*HG797785, \*HG798077, \*HG798237, \*HG797922. *Helichrysum nicolai* N. Kilian, Galbany & Oberpr. (1); Cape Verde, São Nicolau, Alto das Cabaças, *Galbany 2111-3 & Molero* (BC); FN691030, \*HG798078, \*HG798238, \*HG797923. *Helichrysum nicolai* N. Kilian, Galbany & Oberpr. (2); Cape Verde, São Nicolau, Alto das Cabaças, *Galbany 2111-17 & Molero* (BC); FN691031, \*HG798079, \*HG798239, \*HG797924. *Helichrysum nudifolium* (L.) Less.; Kenya, Mount Kenya, *Galbany & Arrabal s. n.* (BC 867834); FJ211456 and FJ211514, \*HG798080, \*HG798240, \*HG797925. *Helichrysum obconicum* DC.; Portugal, Madeira island, *Jardim s. n.* (MADJ); FJ211442 and FJ211500, FJ211580, \*HG798241, \*HG797926. *Helichrysum obductum* H. Bol.; South Africa, Mpumalanga Province, *Romo 14573 & al.* (BC 867780); \*HG797786, \*HG798081, \*HG798242, \*HG797927. *Helichrysum odoratissimum* (L.) Sweet; Tanzania, Olmoti, *Galbany & Arrabal s. n.* (BC 867820); FJ211448 and FJ211506; \*HG798082, \*HG798243, \*HG797928. *Helichrysum opacum* Klatt; South Africa, Mpumalanga Province, *Romo 14593 & al.* (BC 867796); \*HG797787, \*HG798083, --, --. *Helichrysum orientale* (L.) Gaertn.; Greece, Crete, ex Roy. Bot. Gard. Kew (BCN 6098); AY445205, FJ211567, \*HG798244, \*HG797929. *Helichrysum pagophilum* M. D. Hend.; Lesotho, ex Roy. Bot. Gard. Kew (BCN 6100); AY445217, \*HG798084, \*HG798245, \*HG797930. *Helichrysum paleatum* Hilliard; South Africa, Kwazulu-Natal Province, *Romo 14385 & al.* (BC 867652); \*HG797788, \*HG798085, --, --. *Helichrysum patulum* (L.) D. Don (1); South Africa, Western Cape Province, *Romo 14507 & al.* (BC 867729); \*HG797789, \*HG798086, \*HG798246, \*HG797931. *Helichrysum patulum* (L.) D. Don (2); South Africa, Western Cape Province, *Romo 14527 & al.* (BC 867745); \*HG797790, \*HG798087, \*HG798247, \*HG797932. *Helichrysum*



*pendulum* (C. Presl) C. Presl; Spain, Balearic islands, Ibiza, *Sáez s. n.* (BCN 6118); AY445189, FJ211539, \*HG798248, \*HG797933. *Helichrysum petiolare* Hilliard & B. L. Burt; Ex J. Bot. Mar i Murtra, Blanes (BCN 6110); AY445213, \*HG798088, \*HG798249, \*HG797934. *Helichrysum pilosellum* (L. f.) Less.; South Africa, Mpumalanga Province, *Romo 14597 & al.* (BC 867800); \*HG797791, \*HG798089, \*HG798250, \*HG797935. *Helichrysum plantago* DC.; Madagascar, Antananarivo Province, Mt. Ibity, *Bayer MAD-04062 & al.* (CANB 660399); \*HG797792, \*HG798090, --, --. *Helichrysum platycephalum* Bak; Madagascar, Fianarantsoa Province, *Bayer MAD-04022 & al.* (CANB 660359); \*HG797793, \*HG798091, \*HG798251, \*HG797936. *Helichrysum platypterum* DC.; South Africa, Kwazulu-Natal Province, *Romo 14360 & al.* (BC 867634); FJ211458 and FJ211516, \*HG798092, \*HG798252, \*HG797937. *Helichrysum plicatum* DC.; Turkey, Sivas, *Susanna 2419 et al.* (BCN 6129); AY445201, FJ211556, \*HG798253, HM445672. *Helichrysum polycladum* Klatt; South Africa, Mpumalanga Province, *Romo 14598 & al.* (BC 867801); \*HG797794, \*HG798093, \*HG798254, \*HG797938. *Helichrysum populifolium* DC. South Africa, ex Silverhill Seeds (BCN 8218); AY445210, FJ211538, \*HG798255, \*HG797939. *Helichrysum psilolepis* Harv.; South Africa, Eastern Cape Province, *Romo 14461 & al.* (BC 867689); \*HG797795, \*HG798094, \*HG798256, \*HG797940. *Helichrysum reflexum* N. E. Br.; South Africa, Mpumalanga Province, *Romo 14571 & al.* (BC 867778); FJ211445 and FJ211503, \*HG798095, \*HG798257, \*HG797941. *Helichrysum retortum* (L.) Willd.; South Africa, ex Silverhill Seeds (BCN 6112); AY445222, \*HG798096, \*HG798258, \*HG797942. *Helichrysum rosum* (Berg.) Less. var. *rosum*; South Africa, Eastern Cape Province, *Romo 14462 & al.* (BC 867690); \*HG797796, \*HG798097, \*HG798259, \*HG797943. *Helichrysum rosum* (Berg.) Less. var. *arcuatum* Hilliard; South Africa, Eastern Cape Province, *Romo 14494 & al.* (BC 867718); \*HG797797, \*HG798098, \*HG798260, \*HG797944. *Helichrysum rubicundum* (K. Koch) Bornm.; Iran, Azarbaijan, *Termeh & al. s. n.* (IRAN 35924,4); FJ211437 and FJ211495, FJ211573, \*HG798261, \*HG797945. *Helichrysum rugulosum* Less.; South Africa, Free State Province, *Romo 14331 & al.* (BC 867613); FJ211471 and FJ211529, \*HG798099, \*HG798262, \*HG797946. *Helichrysum schimperi* (A. Rich.) Moeser; Tanzania, Olmoti, *Galbany & Arrabal s. n.* (BC 867821); FJ211451 and FJ211509, \*HG798100, \*HG798263, \*HG797947. *Helichrysum selaginifolium* R. Vig. & Humbert; Madagascar, Antananarivo Province, Mt. Ibity, *Bayer MAD-04074 & al.* (CANB 660411); \*HG797798, \*HG798101, \*HG798264, \*HG797948. *Helichrysum setosum* Harv.; Tanzania, Empaakai, *Galbany & Arrabal s. n.* (BC 867824); FJ211467 and FJ211525, \*HG798102, \*HG798265, \*HG797949. *Helichrysum sibthorpii* Rouy; Greece, ex Roy. Bot. Gard. Kew (BCN 6099); AY445203, FJ211561, \*HG798266, \*HG797950. *Helichrysum silvaticum* Hilliard; Mozambique, Licuati Sand Forest, *McMurtry 11424* (Buffelskloof herb.); \*HG797799, \*HG798103, \*HG798267, \*HG797951. *Helichrysum simillinum* DC.; South Africa, Kwazulu-Natal Province, *Romo 14364 & al.* (BC 867637); \*HG797800, \*HG798104, \*HG798268, \*HG797952. *Helichrysum spiralepis* Hilliard & B. L. Burt; South Africa, Kwazulu-Natal Province, *Romo 14372 & al.* (BC 867645); FJ211477 and FJ211535, \*HG798105, --, --. *Helichrysum splendidum* (Thunb.) Less.; Ex Roy. Bot. Gard. Kew (BCN 6102); AY445218, \*HG798106, \*HG798269, \*HG797953. *Helichrysum stellatum* (L.) Less.; South Africa, Northern Cape Province, *Koekemoer 3513* (BC); \*HG797801, \*HG798107, \*HG798270, \*HG797954. *Helichrysum stoechas* (L.) Moench; Spain, Lleida, *Galbany s. n.* (BCN 6114); AY445193, FJ211543, FN649351, \*HG797955. *Helichrysum stuhlmannii* O. Hoffm.; Uganda, Rwenzori Mts., *Roquet s. n.* (BC 867841); FJ211466 and FJ211524, \*HG798108, \*HG798271, \*HG797956. *Helichrysum summo-montanum* Verdoorn; South Africa, Mpumalanga Province, *Burrows 7370* (Buffelskloof herb.); \*HG797802, \*HG798109, \*HG798272, \*HG797957. *Helichrysum sutherlandii* Harv.; South Africa, Kwazulu-Natal Province, *Romo 14370 & al.*

(BC 867643); \*HG797803, \*HG798110, \*HG798273, \*HG797958. *Helichrysum swynnertonii* S. Moore; South Africa, Mpumalanga Province, *Camacho s. n.* (Buffelskloof herb.); \*HG797804, \*HG798111, --, --. *Helichrysum thianschanicum* Regel; Ex Hortus Botanicus Táhor (BCN 10337); AY445200, FJ211554, \*HG798274, \*HG797959. *Helichrysum transmontanum* Hilliard; South Africa, Mpumalanga Province, *Burrows 7543* (Buffelskloof herb.); \*HG797805, \*HG798112, \*HG798275, \*HG797960. *Helichrysum trilineatum* DC.; South Africa, Eastern Cape Province, *Romo 14416 & al.* (BC 867669); \*HG797806, \*HG798113, \*HG798276, \*HG797961. *Helichrysum triplinerve* DC.; Madagascar, Fianarantsoa Province, *Bayer MAD-04057 & al.* (CANB 660394); \*HG797807, \*HG798114, \*HG798277, \*HG797962. *Helichrysum truncatum* Burt Davy; South Africa, Mpumalanga Province, *Romo 14574 & al.* (BC 867781); \*HG797808, \*HG798115, \*HG798278, \*HG797963. *Helichrysum umbraculigerum* Less.; South Africa, Kwazulu-Natal Province, *Romo 14366 & al.* (BC 867639); FJ211450 and FJ211508, \*HG798116, \*HG798279, \*HG797964. *Helichrysum xylocladum* Humbert; Madagascar, Antananarivo Province, Mt. Ibity, *Bayer MAD-04073 & al.* (CANB 660410); \*HG797809, \*HG798117, --, --. *Helichrysum zeyheri* Less.; South Africa, Western Cape Province, *Romo 14542 & al.* (BC 867754); FJ211478 and FJ211536, \*HG798118, --, --. *Helichrysum zwartbergense* H. Bol.; South Africa, Western Cape Province, *Romo 14520 & al.* (BC 867739); HM244707, \*HG798119, \*HG798280, HM445668. *Humeocline madagascariensis* (Humb.) Anderb.; Madagascar, Antananarivo Province, Mt. Ibity, *Bayer MAD-04061 & al.* (CANB 660398); \*HG797810, \*HG798120, \*HG798281, \*HG797965. *Langebergia canescens* (DC.) Anderb.; South Africa, Western Cape Province, *Bayer & Puttock s. n.* (CANB 499988); \*HG797811, \*HG798121, --, --. *Leontopodium alpinum* Cass.; Spain, Huesca, Posets, *Roquet s. n.* (BC); Spain, Huesca, Posets, *Roquet s. n.* (BC); FN645824, FN645625, FN649348, HM445682. *Leysera gnaphalodes* (L.) L.; South Africa, Western Cape Province, *Romo 14546 & al.* (BC 867757); FN645815, FN645636, FN649329, HM445663. *Ozothamnus diosmifolius* (Vent.) DC.; Australia, New South Wales, *Bayer NSW 94-006* (NSW, ALTA); HM244716, HM450851, --, --. *Petalacte coronata* (L.) D. Don; South Africa, Western Cape Province, *Bayer & Chandler SAF-01095* (CANB 634589); \*HG797812, \*HG798122, \*HG798282, \*HG797966. *Pseudognaphalium beneolens* (Davidson) Anderb.; United States of America, California, San Diego Co., *Rebman 10825* (RSA 705579); \*HG797813, \*HG798123, \*HG798283, \*HG797967. *Pseudognaphalium biolettii* Anderb.; United States of America, California, Los Angeles Co., *Sanders 25759* (RSA 712717); \*HG797814, \*HG798124, \*HG798284, \*HG797968. *Pseudognaphalium californicum* (DC.) Anderb.; United States of America, California, Riverside Co., *Boyd 10845* (RSA 701560); HM244709; \*HG798125, \*HG798285, HM445676. *Pseudognaphalium gayanum* (J. Rémy) Anderb.; Chile, region III, Coquimbo, *Carnicero-Campmany s. n.* (SI); \*HG797815, \*HG798126, \*HG798286, \*HG797969. *Pseudognaphalium landbeckii* Phil.; Chile, region VI, Pichilemu, *Carnicero-Campmany s. n.* (SI); \*HG797816, \*HG798127, \*HG798287, \*HG797970. *Pseudognaphalium leucocephalum* (A. Gray) Anderb.; United States of America, California, Orange Co., *Roberts 6088* (RSA 703730); \*HG797817, \*HG798128, \*HG798288, \*HG797971. *Pseudognaphalium luteoalbum* (L.) Hilliard & B. L. Burt; Portugal, Marinha Grande, *Susanna 2435 & Garcia-Jacas* (BCN 6125); AY445227, FN645633, FN649358, HM445674. *Pseudognaphalium macounii* (Greene) Kartesz; United States of America, Utah, Daggett Co., *Goodrich 22823* (RSA 490532); \*HG797821, \*HG798132, \*HG798293, \*HG797975. *Pseudognaphalium oligandrum* (DC.) Hilliard & B. L. Burt; South Africa, Free State Province, *Romo 14336 & al.* (BC 867618); HM244708, \*HM450843, \*HG798289, HM445675. *Pseudognaphalium obtusifolium* (L.) Hilliard & B. L. Burt; United States of America, Iowa, Lucas Co., *Neese 11074* (RSA 299987); \*HG797818, \*HG798129, \*HG798290, \*HG797972. *Pseudognaphalium ramosissimum* (Nutt.) Anderb.; United States

of America, California, San Diego Co., *Sanders 31665* (RSA 713933); \*HG797819, \*HG798130, \*HG798291, \*HG797973. *Pseudognaphalium thermale* (E. E. Nelson) G. L. Nesom; United States of America, California, T26N, R11E, *Ahart 12405* (RSA 716686); \*HG797820, \*HG798131, \*HG798292, \*HG797974. *Raoulia tenuicaulis* Hook.f.; New Zealand, Gisborne, mouth of Motu River, *Smitten s. n.* (CHR 607934); HM244728, HM450865, --, --. *Relhania pungens* L'Hérit.; South Africa, Western Cape Province, *Koekemoer 3427* (BC); FN645814; FN645635; FN649331, HM445662. *Syncarpha mucronata* (P. J. Bergius) B. Nord.; South Africa, Western Cape Province, *Romo 14511 & al.* (BC 867732); FJ211421 and FJ211479, FN645626, FN649360, HM445677. *Vellereophyton dealbatum* (Thunb.) Hilliard & B. L. Burtt; South Africa, Western Cape Province, *Romo 14549 & al.* (BC); FN645832, FN645631, FN649355, HM445681.

**Table S1.** Summary of available chromosome counts for species of the HAP clade included in this study. See the main text for sources of data.

<b>Taxon</b>	<b>Chromosome number</b>
<i>Achyrocline alata</i> DC.	-
<i>Achyrocline satureioides</i> (Lam.) DC.	$n = 14$ ; $2n = 24, 28$
<i>Achyrocline stenoptera</i> (DC.) Hilliard & B. L. Burt	-
<i>Anaphalis aureopunctata</i> Lingelsh. & Borza	-
<i>Anaphalis busua</i> DC.	$n = 21$
<i>Anaphalis flavescens</i> Hand.-Mazz.	-
<i>Anaphalis gracilis</i> Hand.-Mazz.	-
<i>Anaphalis margaritacea</i> (L.) Benth. & Hook. f.	$n = 14, 21, 28$ ; $2n = 26, 27, 28, 56$
<i>Anaphalis nepalensis</i> (Spreng.) Hand.-Mazz.	$n = 14$
<i>Anaphalis virens</i> C. C.Chang	-
<i>Helichrysum abbayesii</i> Humbert	-
<i>Helichrysum acutatum</i> DC.	-
<i>Helichrysum adenocarpum</i> DC.	-
<i>Helichrysum albilanatum</i> Hilliard	-
<i>Helichrysum albobrunneum</i> S. Moore	-
<i>Helichrysum alucense</i> García-Cas., S. Scholz & E. Hernández	-
<i>Helichrysum anomalum</i> Less.	-
<i>Helichrysum appendiculatum</i> (L. f.) Less.	$2n = 14$
<i>Helichrysum argyranthum</i> O. Hoffm.	-
<i>Helichrysum argyrophyllum</i> DC.	-
<i>Helichrysum argyrosphaerum</i> DC.	-
<i>Helichrysum armenium</i> DC. subsp. <i>armenium</i>	-
<i>Helichrysum arwae</i> J. R. I. Wood	-
<i>Helichrysum asperum</i> (Thunb.) Hilliard & B. L. Burt var. <i>albidulum</i> (DC.) Hilliard	$2n = 14$
<i>Helichrysum aureolum</i> Hilliard	-
<i>Helichrysum aureonitens</i> Sch. Bip.	-
<i>Helichrysum aureum</i> (Houtt.) Merrill var. <i>aureum</i>	-
<i>Helichrysum auriceps</i> Hilliard	-
<i>Helichrysum basalticum</i> Hilliard	-
<i>Helichrysum benthamii</i> R. Vig. & Humbert	-
<i>Helichrysum brownei</i> S. Moore	-
<i>Helichrysum callicomum</i> Harv.	-
<i>Helichrysum candolleanum</i> Buek	-
<i>Helichrysum cephaloideum</i> DC.	$2n = 14$
<i>Helichrysum cerastioides</i> DC.	-
<i>Helichrysum chionoides</i> Philipson	$2n = 14$
<i>Helichrysum chionosphaerum</i> DC.	-
<i>Helichrysum chrysargyrum</i> Moeser	-
<i>Helichrysum citrispinum</i> Del. var. <i>citrispinum</i>	-
<i>Helichrysum citrispinum</i> Del. var. <i>hoehnelii</i> (Schweinf.) Hedberg	$2n = 14$
<i>Helichrysum confertifolium</i> Klatt	-

<i>Helichrysum confertum</i> N. E. Br.	-
<i>Helichrysum cooperi</i> Harv.	-
<i>Helichrysum cordifolium</i> DC.	-
<i>Helichrysum crassifolium</i> (L.) D. Don	$2n = 28$
<i>Helichrysum cryptomerioides</i> Baker	-
<i>Helichrysum crispum</i> (L.) D. Don	-
<i>Helichrysum cymosum</i> (L.) D. Don subsp. <i>calvum</i> Hilliard	-
<i>Helichrysum dasycephalum</i> O. Hoffm.	-
<i>Helichrysum devium</i> J. Y. Johnson	$2n = 28$
<i>Helichrysum difficile</i> Hilliard	-
<i>Helichrysum ecklonis</i> Sond.	-
<i>Helichrysum elegantissimum</i> DC.	-
<i>Helichrysum epapposum</i> H. Bol.	-
<i>Helichrysum ephelos</i> Hilliard	-
<i>Helichrysum excisum</i> (Thunb.) Less.	-
<i>Helichrysum felinum</i> Less.	-
<i>Helichrysum flagellare</i> Bak	-
<i>Helichrysum flammeiceps</i> Brenan	-
<i>Helichrysum flanaganii</i> H. Bol.	-
<i>Helichrysum foetidum</i> (L.) Moench	$n = 7; 2n = 14, 28$
<i>Helichrysum formosissimum</i> (Sch. Bip.) A. Rich.	$2n = 14$
<i>Helichrysum forskahlii</i> (J. F. Gmel.) Hilliard & B. L. Burtt var. <i>compactum</i> (Vatke) Mesfin	-
<i>Helichrysum forskahlii</i> (J. F. Gmel.) Hilliard & B. L. Burtt var. <i>forskahlii</i>	$2n = 14, 28$
<i>Helichrysum forsythii</i> Humbert	-
<i>Helichrysum fulvescens</i> DC.	-
<i>Helichrysum galpinii</i> N. E. Br.	-
<i>Helichrysum globosum</i> A. Rich.	$2n = 14$
<i>Helichrysum glomeratum</i> Klatt	-
<i>Helichrysum glumaceum</i> DC.	-
<i>Helichrysum gofense</i> Cufod.	-
<i>Helichrysum gossypinum</i> Sch. Bip.	$2n = 28$
<i>Helichrysum griseolanatum</i> Hilliard	-
<i>Helichrysum gymnocephalum</i> (DC.) Humbert	-
<i>Helichrysum hamulosum</i> [E. Mey. ex] DC.	-
<i>Helichrysum herbaceum</i> (Andr.) Sweet	-
<i>Helichrysum homilochrysum</i> S. Moore	-
<i>Helichrysum ibityense</i> Humbert	-
<i>Helichrysum indicum</i> (L.) Grierson	$2n = 10$
<i>Helichrysum interjacens</i> Hilliard	-
<i>Helichrysum isolepis</i> H. Bol.	-
<i>Helichrysum italicum</i> (Roth) G. Don subsp. <i>italicum</i>	$2n = 28$
<i>Helichrysum italicum</i> (Roth) G. Don subsp. <i>microphyllum</i> (Willd.) Nyman	$n = 14; 2n = 28$
<i>Helichrysum kilimanjari</i> Oliv.	$2n = 14$
<i>Helichrysum lambertianum</i> DC.	-
<i>Helichrysum lecomtei</i> R. Vig. & Humbert	-
<i>Helichrysum lepidissimum</i> S. Moore	-

<i>Helichrysum litorale</i> H. Bol.	$2n = 8$
<i>Helichrysum manopappoides</i> Humbert	-
<i>Helichrysum maranguense</i> O. Hoffm.	-
<i>Helichrysum marginatum</i> DC.	-
<i>Helichrysum mariepsopicum</i> Hilliard	$2n = 14$
<i>Helichrysum melaleucum</i> Rchb. ex Holl	$n = 14; 2n = 28$
<i>Helichrysum melanacme</i> DC.	-
<i>Helichrysum meyeri-johannis</i> Engl.	$2n = 14$
<i>Helichrysum miconiifolium</i> DC.	-
<i>Helichrysum milfordiae</i> Killick	-
<i>Helichrysum mimetes</i> S. Moore	-
<i>Helichrysum mixtum</i> (O. Kuntze) Moeser var. <i>mixtum</i>	$n = 7; 2n = 14$
<i>Helichrysum monizii</i> Lowe	-
<i>Helichrysum monogynum</i> B. L. Burtt & Sunding	$n = 14, 21; 2n = 42$
<i>Helichrysum montanum</i> DC.	-
<i>Helichrysum monticola</i> Hilliard	-
<i>Helichrysum mundtii</i> Harv.	-
<i>Helichrysum mussae</i> Nevski	-
<i>Helichrysum natalitium</i> DC.	-
<i>Helichrysum neoachyroclinoides</i> Humbert	-
<i>Helichrysum nicolai</i> N. Kilian, Galbany & Oberpr.	-
<i>Helichrysum nudifolium</i> (L.) Less.	$2n = 28$
<i>Helichrysum obconicum</i> DC.	$n = 14$
<i>Helichrysum obductum</i> H. Bol.	-
<i>Helichrysum odoratissimum</i> (L.) Sweet	$2n = 14, 28, 42$
<i>Helichrysum opacum</i> Klatt	$2n = 28$
<i>Helichrysum orientale</i> (L.) Gaertn.	$2n = 28$
<i>Helichrysum pagophilum</i> M. D. Hend.	-
<i>Helichrysum paleatum</i> Hilliard	-
<i>Helichrysum patulum</i> (L.) D. Don	$2n = 14$
<i>Helichrysum pendulum</i> (C. Presl) C. Presl	$2n = 28$
<i>Helichrysum petiolare</i> Hilliard & B. L. Burtt	-
<i>Helichrysum pilosellum</i> (L. f.) Less.	-
<i>Helichrysum plantago</i> DC.	-
<i>Helichrysum platycephalum</i> Bak	-
<i>Helichrysum platypterum</i> DC.	-
<i>Helichrysum plicatum</i> DC.	$2n = 56$
<i>Helichrysum polycladum</i> Klatt	-
<i>Helichrysum populifolium</i> DC.	-
<i>Helichrysum psilolepis</i> Harv.	-
<i>Helichrysum reflexum</i> N. E. Br.	$2n = 14$
<i>Helichrysum retortum</i> (L.) Willd.	$2n = 14$
<i>Helichrysum rosum</i> (Berg.) Less.	$n = 14$
<i>Helichrysum rubicundum</i> (K. Koch) Bornm.	$n = 14; 2n = 56$
<i>Helichrysum rugulosum</i> Less.	$2n = 14$
<i>Helichrysum schimperii</i> (A. Rich.) Moeser	-
<i>Helichrysum selaginifolium</i> R. Vig. & Humbert	-
<i>Helichrysum setosum</i> Harv.	$2n = 14$

<i>Helichrysum sibthorpii</i> Rouy	$2n = 28$
<i>Helichrysum silvaticum</i> Hilliard	-
<i>Helichrysum simillinum</i> DC.	-
<i>Helichrysum spiralepis</i> Hilliard & B. L. Burt	$2n = 8$
<i>Helichrysum splendidum</i> (Thunb.) Less.	$2n = 14$
<i>Helichrysum stellatum</i> (L.) Less.	-
<i>Helichrysum stoechas</i> (L.) Moench	$n = 14; 2n = 28$
<i>Helichrysum stuhlmannii</i> O. Hoffm.	-
<i>Helichrysum summo-montanum</i> Verdoorn	-
<i>Helichrysum sutherlandii</i> Harv.	-
<i>Helichrysum swynnertonii</i> S. Moore	-
<i>Helichrysum thianschanicum</i> Regel	$n = 14; 2n = 28$
<i>Helichrysum transmontanum</i> Hilliard	-
<i>Helichrysum trilineatum</i> DC.	-
<i>Helichrysum triplinerve</i> DC.	-
<i>Helichrysum truncatum</i> Burt Davy	-
<i>Helichrysum umbraculigerum</i> Less.	-
<i>Helichrysum xylocladum</i> Humbert	-
<i>Helichrysum zeyheri</i> Less.	-
<i>Helichrysum zwartbergense</i> H. Bol.	$2n = 8$
<i>Humeocline madagascariensis</i> (Humb.) Anderb.	-
<i>Pseudognaphalium beneolens</i> (Davidson) Anderb.	$2n = 14$
<i>Pseudognaphalium biolettii</i> Anderb.	-
<i>Pseudognaphalium californicum</i> (DC.) Anderb.	$n = 14; 2n = 28$
<i>Pseudognaphalium gayanum</i> (J. Rémy) Anderb.	-
<i>Pseudognaphalium landbeckii</i> Phil.	-
<i>Pseudognaphalium leucocephalum</i> (A. Gray) Anderb.	$n = 14; 2n = 28$
<i>Pseudognaphalium luteoalbum</i> (L.) Hilliard & B. L. Burt	$n = 7, 8, 10; 2n = 14, 18, 28$
<i>Pseudognaphalium macounii</i> (Greene) Kartesz	-
<i>Pseudognaphalium oligandrum</i> (DC.) Hilliard & B. L. Burt	-
<i>Pseudognaphalium obtusifolium</i> (L.) Hilliard & B. L. Burt	$n = 14$
<i>Pseudognaphalium ramosissimum</i> (Nutt.) Anderb.	$n = 14; 2n = 28$
<i>Pseudognaphalium thermale</i> (E. E. Nelson) G. L. Nesom	-