

# Phylogeny of the *Inula* group (Asteraceae: Inuleae): Evidence from nuclear and plastid genomes and a recircumscription of *Pentanema*

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**Abstract** The *Inula* complex (Asteraceae: Inulinae) is a monophyletic group which comprises eight genera distributed in Eurasia and Africa: *Amblyocarpum*, *Carpesium*, *Chrysophthalmum*, *Inula*, *Pentanema*, *Rhanteriopsis*, *Telekia*, and *Varthemia*. With the aim to shed light on the circumscription of these genera, phylogenetic analyses were performed with 293 new DNA sequences (ITS region from nrDNA and three plastid spacers from cpDNA: *rps16-trnQ*, *rpl32-trnL*, *ndhF-rpl32*). It is concluded that both *Inula* and *Pentanema* are paraphyletic and that generic delimitations within the *Inula* complex need to be revised. Based on the results of the analyses, together with morphological and karyological data, we argue that the best solution is a new circumscription of the genus *Pentanema* including an amended description and 24 new combinations of former *Inula* species. Resurrection of the names *Codonocephalum*, *Monactinocephalus* and *Vicoa* is proposed to preserve the monophyly of the genera included in the *Inula* complex. Incongruences between nrDNA and cpDNA are documented and discussed.

**Keywords** *Inula*; Inuleae; molecular phylogeny; morphology; *Pentanema*; taxonomy

**Supplementary Material** The Electronic Supplement (Figs. S1, S2) and DNA sequence alignments are available from <https://doi.org/10.12705/671.10.S1> and <https://doi.org/10.12705/671.10.S2>, respectively.

## ■ INTRODUCTION

It is currently accepted that the tribe Inuleae is composed of two major lineages: the Inulinae and the Plucheinae both recognized at the taxonomic level of subtribe by Anderberg & Eldenäs (2007). The species included within subtribe Inulinae have a large oxalate crystal in each cell of the cypsela epidermis, which is a synapomorphic trait for the Inuleae-Inulinae (Eldenäs & al., 1999; Anderberg & al., 2005). The most comprehensive investigation of the relationships of the species included in the Inuleae-Inulinae by Englund & al. (2009) demonstrated that the circumscription of most genera is problematic, and that many of them – including *Inula* L., the main focus of this study – are paraphyletic (see also Nylinder & Anderberg, 2015). *Inula* is composed of ca. 100 species (Anderberg, 2009) distributed across Europe, Africa and Asia and it was recovered in a morphologically diverse clade called the *Inula* complex (i.e., Clade C, Englund & al., 2009), together with the genera *Amblyocarpum* Fisch. & C.A.Mey., *Carpesium* L., *Chrysophthalmum* Sch.Bip., *Pentanema* Cass., *Rhanteriopsis* Rauschert, *Telekia* Baumg., and *Varthemia* DC. These related genera, all of them smaller than *Inula*, are distributed mainly in the Middle East and in Central Asia. There are some characters that allow distinguishing the species included in the complex, which are mainly type of capitulum (heterogamous radiate, heterogamous disciform, or

homogamous discoid; terminology hereafter sensu Roque & al., 2009), presence or absence of receptacular scales and resiniferous canals in the stem, and the morphology of the cypsela. In contrast, there is not a single morphological character allowing unequivocal diagnosis of the *Inula* complex; this heterogeneity among genera regarding morphological traits is exemplified in Table 1.

Some of the species of *Inula* are of economic interest as ornamental garden plants, e.g., *I. britannica* L., *I. ensifolia* L., *I. helenium* L., *I. oculus-christi* L., and *I. orientalis* Lam. The genus is also well-known because of the presence of inulin, an oligosaccharide of high bromatological and therapeutic interest. This compound can be found in almost the entire Asteraceae, and was named after *Inula* because it was first obtained from rhizomes of *I. royleana* DC. (Anderberg, 2009). In fact, the type of *Inula* and Inuleae is *I. helenium* (Anderberg, 1993), the Elecampane, a popular medicinal plant particularly well-known in rural communities.

The circumscription of *Inula* has changed from the initial description of nine species by Linnaeus (1753) to the present broad genus concept that comprises ca. 100 species. Some of the species currently included in *Inula* were described as part of other genera (e.g., *Codonocephalum* Fenzl, *Conyza* L.), while others originally described under *Inula* have been transferred to various other genera (e.g., *Pulicaria dysenterica* (L.) Gaertn., *P. odora* (L.) Rchb.). Beck (1882) in the most comprehensive

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monograph of the European species of *Inula* recognized 21 species and arranged them in four sections based on cypsel shape and pappus insertion. Two of these sections today are commonly accepted as independent genera: *Dittrichia* Greuter (*Inula* sect. *Cupularia* Gren. & Godr.) and *Limbarda* Adans. (*Inula* sect. *Limbarda* (Adans.) DC.), which have been found to belong to other groups outside of the *Inula* complex (Englund & al., 2009). The remaining two sections accepted by Beck were *Inula* sect. *Enula* (Boemer) Duby, that comprised most of the European species of *Inula*, and *Inula* sect. *Corvisartia* (Mérat) DC., which included only *I. helenium*. The genus *Corvisartia* Mérat (1812) was split from *Inula* to accommodate *I. helenium*, based on the fact that the involucre bracts are ovate-trapezoidal in contrast to the linear to ovate-lanceolate bracts present in the remaining species of *Inula*, and also considering the much larger plant size of *I. helenium*. A few authors (Grenier & Godron, 1850; Reichenbach, 1853; Schur, 1866) followed the proposal by Mérat (1812) supporting the taxonomic independence of *Corvisartia* from *Inula*. Molecular analyses have demonstrated that *I. helenium* is phylogenetically closer to other genera of the *Inula* complex such as *Carpesium* or *Telekia*, than to the majority of the species included in *Inula* (Englund & al., 2009). Santos-Vicente & al., (2012) considered that *I. helenium* and its sister species (*I. sect. Corvisartia* sensu Santos-Vicente [pers. obs.] includes *I. helenium*, *I. koeltzii* R.Dawar & Qasier, *I. magnifica* Lipsky, *I. racemosa* Hook.f. and *I. royleana* DC.) could be segregated from the remaining species of *Inula* and transferred to *Corvisartia*. With the aim of limiting the number of nomenclatural changes these authors proposed the name *Inula* for conservation (with a conserved type). However, this proposal

was not recommended by the IAPT Committee (Applequist, 2013) considering that transferring the well-known medicinal species *I. helenium* to the unfamiliar genus *Corvisartia* would cause confusion particularly among non-specialists.

*Pentanema* is a small paraphyletic genus (ca. 20 species) distributed in southern to southwestern Asia and in East Africa (Duman & Anderberg, 1999; Pornpongrungrueng & al., 2007; Anderberg, 2009; Englund & al., 2009; Nylinder & Anderberg, 2015). It was described by Cassini (1818), with *P. divaricatum* Cass. as type, and after *Inula* it is the second-largest genus in the *Inula* complex. A few species, i.e., *P. cernuum* (Dalzell) Ling, *P. indicum* (L.) Ling and *P. ligneum* Mesfin have been shown to be closely related to the *Duhaldea-Caesulia-Blumea* clade (Pornpongrungrueng & al., 2007; Englund & al., 2009; Nylinder & Anderberg, 2015).

In this study, we present a phylogeny of the species included in the *Inula* complex (Englund & al., 2009) with particular emphasis on the European species of *Inula*. For this purpose, we sequenced the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) and three regions of the maternally inherited plastid DNA. To place our results in a wider phylogenetic framework, we completed the newly generated dataset with previously published ITS sequences of species belonging to the *Pulicaria* complex and the *Duhaldea-Caesulia-Blumea* clade (Englund & al., 2009). We also investigated the micromorphology of the cypsel of some European species of *Inula* because it has characters considered relevant in the classification of the group. The aims of this study are to (1) investigate the phylogenetic position of the European species of *Inula* within the *Inula* complex and explore which

**Table 1.** Morphological character states of some representative species of the genera belonging to the *Inula* complex (Englund & al., 2009).

Taxon	Resiniferous canals	Receptacular scales	Cypsel morphology	Type of capitulum
<i>Amblyocarpum inuloides</i> Fisch. & C.A.Mey.	Absent	Epaleate	Slightly compressed, upper part swollen and truncate; glandular; pappus absent	Radiate
<i>Carpesium cernuum</i> L.	Absent	Epaleate	Fusiform, beaked; glandular on beak and at base; pappus absent	Disciform
<i>Chrysophthalmum dichotomum</i> Boiss. & Heldr.	Absent	Paleate	Tetragonous; minutely pubescent, especially near apex; pappus absent	Discoid
<i>Inula helenium</i> L.	Present	Epaleate	Tetragonous; glabrous; pappus connate at the base; ca. 50–80 barbellate bristles	Radiate
<i>Inula hirta</i> L.	Absent	Epaleate	Cylindrical; glabrous; pappus slightly connate; ca. 30 barbellate bristles	Radiate
<i>Inula montana</i> L.	Absent	Epaleate	Oblong; appressed hairy; pappus slightly connate; ca. 30 barbellate bristles	Radiate
<i>Pentanema divaricatum</i> Cass.	Absent	Epaleate	Oblong; appressed hairy; pappus of free bristles; ca. 5 barbellate bristles	Radiate
<i>Rhanteropsis lanuginosa</i> (DC.) Rauschert	Absent	Paleate	Tetragonous; apically strigose-hispid; pappus of free bristles; 2–3 long serrulate bristles	Radiate
<i>Telekia speciosa</i> (Schreb.) Baumg.	Present	Paleate	Cylindrical; glabrous; pappus formed by a rim of minute scales or absent	Radiate
<i>Varthemia persica</i> DC.	Absent	Epaleate	Slightly compressed or almost terate; short appressed hairs; ca. 20 barbellate pappus bristles	Discoid

evolutionary processes are involved in their diversification, (2) investigate monophyletic groups within *Inula* and how *Inula* should be circumscribed in the light of the most recent phylogenetic data and, (3) evaluate and propose the necessary nomenclatural changes.

## ■ MATERIALS AND METHODS

**Plant material.** — Specimens deposited in 30 herbaria were investigated. Two datasets were generated to study the evolutionary relationships within the *Inula* complex. With dataset 1, our aim was to elucidate the placement of the European species of *Inula* within the *Inula* complex. For this objective, we sequenced the ITS region of nrDNA for 68 accessions (31 taxa) and obtained 31 additional sequences (28 additional taxa) from GenBank. The sampling covered ca. 25% of the species currently accepted under *Inula* (i.e., all European and North African species, plus *I. auriculata* Wall., *I. grandis* Schrenk, *I. mariae* Bordz. *I. orientalis* and *I. peacockiana* (Aitch. & Hemsl.) Korovin from Asia, and *I. confertiflora* A.Rich., *I. paniculata* (Klatt) Burt Davy and *I. shirensis* Oliv. as African representatives), as well as most species of the related genera from the complex (i.e., *Carpesium* was represented by 6 species [25 species in the genus]; *Chrysophthalmum* by 3 species [5 species in the genus]; *Rhanteriosis* by 2 species [4 species in the genus], plus *Telekia* and *Varthemia*, which are monospecific genera), except for *Amblyocarpum inuloides* Fisch. & C.A.Mey., a monospecific genus from the Caspian Sea area. A few species were included as representatives of the *Pulicaria* complex and the *Duhaldea-Caesulia-Blumea* clade. Finally, *Stenachaenium campestre* Baker which belongs to Inuleae-Plucheinae was used as outgroup taxon to root the tree.

In the case of dataset 2, we analyzed the ITS region plus three plastid DNA regions (*rps16-trnQ*, *rpl32-trnL*, *ndhF-rpl32*), corresponding to 75 representative specimens of 34 species of the *Inula* complex included in dataset 1. Our aim was to compare possibly incongruent gene tree topologies obtained from plastid and nuclear DNA markers. In this case *Pulicaria dysenterica* from the *Pulicaria* complex and *Pentanema indicum*, which earlier had been found to belong in the *Duhaldea-Caesulia-Blumea* clade (Englund & al., 2009), was used as outgroup.

Leaf material was also collected in the field between 2004 and 2015 and stored in silica gel. Additional DNA extractions were made from herbarium material whenever necessary. Voucher information, the source of material and GenBank accession numbers are given in Appendix 1.

**DNA extraction and amplification.** — DNA extraction from dried silica-gel material was done following CTAB protocol (Doyle & Doyle, 1987) with slight modifications (after centrifugation the DNA pellet was washed with 70% ethanol, dried at 37°C and resuspended in TE buffer). The amount of leaf material used was 20–25 mg. For herbarium material the DNEasy plant DNA Extraction kit from Qiagen (Valencia, California, U.S.A.) was used and the manufacturer's instructions were followed.

The polymerase chain reaction (PCR) was performed in an Eppendorf thermocycler (Eppendorf Mastercycler ProS). The ITS region was amplified with the primers LEU1 (Vargas & al., 1998) and ITS4 (White & al., 1990) under the following PCR conditions: 2:30 min of preheating at 94°C followed by 40 cycles of 30 s at 94°C denaturation, 30 s at 53°C annealing and 1:15 min at 72°C extension, with a final step of 10 min at 72°C and 4°C holding. The PCR mix used for the ITS region included: 5 µl of 5× Green GoTaq buffer, 0.5 µl of dNTP 10 mM (Fermentas, Waltham, Massachusetts, U.S.A.), 0.83 µl of primer F and R 10 mM, 0.17 µl of Go Taq Polymerase (Promega, Madison, Wisconsin, U.S.A.), 1.25 µl of DMSO (Fisher Scientific, Hampton, New Hampshire, U.S.A.), 0.25 µl of BSA 1 mg/ml (New England Biolabs, Ipswich, Massachusetts, U.S.A.), 14.17 µl of ddH<sub>2</sub>O and 2 µl of DNA (18 ng/µl) in a reaction final volume of 25 µl. The products of the amplifications were checked in a TBE 1× 1% agarose gel, with 0.01% SyberSafe. All PCR products were purified with ExoSap-IT (Affymetrix, Santa Clara, California, U.S.A.) exonuclease and sent to the European section of Macrogen for Sanger sequencing in both directions.

For plastid non-coding regions we used the following PCR master-mix per sample: 5 µl of 5× Green GoTaq buffer (Promega), 0.5 µl of dNTPs 10 mM (Fermentas), 0.83 µl of each primer 10 mM (MWG Operon), 0.17 µl of Go Taq Polymerase (Promega), 15.17 µl of ddH<sub>2</sub>O and 2 µl of DNA dilution at 18.2 ng/µl with a final volume of 25 µl. To amplify the *rps16-trnQ* region the primers rps16x1 and trnQUUG of Shaw & al. (2007) were used, with the following PCR conditions: 2 min of preheating at 95°C, followed by 35 cycles of 30 s at 95°C denaturation, 30 s at 55°C annealing and 80 s at 72°C extension with a final extension of 5 min at 72°C and 4°C holding. The *rpl32-trnL* region was amplified with the primers rpl32-F and trnLUAG (Shaw & al., 2007) with the same conditions indicated by Shaw & al. (2007) with minor modifications: initial preheating at 85°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 65°C for 4 min with a ramp of +0.3°C/s to 65°C and extension at 65°C for 5 min followed by a final extension of 5 min at 65°C and 4°C holding. The *ndhF-rpl32* region was amplified with the primers ndhF and rpl32-R from Shaw & al. (2007) under the following PCR conditions: initial preheating for 5 min at 80°C, 10 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min with a ramp of 3°C/s to 65°C temperature of extension for 4 min followed by 20 cycles with an annealing temperature of 48°C, plus other 20 cycles at 46°C annealing and a final extension of 5 min at 65°C and 4°C holding.

**Alignment.** — Sequences were edited in Geneious v.5.5.9 (Biomatters, 2013) and aligned using the algorithm MUSCLE. Each region was manually checked. Ambiguous regions were excluded from the alignment using the software Gblocks v.0.91b (Castresana, 2000; Talavera & Castresana, 2007) under the following relaxed conditions: Minimum length of a block “5”, Allowed Gap positions “With half”, Minimum number of sequences for a flank position “40” and the Maximum number of contiguous non conserved positions “10”. These parameters allowed the presence of some informative gaps in the alignments. Indels were coded according to the “simple coding” method

of Simmons & Ochoterena (2000) as implemented in SeqState v.1.4.1 (Müller, 2005). They were added to the data as a binary matrix and consequently gaps were treated as missing data.

**Analysis of the secondary structure.** — Secondary structure of the ribosomal ITS sequences were examined to detect the presence of pseudogenes in the dataset. The analysis was carried out using RNAstructure v.5.7 (Reuter & Mathews, 2010), looking for the functional presence of the hairpin conserved motif GCCRY-(4n to 7n)-GYGYCAAGAA (Liu & Schardl, 1994).

**Test of recombination.** — Recombination between nucleotide sequences can occur after hybridization or as an artifact, during PCR amplification (Lemey & Posada, 2009). Evidence for recombination in the ITS sequences was searched for using RDP4 v.4.43 (Martin & al., 2010) using *P*-values set to 0.05 under the tests RDP, GENECONV and MaxChi. The multiple comparison corrections were performed with the tests BootsScan and SiScan.

**Phylogenetic reconstruction.** — The best available evolutionary model was calculated for each marker and dataset with jModelTest 2 v.2.1.4 (Darriba & al., 2012) under the Akaike information criterion (AIC) (Akaike, 1973) (Table 2). For dataset 1, Bayesian (BA), maximum likelihood (ML) and maximum parsimony (MP) analyses were carried out. In the case of dataset 2, the three plastid markers were concatenated and only BA was performed. Since topological incongruences between the nrDNA and cpDNA trees were detected, analyses using a plastid and nuclear concatenated matrix were not performed.

The Bayesian analyses were performed with MrBayes v.3.2.0 (Ronquist & al., 2012). MCMCMC were carried out for dataset 1 and dataset 2 in two independent runs. Each run was performed with four chains (one cold and three hot), starting with random trees, computing 20 million generations and sampling each 2000th tree. After discarding a burn-in of 4000 trees (20% of all sampled trees) from each run, a majority-rule consensus tree was calculated. Traces were visually inspected in Tracer v.1.6 (Rambaut & al., 2014) to ensure that the effective sample sizes (ESSs) of all parameters were >200, as recommended by the authors, and to check the convergence of parameter estimates across runs. Nodes with Bayesian posterior probabilities (BPP)  $\geq 0.95$  were considered to be strongly or significantly supported (Huelsenbeck & Rannala, 2004), and branches receiving <0.90 BPP were collapsed.

ML analysis was carried out using PhyML v.3.0 (Guindon & al., 2010) as implemented in ATGC Montpellier bioinformatics platform (<http://www.atgc-montpellier.fr/phyml/>). Tree improvement was performed under the SPR (Subtree Pruning Regrafting) and NNI (Nearest Neighbor Interchange) algorithms, and branch support was calculated with the approximate Likelihood Ratio Test aLRT SH-like method (Anisimova & Gascuel, 2006). Branches receiving <0.90 support were collapsed.

MP analysis was carried out using TNT v.1.5 (Goloboff & al., 2008). Traditional search option was applied in an initial run of 20,000 random addition sequence RAS replicates, and using tree bisection-reconnection (TBR) branch-swapping saving 10 trees per replicate. A majority-rule consensus tree was obtained from 264 best-score trees, and jackknife (JK) (Kopuchian & Ramírez, 2010) support values were calculated with 2000 RAS replicates using TBR branch-swapping. Branches receiving JK values <70% were collapsed. Consistency index (CI) and retention index (RI) were obtained using PAUP\* v.4.0b10 (Swofford, 2002).

Additionally, a consensus network was performed with SplitsTree v.4 (Huson & Bryant, 2006) in order to summarize the information provided by the ITS and cpDNA markers. A consensus network is a useful tool to visualize information provided by incongruent markers, and to detect the main spots of incongruence among tree topologies. The method requires that the input trees are inferred from the same dataset. In our case, the consensus network was built using the Bayesian topologies obtained from the independent analyses of the nuclear and plastid matrices of dataset 2. Branches of the input topologies with BPP support <95 were collapsed in order to keep the statistical strength of the analysis.

Names provided at the tips of branches in the phylogenetic reconstructions refer to the classification and nomenclatural treatment accepted here, followed (in parentheses) by the traditional names

**Microphotography with scanning electron microscopy (SEM).** — Mature cypselae of *Inula helenioides* DC., *I. helenium*, *I. montana* L. and *I. salicina* L. were collected in 2016 and preserved in FAA (formaldehyde–acetic acid–alcohol). Cypselas of *Pentanema divaricatum* and *Varthemia persica* DC. were sampled from herbarium sheets and rehydrated with

**Table 2.** Main sequence characteristics, number of parsimony-informative characters of dataset 1 and nucleotide substitution models for each marker.

	Dataset 1		Dataset 2		
	ITS	ITS	<i>rps16-trnQ</i>	<i>rpl23-trnL</i>	<i>ndhF-rpl32</i>
Number of individuals	99	75	75	75	75
Sequence length [bp]	668–753	546–589	740–764	776–817	612–667
Sequence aligned length [bp]	719	589	764	817	667
Number of coded indels	75	15	12	22	20
Identical sites	12.5%	28.5%	81.3%	69.4%	71.8%
Number of parsimony-informative characters	272				
Model of molecular evolution	SYM+I+G	012310+I+G	001001+G+F	012010+G+F	001001+G+F

water. Details about plant material used for SEM are available in Appendix 2. Samples were dehydrated through an ethanol series (30, 50, 70, 80, 90, 95, 98, 100 and 100% again). Critical point drying was done with a CPD7501 dryer (Polarum Quorum Technologies) in a CO<sub>2</sub> atmosphere at 38°C and 1500 PSI (pound force/square inch). The samples were then mounted on stubs and coated with gold using a BALZER SCD040 ion sputter. Samples were observed with a HITACHI S-3000N scanning electron microscope.

## ■ RESULTS

**Sequence characteristics.** — A total of 293 sequences were newly generated for this study, 68 of ITS and 225 of plastid markers. Table 2 summarizes alignment characteristics and statistics for all markers and datasets. Higher nucleotide variation rates were detected in the ITS region than in the plastid regions. All ITS sequences had the characteristic conserved hairpin structure in the motif of Liu & Schardl (1994). GC content in ITS1 varied from 31% to 55.2% (average 47.1%), while in ITS2 it varied between 36.3% and 54.2% (average 45.6%). The average between the difference of the GC content values in ITS1 and ITS2 for each sample was 1.48 (more details in Table 2). No recombination among the tested sequences was found using RDP4.

**Major clades in the ITS analysis.** — The Bayesian, ML (Electr. Suppl.: Fig. S1) and MP (Electr. Suppl.: Fig. S2) analyses of dataset 1 resulted in highly congruent topologies. Therefore only the Bayesian topology is shown here (Fig. 1) with, ML and MP support values added. Similarly, only the ITS tree of dataset 1 is discussed, as the ITS analysis of dataset 2 (Fig. 2, left) does not differ in topology and has only slight differences in PP values.

Subtribe Inulinae (BPP 1.00, aLRT 1.00, JK 100), the *Pulicaria* complex (BPP 1.00, aLRT 0.97, JK –), and the *Inula* complex (BPP 1.00, aLRT –, JK –) are recovered as monophyletic groups, at least in the BA, which is in congruence with former studies (Eldenäs & al., 1998; Anderberg & al., 2005; Englund & al., 2009). *Duhaldea cappa* (Buch.-Ham. ex D. Don) Pruski & Anderb., *Pentanema indicum*, *Caesulia axillaris* Roxb. and *Blumea balsamifera* DC. represent the early-branching *Duhaldea-Caesulia-Blumea* clade (BPP 1.00, aLRT 0.97, JK 99). This clade is in a sister position to a trichotomy formed by the *Pulicaria* complex, the *Inula* complex and one accession of *Buphthalmum salicifolium* L. *Pulicaria* is recovered as polyphyletic, which is in agreement with former results by Anderberg & al. (2005) and Englund & al. (2009). Different species of *Pulicaria* are scattered among other genera represented in our study by *Pallenis (Asteriscus) maritima* (L.) Greuter, *Pallenis spinosa* (L.) Cass., *Rhanterium epapposum* Oliv., *Limbarda crithmoides* (L.) Dumort., *Jasonia tuberosa* (L.) DC., *Dittrichia viscosa* (L.) Greuter, and *Iphiona scabra* DC. ex Decne.

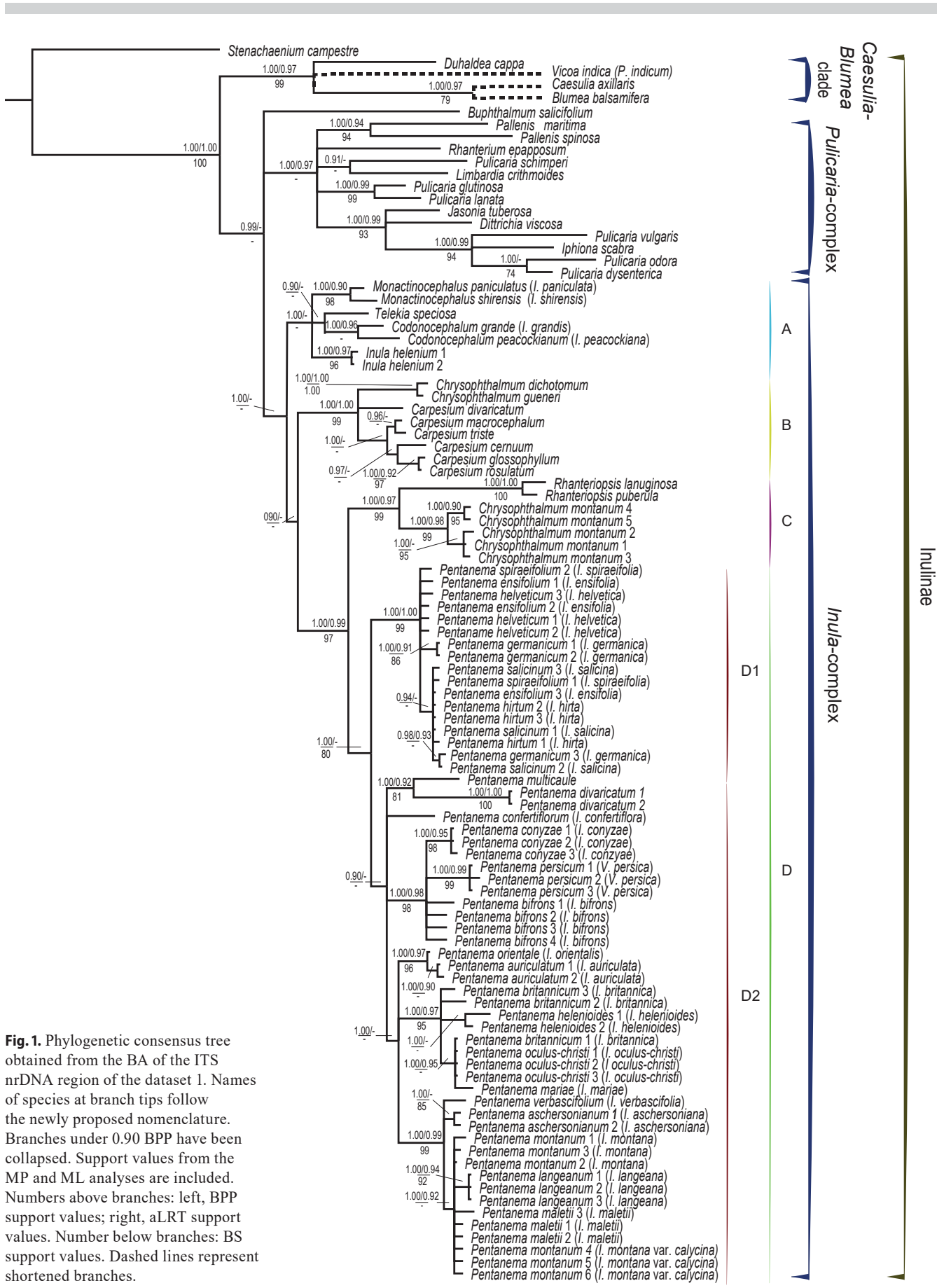
In the *Inula* complex, four different clades can be distinguished. Clade A (BPP 1.00, aLRT –, JK –) is poorly supported as sister to the rest of the complex (BPP 0.90, aLRT –, JK –) and includes *I. helenium* plus four other species of *Inula*

(i.e., *I. grandis*, *I. paniculata*, *I. peacockiana*, *I. shirensis*). *Inula paniculata* and *I. shirensis* from southern and central Africa are recovered as one subclade (BPP 1.00, aLRT 0.90, JK 98) that is placed in a trichotomy within clade A. *Inula grandis* and *I. peacockiana*, distributed in Asia with Iran as a center of distribution, are recovered together in a sister position to *Telekia speciosa* (Schreb.) Baumg. (BPP 0.90, aLRT –, JK –). Finally, the last subclade of the trichotomy (BPP 1.00, aLRT 0.97, JK 96) is formed by *I. helenium*.

Clade B (BPP 1.00, aLRT 1.00, JK 99) includes two species of *Chrysophthalmum* from western Turkey (i.e., *Ch. gueneri* Aytaç & Anderb., *Ch. dichotomum* Boiss. & Heldr.), which are recovered in an unresolved position with respect to six species of *Carpesium* (i.e., *C. divaricatum* Siebold & Zucc., *C. macrocephalum* Franch. & Sav., *C. triste* Maxim., *C. cernuum* L., *C. glossophyllum* Maxim., *C. rosulatum* Miq.).

A sister-group relationship between clade B and clades C+D receives poor Bayesian support (BPP 0.90, aLRT –, JK –). Clades C and D are well supported as sister groups (BPP 1.00, aLRT 0.99, JK 97). Clade C (BPP 1.00, aLRT 0.97, JK 99) contains two species of *Rhanteriosis* (*R. puberula* (Boiss. & Hausskn.) Rauschert, *R. lanuginosa* (DC.) Rauschert) clearly recovered as monophyletic (*Rhanteriosis* comprises four species) and five accessions of *Chrysophthalmum montanum* (DC.) Boiss.

Finally, clade D (BPP 1.00, aLRT –, JK 80) contains the European species of *Inula* plus other Asian and African taxa such as *Pentanema multicaule* Boiss., *P. divaricatum*, *Varthemis persica*, *Inula auriculata*, *I. mariae*, *I. orientalis*, *I. confertiflora* and *I. maletii* Maire. Clade D comprises two sister subclades. Subclade D1 (BPP 1.00, aLRT 1.00, JK 99) contains the diploid species *Inula spiraeifolia* L., *I. ensifolia* L., *I. helvetica* Weber, *I. germanica* L., *I. salicina* and *I. hirta* L., a group of species already recognized as closely related to each other by several authors, even based only on morphological characters (Beck, 1882; Anderberg, 1991; Englund & al., 2009). The poorly supported subclade D2 (BPP 0.90, aLRT –, JK –) is composed mainly of polyploid taxa arranged in four groups. The first (BPP 1.00, aLRT 0.92, JK 81) includes two species of *Pentanema* (*P. multicaule*, *P. divaricatum*). Thus, not only the genus is recovered as paraphyletic in agreement with previous studies (Englund & al., 2009), but also the currently listed type of *Pentanema* appears nested within the species of *Inula* that compose subclade D2. The second group in this polytomy is one accession of *I. confertiflora*, a species from Ethiopia. The third (BPP 1.00, aLRT 0.98, JK 98) is a subclade containing specimens of *I. conyzae* (Griess.) DC., *I. bifrons* L. and *V. persica*, the latter a monospecific genus distributed in the Middle East, also recovered close to *I. conyzae* and *I. bifrons* (from *I.* subsect. *Breviligulatae* Beck) by Englund & al. (2009). Last, the fourth group (BPP 1.00, aLRT –, JK –) within subclade D2 is composed by three minor clades: The first one (BPP 1.00, aLRT 0.97, JK 96) includes *I. orientalis*, which is widespread in Asia, plus *I. auriculata*, a species restricted to the Caucasus region; the second one (BPP 1.00, aLRT 0.97, JK 95) comprises *I. britannica*, *I. helenioides*, *I. oculus-christi* and *I. mariae*, and the last minor clade (BPP 1.00, aLRT 0.99, JK 99) is composed



**Fig. 1.** Phylogenetic consensus tree obtained from the BA of the ITS nrDNA region of the dataset 1. Names of species at branch tips follow the newly proposed nomenclature. Branches under 0.90 BPP have been collapsed. Support values from the MP and ML analyses are included. Numbers above branches: left, BPP support values; right, aLRT support values. Number below branches: BS support values. Dashed lines represent shortened branches.

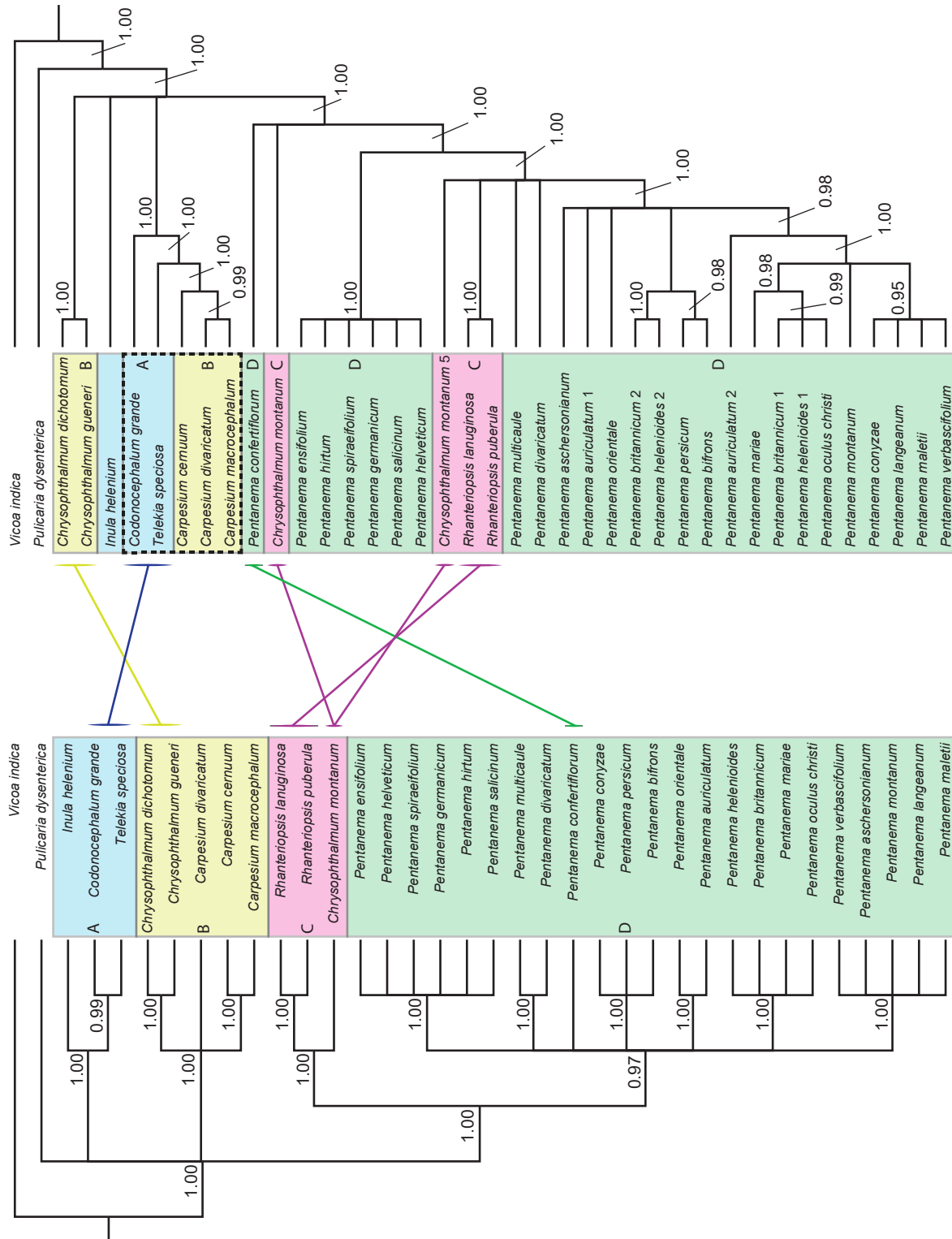
of *Inula verbascifolia* Hausskn., and *I. aschersoniana* Janka from the Balkan Peninsula plus *I. montana*, *I. langeana* Beck and *I. maletii*, which are distributed throughout the western Mediterranean region.

**Incongruence between nrDNA and cpDNA gene-trees.** —

The incongruent phylogenetic signal is shown in Fig. 2 by comparing the topologies obtained from the Bayesian analyses

of the ITS and the three plastid regions of dataset 2. In Fig. 2, branches corresponding to the same species have been collapsed, in order to represent a species by a single tip whenever possible. Only the main differences between the two topologies are described here.

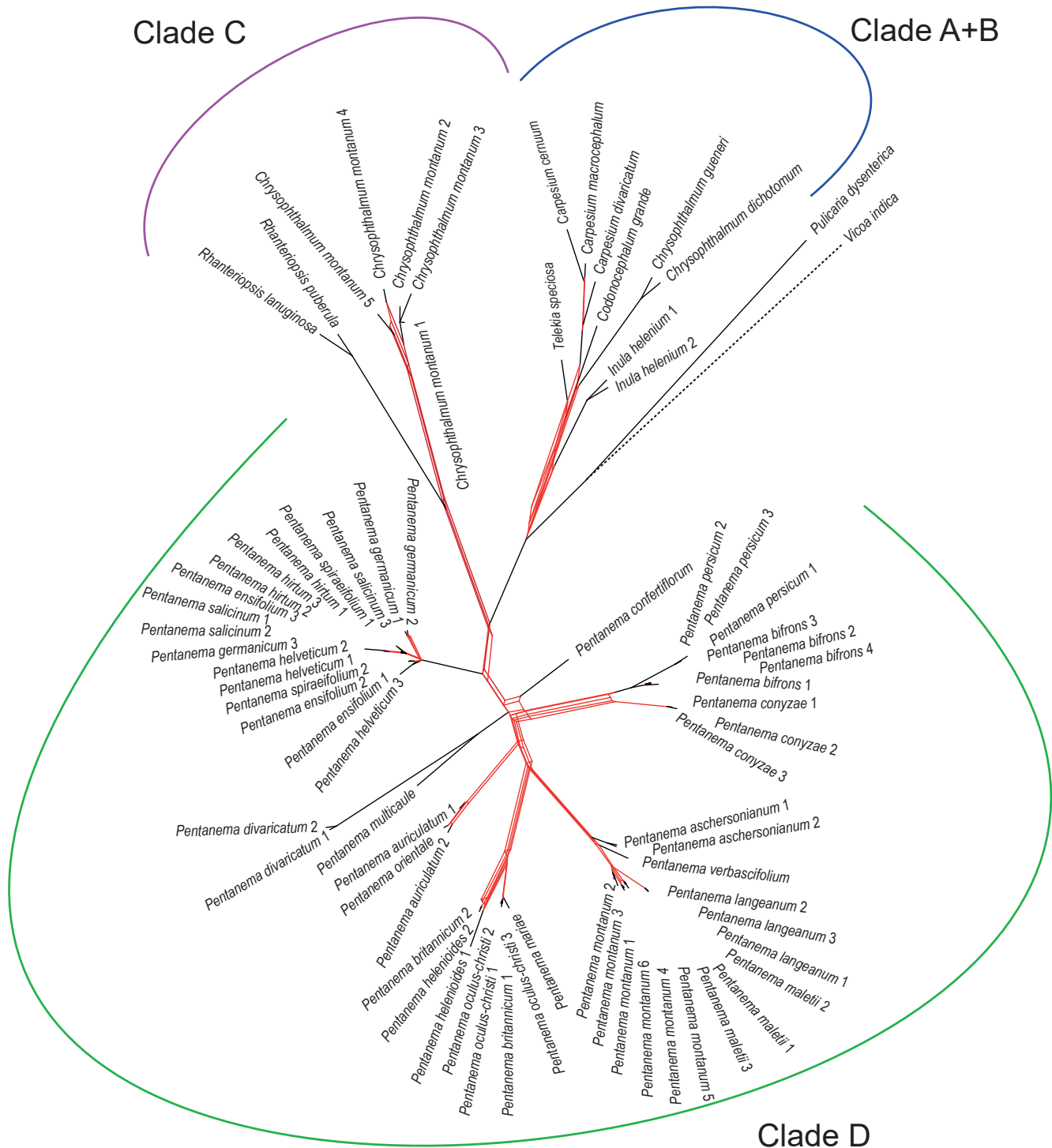
In the analysis of ITS sequences, *Inula grandis*, *I. helenium* and *Telekia speciosa* are recovered in a single clade, while in



**Fig. 2.** Phylogenetic consensus trees obtained from the analysis of dataset 2. **Left,** Topology obtained from the BA of the ITS nrDNA region; **Right,** Topology obtained from the BA of concatenated plastid markers. Branches corresponding to the same species have been collapsed in order to represent a species by a single tip whenever possible. Letters indicate main clades recovered in the ITS nrDNA analysis of dataset 1. Names of species at branch tips follow the newly proposed nomenclature. Lines indicate the main incongruences between topologies. Numbers above branches: BPP support values. Branches with BPP support value lower than 0.90 have been collapsed.

the cpDNA topology, *T. speciosa* and *I. grandis* are successive sisters to the species of *Carpesium*. In the cpDNA tree *I. helenium* is placed in an unresolved position with respect to *Carpesium* + *Telekia* + *I. grandis* clade and the monophyletic group comprising *Chrysophthalmum dichotomum* and *Ch. gueneri*. *Rhanteropsis lanuginosa* and *R. puberula* appeared

in an unresolved position within subclade D in the cpDNA tree, but are members of clade C in the ITS reconstruction. Moreover, *Ch. montanum* and *I. auriculata* are recovered in the ITS topology as monophyletic, in contrast with the plastid topology (Fig. 2). Neither *I. britannica* nor *I. helenioides* are recovered as monophyletic in the cpDNA reconstruction.



**Fig. 3.** Consensus network obtained from the analysis of the ITS and cpDNA trees shown in Fig. 2. Highlighted edges represent incongruities between markers. Dashed lines represent shortened branches.



Finally, *I. conyzae*, a species that has been traditionally related to *I. bifrons* (Beck, 1882; Anderberg, 1991), which is confirmed by our ITS data, appears as closely related to *I. langeana*, *I. mal-etii* and *I. verbascifolia* in the cpDNA topology.

The consensus network (Fig. 3) obtained from the ITS and plastid DNA trees of dataset 2 reveals that the phylogenetic history of the *Inula* complex involved reticulate evolution to some extent, mainly limited to clades C and D. The network shows three main groups. The first includes clades A and B, which reflects the incongruent phylogenetic signal between the two markers in these clades. The second group corresponds to clade C and the third to clade D.

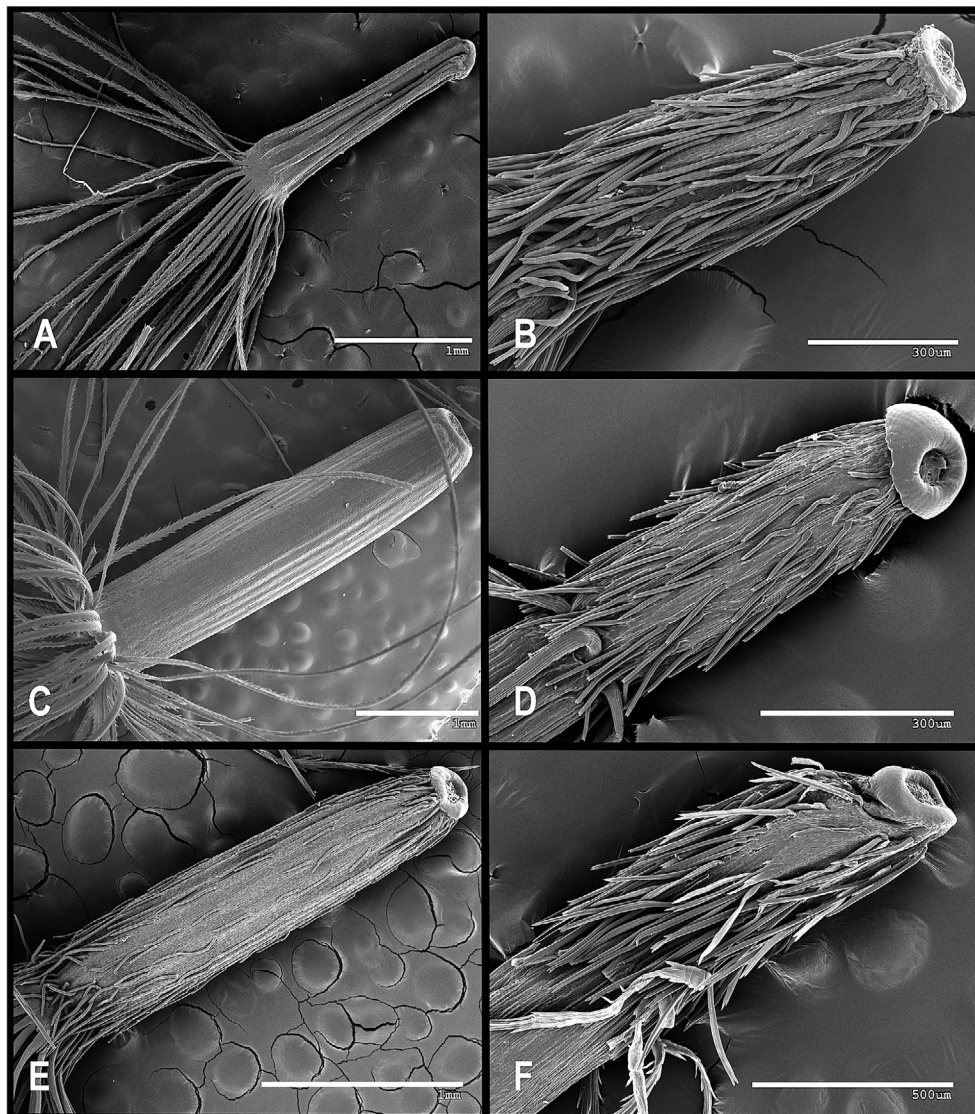
**Cypselae examination using SEM.** — The morphology of the cypselae has been traditionally considered as a first-order character in the taxonomy of the Asteraceae. Thus, detailed microphotographs of the cypselae corresponding to selected species recovered in the *Inula* complex were performed. One individual of *I. helenioides*, *I. helenium*, *I. montana*, *I. salicina*, *Pentanema divaricatum* and *Varthemia persica* was selected, given that these species are representative of the main clades

recovered in our analysis. Pictures are presented in Fig. 4. While *I. helenium* has prismatic (tetragonous) and glabrous cypselae, 3–5 mm in length, the remaining species studied have cylindrical (oblong), glabrous to densely hairy cypselae, 0.5–3 mm long.

## ■ DISCUSSION

This study represents the most extensive phylogeny with a focus on the European species of *Inula*. In congruence with previous results (Anderberg & al., 2005; Pornpongrueng & al., 2007; Englund & al., 2009; Santos-Vicente, 2013; Nylinder & Anderberg, 2015), it demonstrates that the *Inula* complex is monophyletic, and that several genera within it, such as *Chrysophthalmum* (3 spp.), *Pentanema* and *Inula*, are paraphyletic. Further studies including two unsampled species of *Rhanteropsis* are needed to test the monophyly of that genus.

The nomenclatural changes made in this paper are not only based on our present results, but also on results from



**Fig. 4.** Cypselae microphotography (SEM). **A**, *Inula salicina*, DG788; **B**, *Inula helenioides*, DG807; **C**, *Inula helenium*, DG808; **D**, *Pentanema divaricatum*, E00100893; **E**, *Inula montana*, SA1162; **F**, *Varthemia persica*, E00591725.

analyses of the broader sampling of Englund & al. (2009) that covers most of the morphological variation in the *Inula* complex. These insights together with our present results lead to the following conclusions and the subsequently amended taxonomy.

**Circumscription of *Inula*.** — As previously discussed, one way to resolve the paraphyletic status of *Inula* minimizing the number of nomenclatural changes would have been to transfer *I. helenium* to *Corvisartia*, keeping the name *Inula* for the species in clade D. However, this option (Santos-Vicente & al., 2012) was not favoured by the Nomenclature Committee for Vascular Plants (Appelquist, 2013).

Another alternative would be to adopt a wide concept of *Inula*, equivalent to the entire *Inula* complex (i.e., clades A, B, C and D) and synonymizing a number of genera, but this would make *Inula* very heterogeneous from a morphological point of view. Considering that the base chromosome number of the taxa included in clade A is  $x = 10$ , while in clade D it is  $x = 8$ , a large *Inula* would be also karyologically heterogeneous.

We argue that by preserving the name *Inula* for *Inula helenium* and related taxa such as *I. magnifica*, *I. racemosa*, or *I. royleana*, *Inula* circumscribed in this way would correspond with *Inula* s.str. sensu Anderberg (1991), which includes large herbs with resin canals in the stem and radiate capitula.

*Telekia speciosa* a species showing resin canals in the stem, appears as closely related to *Inula* s.str. in all phylogenetic reconstructions available (Anderberg & al., 2005; Englund & al., 2009), but it differs morphologically from the species included in this group, because the capitula have receptacular scales and fruits lack a pappus.

*Inula peacockiana* and *I. grandis*, both with resin canals, are recovered as well-supported sister species. We propose to resurrect for them the available combinations in *Codonocephalum*, i.e., *Codonocephalum peacockianum* Aitch. & Hemsl. and *C. grande* (Schrenk ex Fisch. & C.A.Mey.) B.Fedtsch.

Finally, *Inula shirensis* and *I. paniculata* have traditionally been included into the “*Inula glomerata* group” (Anderberg, 1991; Englund & al., 2009), which is composed of species distributed in tropical Africa and Madagascar and mainly characterized by the presence of disciform capitula (e.g., *I. glomerata* Oliv. & Hiern, *I. mannii* (Hook.f.) Oliv. & Hiern, *I. perrieri* (Humbert) Mattf., *I. speciosa* O. Hoffm.). Our proposal here is again resurrecting the old name *Monactinocephalus paniculatus* Klatt for *I. paniculata*. As regards *I. shirensis*, the species represents an exception within the “*I. glomerata* group” due to the presence of rayed capitula, a character state that may have evolved secondarily. In this case, the following combination would be necessary:

***Monactinocephalus shirensis*** (Oliv.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula shirensis* Oliv. in Hooker’s Icon. Pl. 14: t. 1399. 1882.

**A new circumscription of *Pentanema* Cass.** — *Pentanema* has traditionally been diagnosed by the presence of few pappus bristles (5–20). With the exception of the number of bristles, there is no other robust morphological trait for distinguishing

between *Inula* and *Pentanema*. In its current circumscription *Pentanema* is also a paraphyletic genus (Anderberg, 1991; Duman & Anderberg, 1999; Anderberg & al., 2005; Englund & al., 2009; and polyphyletic according to Pornpongrungrueng & al., 2007). Both genera are considered residual taxonomic hodgepodes (after Duman & Anderberg, 1999) within the *Inula* complex, because they are diagnosed by the absence of certain traits which are characteristic of other smaller or monospecific genera (e.g., *Chrysophthalmum*, *Rhanterioopsis*, *Telekia*; Duman & Anderberg, 1999). Considering that the type of *Pentanema* is *P. divaricatum*, that is recovered within clade D together with the remaining European and North African species of *Inula*, and that the name *Pentanema* has priority over *Varthemia* (also within clade D), the option we favour is to transfer the species of *Inula* included in clade D as well as *V. persica* to *Pentanema*. The latter species resembles many Central Asian species of *Pentanema*, albeit with a more elongated growth habit.

Given that *P. indicum* is not a member of clade D, but of the *Duhaldea-Caesulia-Blumea* clade and considering also that it has a chromosome base number different from the remaining species included in *Pentanema* as the genus is here circumscribed ( $x = 9$  instead of  $x = 8$ ), we propose the combination *Vicoa indica* (L.) DC. (traditionally included in *Inula* sect. *Vicoa* (Cass.) O.Hoffm.) as a name for this species.

*Pentanema* in the newly proposed circumscription is described as follows:

***Pentanema* Cass.** in Bull. Sci. Soc. Philom. Paris 1818: 74. 1818 ≡ *Inula* sect. *Pentanema* (Cass.) Boiss., Fl. Orient. 3: 199. 1875.

Dwarf shrubs, perennial or annual herbs, rarely biennial, generally rhizomatous; indumentum variable from glabrous-glabrescent to tomentose. *Stems* erect, not winged, without resin canals. *Leaves* alternate, ± linear to ovate or elliptic, entire to serrate-crenate, petiolate to amplexicaul or decurrent. *Capitula* rarely solitary, generally many in terminal corymbs, generally heterogamous radiate to minutely radiate, or in few species heterogamous disciform, or homogamous discoid. *Involucral bracts* imbricate, usually unequal, in many rows, linear to ovate-lanceolate. *Receptacle* flat to ± convex, alveolate, generally glabrous, epaleate. *Marginal florets* female, radiate, with a three-toothed corolla, clearly longer than the involucre, sometimes as long as the involucre, exceptionally slightly shorter, almost tubular in some species, bright yellow, rarely purplish. *Disc florets* hermaphrodite, corolla with a five-toothed corolla, brightly yellow, rarely with purplish tinge. *Anthers* ecalcarate, with branched tails; endothecial tissue radial. *Style* not thickened, branches wide with acute sweeping-hairs not reaching the furcation. *Cypselae* homomorphic, ellipsoid or cylindrical, ribbed, glabrous or hairy, with twin hairs on the entire surface or only apically; epidermis cells with one elongated crystal each. Pappus of barbellate, capillary bristles, (5)8–35(45), one-rowed, exceptionally a few isolated innermost bristles, free or rarely slightly connate at the base.

**Distribution.** — Ca. 40 species distributed in SW, S, C Asia, Africa and Europe.

*Chromosome number.* – Chromosome base number  $x = 8$ .  
*Etymology.* – *Pentanema*, gr., n-, -*pén*te- five, and -*nema*- filament (number of pappus bristles).

The following combinations under *Pentanema* are required:

*Pentanema aschersonianum* (Janka) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula aschersoniana* Janka in Oesterr. Bot. Z. 22: 179. 1872.

*Pentanema auriculatum* (Boiss. & Balansa) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula auriculata* Boiss. & Balansa in Boissier, Diagn. Pl. Orient., ser. 2, 3: 13. 1856.

*Pentanema bifrons* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula bifrons* L., Sp. Pl., ed. 2: 1236. 1763.

*Pentanema britannicum* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula britannica* L., Sp. Pl.: 882. 1753.

*Pentanema confertiflorum* (A.Rich.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula confertiflora* A.Rich., Tent. Fl. Abyss. 1: 399. 1848.

*Pentanema conyzae* (Griess.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Aster conyzae* Griess., Kleine Bot. Schr.: 122. 1836.

*Pentanema ensifolium* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula ensifolia* L., Sp. Pl.: 833. 1753.

*Pentanema germanicum* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula germanica* L., Sp. Pl.: 883. 1753.

*Pentanema helenioides* (DC.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula helenioides* DC. in Candolle & Lamarck, Fl. Franc., ed. 3, 5 [= vol. 6]: 470. 1815.

*Pentanema helveticum* (Weber) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula helvetica* Weber, Pl. Min. Cogn. Decuria: 17. 1784.

*Pentanema hirtum* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula hirta* L., Sp. Pl.: 883. 1753.

*Pentanema langeanum* (Beck) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula langeana* Beck, Europ. Inula-Art.: 49. 1882.

*Pentanema maletii* (Maire) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula maletii* Maire in Bull. Soc. Hist. Nat. Afrique N. 13: 40. 1922.

*Pentanema mariae* (Bordz.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula mariae* Bordz. in Zap. Kievsk. Obshch. Estestvoisp. 25: 115. 1915.

*Pentanema montanum* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula montana* L., Sp. Pl.: 884. 1753.

*Pentanema oculus-christi* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula oculus-christi* L., Sp. Pl.: 881. 1753.

*Pentanema orientale* (Lam.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula orientalis* Lam., Encycl. 3: 255. 1789.

*Pentanema persicum* (DC.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Varthemia persica* DC., Prodr. 5: 473. 1836.

*Pentanema salicinum* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula salicina* L., Sp. Pl.: 882. 1753.

*Pentanema spiraeifolium* (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Inula spiraeifolia* L., Syst. Nat., ed. 10: 1219. 1759.

*Pentanema verbascifolium* (Willd.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** ≡ *Conyza verbascifolia* Willd., Sp. Pl. 3: 1924. 1803.

Several characters allow distinguishing *Inula helenium* from the species of *Pentanema* belonging to clade D. *Inula helenium* has resiniferous canals in the stem, while the species studied of *Pentanema* lack them. The external bracts of the involucre in *I. helenium* are ovate and bigger than those of *Pentanema*, which are linear to ovate-lanceolate and smaller. The shape and size of the cypselae are also different; in *I. helenium* they are prismatic (tetragonous, Fig. 4C) and up to 3–4(5) mm long, while in *Pentanema* they are terate or slightly compressed (Fig. 4), 0.5–3 mm long. The number of pappus bristles is higher in *I. helenium* (50–80, usually ca. 65) than in *Pentanema* (<45). *Inula helenium* has a base chromosome number of  $x = 10$ , while this number is  $x = 8$  in the species of *Pentanema* from clade D, which represents a clear synapomorphy for the group. Finally, while *I. helenium* is a large plant up to ca. 200 (250) cm high, only a few species of *Pentanema*, such as *P. bifrons*, *P. conyzae*, *P. helveticum*, *P. langeanum* and *P. maletii* grow to up to 125 cm high, and the remaining species are always smaller.

**Species monophyly and main lineages within *Pentanema*.**

— By including at least two samples per species, monophyly of most of them has been demonstrated. Only *P. auriculatum*, *P. britannicum* and *P. helenioides* were recovered as non-monophyletic (Fig. 1), which can be explained mainly by the low molecular variability found.

The species within subclade D1 are characterized by their long ray flowers, glabrous cypselae and xeromorphic leaves. This group is composed only of diploids and corresponds with Beck's (1882) group *leiocarpae*, a group without taxonomic rank considered by Beck into *I.* subsect. *Longeligulatae* Beck, and with the *I. salicina* group of Anderberg (1991).

*Pentanema divaricatum* and *P. multicaule* were recovered as sister species placed in an unresolved position within subclade D2 (Fig. 1). While *P. divaricatum* is a delicate annual found in the Middle East and Central Asia, *P. multicaule* is a suffruticose perennial endemic to the Middle East. Former studies (Anderberg & al., 2005; Englund & al., 2009) placed *P. glanduligerum* (Krasch.) Gorschk. and *P. vestitum* (Wall. ex DC.) Ling into the same clade as *P. divaricatum* and *P. multicaule*. The Turkish endemic *P. alanyense* H.Duman & Anderb. was recovered (Anderberg & al., 2005; Pornpongrungrueng & al., 2007; Englund & al., 2009) close to *Chrysophthalmum dichotomum* and *Ch. gueneri*. Part of the remaining *Pentanema* species are endemic suffrutices distributed in Central Asia (Pamir-Alay region and Afghanistan mainly). Although these groups of species are morphologically similar to *P. multicaule* or *P. glanduligerum*, further studies are required to resolve their phylogenetic position.

We also suggest to use the name *Vicoa cernua* Dalzell instead of *P. cernuum*. For the same reason, the following combination for *P. ligneum* is mandatory:

***Vicoa lignea*** (Mesfin) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** = *Pentanema ligneum* Mesfin in Kew Bull. 50: 404. 1995.

Subclade D2 comprises different subgroups. One is composed of *P. conyzae*, *P. bifrons* and *P. persicum*. Beck (1882) included *I. conyzae* and *I. bifrons* within *I.* subsect. *Breviligulatae*, because they have short ray flowers. Anderberg (1991) also recognized this morphological group and included *I. decurrens* Popov and *I. thapsoides* Spreng. in it. Our molecular phylogeny identifies *P. persicum*, the unique species within clade D that has homogamous, discoid capitula, as a member of this group (in accordance with Englund & al., 2009). The ray flowers of *P. conyzae* and *P. bifrons* are never longer than the internal involucre bracts (only rarely they exceed the bracts by 1–2 mm). A trend towards length reduction of ray flowers is observed within this group, with *P. persicum* representing the maximum reduction.

Also within subclade D2 a subgroup subdivided into three lineages was recovered. The first is composed of *P. orientale* and *P. auriculatum*, a species pair that have hairy ray flowers and leaves. Based on morphology Beck (1882) and Anderberg (1991) considered that *P. orientale* is related to the species recovered within subclade D1, because they have long ray flowers

and glabrous cypselae. *Pentanema auriculatum*, an endemic species from the Caucasus region, had been considered to be closely related to *P. oculus-christi* (Grierson, 1975; Gosrchkova, 1999) due to its morphology and distribution. A second lineage is composed of the polyploid species *P. britannicum*, *P. helenioides*, and *P. oculus-christi*, that together with *P. mariaea* (unknown ploidy level) form a group of species that have frequently been confused. This is probably due to the fact that the characters traditionally used for species identification (i.e., density of the indumentum of the leaves, number and size of capitula) are not clear. However, there are other morphological features which are more useful to identify these taxa, such as the position of the outer involucre bracts, the presence of multicellular hairs in the ray flowers, and the size of the cypselae. This second lineage corresponds to *lasiocarpae*, another group considered by Beck into *I.* subsect. *Longeligulatae* that matches also with the *I. britannica* clade of Anderberg (1991). Beck (1882) included *P. montanum* in this subsection, but this species in our analysis (Figs. 1–3) is recovered within the third lineage, together with *P. langeanum*, *P. maletii*, *P. verbascifolium* and *P. aschersonianum*. This third lineage groups together species endemic to the Mediterranean region and of variable ploidy levels, which had not been included until now in DNA sequence based phylogenetic analysis. *Pentanema langeanum* is endemic to C and N Iberian Peninsula, while *P. maletii* is only represented in the Middle Atlas Mountains of Morocco. *Pentanema verbascifolium* and *P. aschersonianum* are representatives of a species complex characterized by the presence of a more or less dense whitish sericeous indumentum covering the plant, distributed in the Balkan Peninsula and southern Italy. Finally, *P. montanum* is distributed from the Iberian Peninsula to Italy and North Africa and together with *P. verbascifolium* and *P. aschersonianum* are the diploid representatives of this group.

*Amblyocarpum* is the only remaining genus belonging to the *Inula* complex that was not included in our analysis. The phylogenetic position of this monotypic genus has, however, been demonstrated by Englund & al. (2009) and Nylander & Anderberg (2015) who recovered it in the *Pentanema* group; thus, we propose the following combination:

***Pentanema inuloides*** (Fisch. & C.A.Mey.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort., **comb. nov.** = *Amblyocarpum inuloides* Fisch. & C.A.Mey., Index Sem. Hort. Petrop. 3: 30. 1837.

**Phylogenetic analysis and incongruence.** — Several cases of incongruence have been found between the nuclear and plastid DNA datasets (Figs. 2, 3). Both the limitations in the use of molecular markers in phylogenetic reconstructions (Naciri & Linder, 2015) and the different problems derived from the use of multi-copy nrDNA markers (Alvarez & Wendel, 2003; Nieto-Feliner & Roselló, 2007) are well-known. Regarding our datasets, neither recombination, nor pseudogenes have been found in the nrDNA sequences analyzed and none of the ITS sequences showed lower GC contents than expected, so that these properties can be excluded as putative sources of the incongruence found. Poor taxon sampling (i.e., phylogenetic

error; particularly regarding clade A, which is not the particular focus of this study) and sampling error (i.e., low proportion of informative characters and consequent low resolution levels) may be producing part of the observed incongruence. Thus, the relationships between clades A and B, particularly among the species of *Carpesium* and *Chrysophthalmum*, are not resolved either in the nrDNA tree (dataset 2) or in the cpDNA phylogeny (Fig. 2). The low levels of resolution obtained using these markers may also explain the different positions of some samples in the cpDNA reconstruction as in the cases of *Telekia speciosa* and *Codonocephalum grande* (Fig. 2). Similarly, the low proportion of informative characters may be affecting the positions of the different samples representing the same species, as observed in *Pentanema aschersonianum*, *P. britannicum* or *P. helenioides* (Fig. 2).

Further sources of incongruence (Wendel & Doyle, 1998; Naciri & Linder, 2015) such as hybridization and/or incomplete lineage sorting (ILS) are much more difficult to detect based only on the available data (Holland & al., 2008; Degnan & Rosenberg, 2009). Additional analyses and information (e.g., population sizes and dating of divergence events) are required to evaluate the role of ILS in the evolution of the complex. Regarding hybridization, Englund & al. (2009) found incongruent topologies between plastid and nuclear markers affecting *Pentanema confertiflorum*, *Rhanteriopsis lanuginosa* and *Telekia speciosa* and argued that this could be due to ancient hybridization events. Our results would reinforce this view and add *Chrysophthalmum montanum* and *Rhanteriopsis puberula* as further examples of species hypothetically affected by this processes.

Considering the impact of polyploidy in the evolution of subclade D2 and the existence of mixed-ploidy populations (e.g., *Pentanema langeanum*; Santos-Vicente, 2013), the reticulate patterns observed in this clade may be best explained by hybridization and introgression. *Pentanema conyzae* appears closely related to *P. langeanum* in the plastid DNA phylogeny. In addition the two species are morphologically similar and are placed in the nrDNA tree within the lineage that groups species endemic to the Mediterranean region and, particularly, close to those from the Western Mediterranean area (i.e., *P. montanum*, *P. maletii*, *P. helenioides*). *Pentanema conyzae* is a tetraploid species related to *P. langeanum* that shows ploidy levels ranging from pentaploid to hexaploid (Santos-Vicente & al., 2009; 2013). Polyploidization events may have played an important role in the evolution of this group and future studies with other markers will specifically address these issues.

## ■ AUTHOR CONTRIBUTION

DGL, MMO and ER conceived this study. DGL and MSV produced the data. DGL analysed the data. DGL wrote the first draft which was enhanced and complemented by AAA, MMO and ER. — AAA, <https://orcid.org/0000-0003-1822-5235>, [arne.anderberg@nrm.se](mailto:arne.anderberg@nrm.se); DGL, <https://orcid.org/0000-0003-2758-8441>, [larruscain@usal.es](mailto:larruscain@usal.es); ER, <https://orcid.org/0000-0003-1769-0426>, [erico@usal.es](mailto:erico@usal.es); MMO, <https://orcid.org/0000-0002-3887-2416>, [mmo@usal.es](mailto:mmo@usal.es); MSV, [m.santos@usal.es](mailto:m.santos@usal.es)

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**Appendix 1.** Specimens collected with voucher information (country, locality, coordinates – if available –, altitude, date, collector and number), herbarium number or code (in brackets) and GenBank accession numbers (four codes per sample in the following order: ITS, *rps16-trnQ*, *rpl32-trnL*, *ndhF-rpl32*; or three codes per sample in the following order: *rps16-trnQ*, *rpl32-trnL*, *ndhF-rpl32*). Additional sequences obtained from GenBank (ITS sequences) are presented below.

*Carpesium cernuum* L.: Cult. Bergius Botanic Garden, Stockholm, *Englund 04-004* (S), KY696283\*, KY696334\*, KY696409\*, KY696484\*. *Carpesium divaricatum* Sieb. & Zucc.: TAIWAN: Chung & Anderberg 1422 (S), KY696335\* KY696410\* KY696485\*. *Carpesium macrocephalum* Franch. & Sav.: Cult. Bergius Botanic Garden, Stockholm, *Englund 04-005* (S), KY696345\*, KY696420\*, KY696495\*. *Chrysophthalmum dichotomum* Boiss. & Heldr.: TURKEY: Duran 3018 (S), KY696336\*, KY696411\*, KY696486\*. *Chrysophthalmum gueneri* Aytaç & Anderb.: TURKEY: Duman 7072 (S), KY696339\*, KY696414\*, KY696489\*. *Chrysophthalmum montanum* (DC.) Boiss.: TURKEY: Malatya, 31 km NE Gölbasi, ca. 4 km SW Erkenek, 1300 m, 18-VII-1978, F. Ehrendorfer, F. Sorger, D. Fürtkrantz, M.A. Fischer & A. Öztürk 787-65B-3 (WU), KY696289\*, KY696344\*, KY696419\*, KY696494\*. Nigde, Ala Gagliari, Karamük Bogazi ravine south Demirkazik Köy, 37°42'N 35°25'E, 1750 m, 14-VIII-1992, P. Hein AG 284-43 (B 10 0158349), KY696285\*, KY696340\*, KY696415\*, KY696490\*. Adana, Pozanti, 3 miles north on eastward slopes of Toro Dagi, 13-VII-1991, *Aberdeen Univ. Amanus Expedition A251* (E00591564), KY696286\*, KY696341\*, KY696416\*, KY696491\*. Adana, Pozanti, 3 miles north of the eastward slopes of Toros Dagi, 1100 m, 13-VII-1991, *Aberdeen Univ. Amanus Expedition A118* (E00591565), KY696287\*, KY696342\*, KY696417\*, KY696492\*. IRAK: Reching 13-15169 (S), KY696288\*, KY696343\*, KY696418\*, KY696493\*. *Inula auriculata* Boiss. & Balansa: ARMENIA: Aragatsotn, Aragat Mt., Ghazaravan, L. Medina & al. LM2608 (SALA 136002), KY696292\*, KY696349\*, KY696424\*, KY696499\*. Vayots Dzor, Vardenis slopes, ca. 5 km of the Selim's cross, 39°56'30"N 45°14'07"E, 2100 m, 23-VI-2005, A. Herrero & al. AH2654 (SALA 136003), KY696559\*, KY696348\*, KY696423\*, KY696498\*. *Inula bifrons* L.: BULGARY: Rhodopes Centrales, Buynovsko Gorge, 41°38'30"N 24°20'03"E, 1040 m, 02-VII-2004, C. Navarro & al. CN4778 (SALA 135422), KY696296\*, KY696350\*, KY696425\*, KY696500\*. FRANCE: Alpes-de-Haute-Provence, Reillanne, M. Santos Vicente & L. Delgado MS777 (SALA 135421), KY696294\*, KY696351\*, KY696426\*, KY696501\*. ITALY: Alessandria, Tortona, slopes of the Tortona's castle, 32TMQ8971, 44°53'38.2"N 08°52'12.1"E. 368 m, 28-VII-2006, M. Santos Vicente & L. Delgado MS783 (SALA 136031), KY696295\*, KY696352\*, KY696427\*, KY696502\*. SPAIN: Segovia, Sacramenia, Coto de San Bernardo, 30TVL2394, 900 m, 10-VIII-2007, M. Santos Vicente & L. Delgado MS948 (SALA 135422), KY696296\*, KY696362\*, KY696437\*, KY696512\*. AUSTRIA: Niederösterreich, Marchegg, M. Santos-Vicente & L. Delgado MS810 (SALA 136030), KY696560\*, KY696355\*, KY696430\*, KY696505\*. GREECE: Kalomira, 39°44'48.5"N 21°23'46.3"E. 1032 m, 25-VI-2009, M. Santos Vicente, X. Giráldez, M.M. Martínez Ortega & B. Rojas Andrés MS1263 (SALA 160094), KY696297\*, KY696354\*, KY696429\*, KY696504\*. *Inula confertiflora* A.Rich.: ETHIOPIA: Hylander & al. 288 (UPS), KY696359\*, KY696434\*, KY696509\*. *Inula conyzae* (Griess.) DC.: ITALY: Trieste, Duino castle, M. Santos Vicente & L. Delgado MS789 (SALA 136026), KY696562\*, KY696362\*, KY696437\*, KY696512\*. SPAIN: Burgos: Panizares, 30TVN63, 750 m, M. Santos Vicente & M.M. Martínez Ortega MS1220 (SALA 136119), KY696300\*, KY696360\*, KY696435\*, KY696510\*. Santander, Valdeprado del Río, Arcera, 30TVN1347, 42°52'14.8"N 04°03'38.0"W, 824 m, 04-VIII-2005, M. Santos Vicente & L. Delgado MS625 (SALA 135803), KY696301\*, KY696361\*, KY696436\*, KY696511\*. *Inula ensifolia* L.: AUSTRIA: Wien, Mödling, pr. Eichkogel, 33UWP9624, 48°03'57.1"N 16°17'33.3"E, 320 m, 21-VII-2005, M. Santos Vicente, L. Delgado, M.M. Martínez Ortega & L.M. Muñoz Centeno MS608 (SALA 136034), KY696303\*, KY696365\*, KY696440\*, KY696515\*. ITALY: Udine, Gemona del Friuli, Quarman Mt., M. Santos Vicente, Delgado, M.M. Martínez Ortega & L.M. Muñoz Centeno MS600 (SALA 135420) KY696563\*, KY696364\*, KY696439\*, KY696514\*. TURKEY: Kiklareli, 6 km from Vize to Kömürköy-Alk, 41°35'41"N 27°49'27"E, 388 m, 23-VI-2009, B. Rojas Andrés, X. Giráldez, M.M. Martínez Ortega & M. Santos Vicente BR46 (SALA 160095), KY696302\*, KY696363\*, KY696438\*, KY696513\*. AUSTRIA: Wien, Mödling, road to Eichkogel, 33UWP9623, 48°03'43.2"N 16°17'30.6"E, 322 m, 21-VII-2005, M. Santos Vicente, L. Delgado, M.M. Martínez Ortega & L.M. Muñoz Centeno MS614 (SALA 136035), KY696305\*, KY696367\*, KY696442\*, KY696517\*. ARMENIA: Syunik, Nerkin Hand vil, Gonzalo & al. RG301 (SALA 136006), KY696564\*, KY696368\*, KY696443\*, KY696518\*. BULGARY: Plovdiv, Popovitsa, surroundings of Popovitsa, 42°07'24.5"N 25°04'24.8"E, 212 m, 18-VI-2009, M. Santos Vicente, X. Giráldez, M.M. Martínez Ortega & B. Rojas Andrés MS1225 (SALA 160096), KY696304\*, KY696366\*, KY696441\*, KY696516\*. *Inula grandis* Schrenk: AFGHANISTAN: Badakshan, 30.0 km south Keshim, 36.5527°N 70.1817°E, 2101 m, 23-VI-2009, PEACE PROJECT, 2055 (E00392438), KY696306\*, KY696369\*, KY696444\*, KY696519\*. *Inula helenioides* DC.: SPAIN: La Rioja, Lagunilla del Jubera, towards Zenzano, M. Santos Vicente, A. Abad, S. Andrés-Sánchez & M.M. Martínez Ortega MS759 (SALA 136023), KY696568\*, KY696370\*, KY696445\*, KY696520\*. Soria, Burgo de Osmo-Ciudad de Osmo, Santiuste, 30TWM0308, 41°37'28.3"N 02°57'35.7"W, 950 m, 23-VIII-2007, M. Santos Vicente & L. Delgado MS953 (SALA 135800), KY696307\*, KY696371\*, KY696446\*, KY696521\*. *Inula helenium* (L.) Mérat: ARMENIA: Vayots Dzor, Ger-Ger, A. Herrero & al. AH2848 (SALA 136001), KY696284\*, KY696337\*, KY696412\*, KY696487\*. SPAIN: Soria, Abejar, road to Molinos de Duero, 30TWM1728, 41°48'30.8"N 02°47'25.4"W, 1100 m, 23-VIII-2007, M. Santos Vicente, L. Delgado & C. Molina Martín MS957 (SALA 135423), KY696565\*, KY696338\*, KY696413\*, KY696488\*. *Inula helvetica* Weber: SPAIN: Álava, Lantarón, Puentelarrá, M. Santos Vicente, A. Abad, S. Andrés Sánchez & M.M. Martínez Ortega MS752 (SALA 135459), KY696567\*, KY696377\*, KY696452\*, KY696527\*. Burgos, Jurisdicción de San Zadornil, San Millán de San Zadornil, Omecillo river, 30TVN8745, 42°51'28.1"N 03°09'21.9"W, 610 m, 12-VII-2004, M. Santos Vicente, S. Andrés Sánchez & J.A. Sánchez Agudo MS407 (SALA 135417), KY696311\*, KY696376\*, KY696451\*, KY696526\*. Lérida, Espot, entre el pueblo y el Camping Voraparc, 31TCH4237716383, 5-IX-2009, D. Pinto Carrasco, P. Marcos Villaverde & E. Rico DP620 (SALA 135669), KY696310\*, KY696375\*, KY696450\*, KY696525\*. *Inula hirta* L.: AUSTRIA: Wien, Mödling, Eichkogel, 33UWP9624, 48°03'49.9"N 16°17'33.8"E, 351 m, 21-VII-2005, M. Santos Vicente, L. Delgado, M.M. Martínez Ortega & L.M. Muñoz Centeno MS612 (SALA 136039), KY696566\*, KY696373\*, KY696448\*, KY696523\*. BULGARY: Sliven, Natural parl of Sinite Kamani, 42°44'04.4"N 26°17'24.2"E, 834 m, 20-VI-2009, B. Rojas Andrés, X. Giráldez, M.M. Martínez Ortega & M. Santos Vicente BR35 (SALA 160097), KY696308\*, KY696372\*, KY696447\*, KY696522\*. ITALY: Udine, between Cividale de Friuli and Castelmonte, M. Santos Vicente & L. Delgado MS792 (SALA 136028), KY696309\*, KY696374\*, KY696449\*, KY696524\*. *Inula langeana* Beck: SPAIN: Álava, Lantarón, Puentelarrá, M. Santos Vicente, A. Abad, S. Andrés-Sánchez & M.M. Martínez-Ortega MS755 (SALA 136022), KY696313\*, KY696380\*, KY696455\*, KY696530\*. Burgos, Panizares, 30TVN63, 750 m, M. Santos Vicente & M.M. Martínez-Ortega MS1220 (SALA 136119), KY696312\*, KY696378\*, KY696453\*, KY696528\*. Burgos, Jurisdicción de San Zadornil, San Millán de San Zadornil, Alto Yerdos, 30TVN8943, 42°50'43.8"N 03°07'58.6"W, 645 m, 12-VII-2004, M. Santos Vicente, S. Andrés Sánchez & J.A. Sánchez Agudo MS408 (SALA 136047), KY696569\*, KY696379\*, KY696454\*, KY696529\*. *Inula maletii* Maire: MOROCCO: Tadla-Azilal, Col de Tanout ou Fillal, A. Quintanar & al. AQ2153 (SALA 136007), KY696570\*, KY696383\*, KY696458\*, KY696533\*. Middle Atlas, Ifrane, Tizi-n-Tretten, 33°27'32"N 05°02'09"W, 1980 m, 10-VII-2006, A. Herrero & al. AH3347 (SALA 136015), KY696314\*, KY696381\*, KY696456\*, KY696531\*. Beni Mellal, El Ksiba, 32°30'36"N,

## Appendix 1. Continued.

06°00'04"W, 1878 m, 05-VII-2006, *A. Quintanar & al. AQ2043* (SALA 136016), KY696315\*, KY696382\*, KY696457\*, KY696532\*. *Inula mariae* Bordz.: ARMENIA: Syunik, pr. Vil. Tandzatap and Ahavni, *Gonzalo & al. RG109* (SALA 136008), KY696571\*, KY696384\*, KY696459\*, KY696534\*. *Inula montana* var. *calycina* (Presl) Batt. MOROCCO: Marrakech-Tensift-Al Haouz, Adrar-n-Oukaïmeden, *A. Herrero & al. AH3113* (SALA 136009), KY696561\*, KY696356\*, KY696431\*, KY696506\*. High Atlas, Oukaïmeden, 31°11'52"N, 07°51'55"W, 30-VI-2006, *A. Herrero & al. AH3215* (SALA 136064), KY696298\*, KY696357\*, KY696432\*, KY696507\*. Circle of Jaffar, slope N, 32°32'17"N, 04°54'01"W, 2160 m, 07-VII-2006, *S. Castroviejo & al. SC17917* (MA745022), KY696299\*, KY696358\*, KY696433\*, KY696508\*. *Inula montana* L. var. *montana*: FRANCE: Bouches-du-Rhône, Le Tholonet, Aix-en-Provence, 31TGJ0421, 43°31'10.0"N, 05°31'47.6"E, 253 m, 27-VII-2006, *M. Santos Vicente & L. Delgado MS778* (SALA 136041), KY696318\*, KY696387\*, KY696462\*, KY696537\*. SPAIN: León, Cistierna, Valmartino, near the river, 30TUN2839, 42°47'26.9"N 05°05'45.1"W, 1090 m, 03-VIII-2005, *M. Santos Vicente & L. Delgado MS619* (SALA 136076), KY696317\*, KY696386\*, KY696461\*, KY696536\*. Segovia, Sepúlveda, road to San Frutos del Duratón, 30TVL3571, 41°17'55"N 03°45'17"W, 986 m, 15-VI-2005, *M.M. Martínez Ortega, X. Giráldez & L.M. Muñoz Centeno MO1832* (SALA 136013), KY696316\*, KY696385\*, KY696460\*, KY696535\*. *Inula oculus-christi* L.: AUSTRIA: Wien, Mödling, Eichkogel, *M. Santos Vicente, L. Delgado, M.M. Martínez-Ortega & L.M. Muñoz-Centeno MS610* (SALA 136019), KY696572\*, KY696389\*, KY696464\*, KY696539\*. Wien, Mödling, Eichkogel, 21-VII-2005, *M. Santos Vicente, L. Delgado, M.M. Martínez Ortega & L.M. Muñoz Centeno MS613* (SALA 160385), KY696320\*, KY696390\*, KY696465\*, KY696540\*. BULGARY: Plovdiv, between Oreshets and Dobrostan, near to the road, 41°53'44.2"N 24°55'58.0"E, 1109 m, 18-VI-2009, *M. Santos Vicente, S. Andrés Sánchez, X. Giráldez, M.M. Martínez Ortega & B. Rojas Andrés MS1222* (SALA 160098), KY696319\*, KY696388\*, KY696463\*, KY696538\*. *Inula orientalis* Lam.: GEORGIA: Mtskheta-Mtianeti, Kazbegi, east Kazbegi, 42°39'N 44°40'E, 1850 m, 20-VIII-1997, *P. Schönswetter & A. Trisbsch 39* (WU), KY696321\*, KY696323\*, KY696394\*, KY696466\*, KY696541\*. ARMENIA: Tavush, Haghartsin monastery, *Castroviejo & al. SC17478* (SALA 136012), KY696573\*, KY696392\*, KY696467\*, KY696542\*. Tavush, between Haghartsin village and cross road to Chambarak, 40°46'12"N 45°00'33"E, 945 m, 21-VI-2005, *S. Castroviejo & al. SC17645* (SALA 136014), KY696322\*, KY696393\*, KY696468\*, KY696543\*. SPAIN: Burgos, Trespaderne, up to the Tejada's castle, 30TVN6638, 42°47'53.8"N, 03°24'16.8"W, 620 m, 25-VI-2005, *M. Santos Vicente & L.P. Gavilán MS588* (SALA 136081), KY696323\*, KY696394\*, KY696469\*, KY696544\*. *Inula spiraeifolia* L.: ITALY: Trieste, Lamiano, Monfalcone, *M. Santos Vicente & L. Delgado MS791* (SALA 136027), KY696325\*, KY696396\*, KY696471\*, KY696546\*. Trieste, Duino, Duino's castle, 33TUL9269, 45°46'19.5"N 13°36'52.8"E, 75 m, 30-VII-2006, *M. Santos Vicente & L. Delgado MS788* (SALA 136091), KY696324\*, KY696395\*, KY696470\*, KY696545\*. *Inula verbascifolia* Hausskn. GREECE: Peloponesus, Lakonia, Taigetos mts., Lagadha gorge, 37°05'38"N 22°20'08"E, 500 m, VI/VIII-2008, *C. Aedo & al. CA14233* (SALA 140779), KY696326\*, KY696397\*, KY696472\*, KY696547\*. *Inula verbascifolia* subsp. *archersoniana* (Janka) Tutin.: BULGARY: Plovdiv, Bačkov, natural park of Červenata Stena ("The Red Wall"), 41°56'16.7"N 24°51'46.3"E, 526 m, 18-VI-2009, *M. Santos Vicente, X. Giráldez, M.M. Martínez Ortega & B. Rojas Andrés MS1220bis* (SALA 160091), KY696290\*, KY696346\*, KY696421\*, KY696496\*. Plovdiv, between Oreshets and Dobrostan, near the road, 41°53'47.3"N 24°55'53.6"E, 1128 m, 18-VI-2009, *M. Santos Vicente, X. Giráldez, M.M. Martínez Ortega & B. Rojas Andrés MS1223* (SALA 160092), KY696291\*, KY696473\*, KY696548\*. *Pentanema divaricatum* Cass.: OMAN: Musandam Khasba, near recharge dam, 26°12'N 56°15'E, 0–250 m, 13-III-1994, *I. McLeish 3506* (E00100893), KY696328\*, KY696399\*, KY696474\*, KY696549\*. IRAN: *Rechinger 19401* (S), KY696327\*, KY696398\*, KY696473\*, KY696548\*. *Pentanema indicum* (L.) Ling: NEPAL: Karnali zone, Humla District, north side of Thanke Khola, 29°44'59"N 82°03'19"E, 1660 m, 14-VI-2008, *C.A. Pendry, S. Baral, S. Noshiro, P.P. Kurmi, B. Dell, B. Adhikari JRS B132* (E00397278), KY696329\*, KY696401\*, KY696476\*, KY696551\*. *Pentanema multicaulis* Boiss.: IRAN: *Karis 732* (S), KY696402\*, KY696477\*, KY696552\*. *Pulicaria dysenterica* (L.) Bernh.: FRANCE: Bouches-du-Rhône, Vauvernargues, *M. Santos Vicente & L. Delgado MS779* (SALA 136025), KY696574\*, KY696400\*, KY696475\*, KY696550\*. *Rhanteropsis lanuginosa* (DC. & Boiss.) Rauschert: SYRIA: Palmyra, Jebel al Todmor, 90 km SW Palmyra, 900 m, 9-VIII-1983, *W. Frey & H. Kürschner 83-666* (E00586802), KY696330\*, KY696403\*, KY696478\*, KY696553\*. *Rhanteropsis puberula* (Boiss. & Hausskn.) Rauschert: IRAN: Loristan, Dow Rud, 1500–1600 m, 17-VI-1974, *K.H. Rechinger 48198* (S), KY696331\*, KY696404\*, KY696479\*, KY696554\*. *Telekia speciosa* (Schreb.) Baumg.: SLOVENIA: Postojna, between Postojna and Cerkljansko lake, *M. Santos Vicente & L. Delgado MS806* (SALA 136029), KY696575\*, KY696405\*, KY696480\*, KY696555\*. *Varthemia persica* D.C.: IRAN: Fars, S.E. of Tal-i-Khusrovi, 2470 m, 23-VII-1966, *J.C. Archibald 2835* (E00591725), KY696333\*, KY696408\*, KY696483\*, KY696558\*. Origin unknown, *Akhani, 11698* (W), KY696406\*, KY696481\*, KY696556\*. AFGHANISTAN: Kabul, Salam Pass, 35°20'N 69°10'E, 26-VIII-1965, *J.D.A. Stainton 5069* (E00591722), KY696332\*, KY696407\*, KY696482\*, KY696557\*.

## Additional ITS sequences from GenBank:

*Pallenis maritima* (L.) Greuter: U84772.1. *Blumea balsamifera* (L.) DC.: voucher ZY130604, KF443296.1. *Buphthalmum salicifolium* L.: voucher *Anderberg & Anderberg 7292* (S), EF210964.1. *Caesulia axillaris* Roxb.: voucher *Pandry 3021* (S), EU195642.1. *Carpesium divaricatum* Sieb. & Zucc.: TAIWAN: *Chung & Anderberg 1422* (S), EF210965. *Carpesium glossophyllum* Maxim.: voucher ANH 00090813, JQ062521.1. *Carpesium macrocephalum* Franch. & Sav.: *Englund 04-005* (S), FM995368. *Carpesium rosulatum* Miq.: voucher KH 42142, JQ062523.1. *Carpesium triste* Maxim.: voucher KWNW 070450, JQ062525.1. *Chrysophthalmum dichotomum* Boiss. & Heldr.: TURKEY: *Duran 3018* (S), FM995369. *Chrysophthalmum gueneri* Aytaç & Anderb.: TURKEY: *Duman 7072* (S), FM995370. *Dittrichia viscosa* (L.) Greuter: *E.J. Tepe 1143*, GU818558.1. *Duhaldea cappa* (Buch.-Ham. ex D. Don) Pruski & Anderb.: KP092568.1. *Inula confertiflora* A.Rich.: ETHIOPIA: *Hylander & al. 288* (UPS), FM995375. *Inula japonica* Thunb.: voucher PS0698MT05, GU724300.1. *Inula paniculata* (Klatt) Burt Davy: *Hylander & al. 231* (UPS), FM995378.1. *Inula peacockiana* (Aitch. & Hemsl.) Korovin: *Rechinger & Rechinger 4956* (S), FM995379.1. *Inula shirensis* Oliv.: *Milne-Redhead & Taylor 10276* (B), FM995380.1. *Iphiona scabra* DC. ex Decne.: AF545823.1. *Jasonia tuberosa* (L.) DC.: U84779.1. *Limbarda crithmoides* (L.) Dumort.: *Anderberg*, (S), FM995387.1. *Pallenis spinosa* (L.) Cass.: *Karis 951* (S), EF210968.1. *Pentanema multicaulis* Boiss.: IRAN: *Karis 732* (S), F2908982. *Pulicaria glutinosa* (Boiss.) Jaub & Spach: *FSI19* (KSU), KF815511. *Pulicaria lanata* E. Gamal-Eldin: AY165044.1. *Pulicaria odora* (L.) Rechb.: *M. Ait Lafkih 110*, HE602393.1. *Pulicaria schimperi* DC.: *FSI92* (KSU), KF815513.1. *Pulicaria vulgaris* Gaertn.: KJ004326.1. *Rhanterium epapposum* (Oliv.): *Nilsson & al.* (S), EF210970.1. *Stenachaenium campestre* Baker: *Englund 05-001* (S), EF210931.1. *Varthemia persica* DC.: IRAN: *Akhani 11698* (W), FM995408.

## Appendix 2. Details about plant material used for SEM (country, locality, coordinates, altitude, date, collector and voucher).

*Inula helenioides* DC.: SPAIN: Soria, Hinojosa de la Sierra, 41°51'38.7"N 02°35'52.6"W, 1032 m, 18-VIII-2016, *D. Gutiérrez Larruscain, S. Andrés Sánchez & D. Pinto Carrasco DG807* (SALA160634). *Inula helenium* L.: SPAIN: Soria, Abejar, 41°48'30"N 02°47'25"W, 1100 m, 18-VIII-2016, *D. Gutiérrez Larruscain, S. Andrés Sánchez & D. Pinto Carrasco DG808*. *Inula montana* L.: SPAIN: Zaragoza, Nombrevilla, 41°06'26.7"N 01°19'36.5"W, 968 m, 18-VII-2016, *S. Andrés Sánchez, D. Gutiérrez Larruscain & M.M. Martínez Ortega SA1162*. *Inula salicina* L.: SPAIN: Toledo, San Pablo de los Montes, 30SUJ1936 76587, 980 m, 16-VII-2016, *D. Gutiérrez Larruscain, S. Andrés Sánchez, V. Arán, N. López González & M.M. Martínez Ortega DG788*. *Pentanema divaricatum* Cass.: OMAN: Musandam Khasba, near recharge dam, 26°12'N 56°15'E, 0–250 m, 13-III-1994, *I. McLeish 3506* (E00100893). *Varthemia persica* DC.: IRAN: Fars, S.E. of Tal-i-Khusrovi, 2470 m, 23-VII-1966, *J.C. Archibald 2835* (E00591725).