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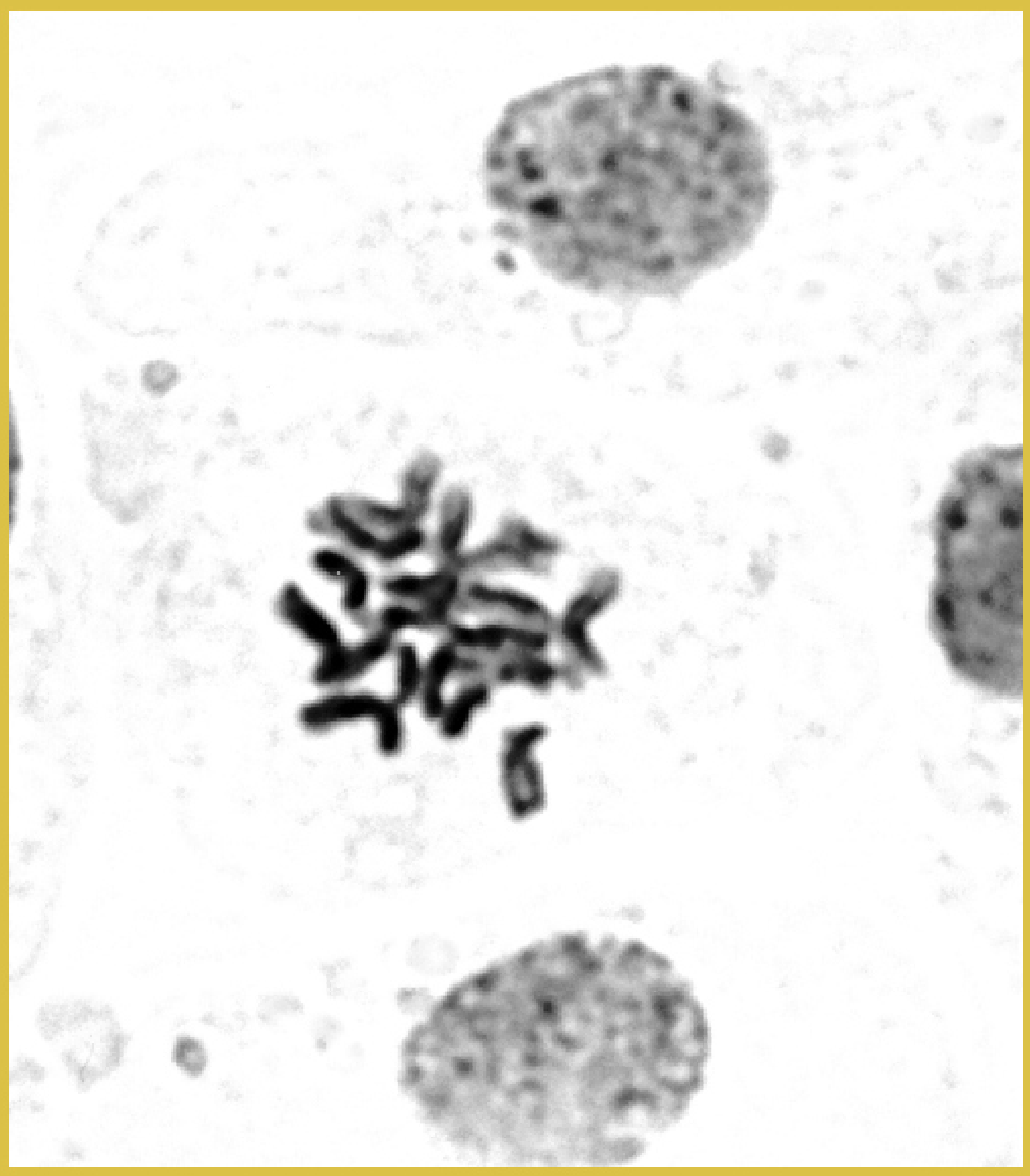
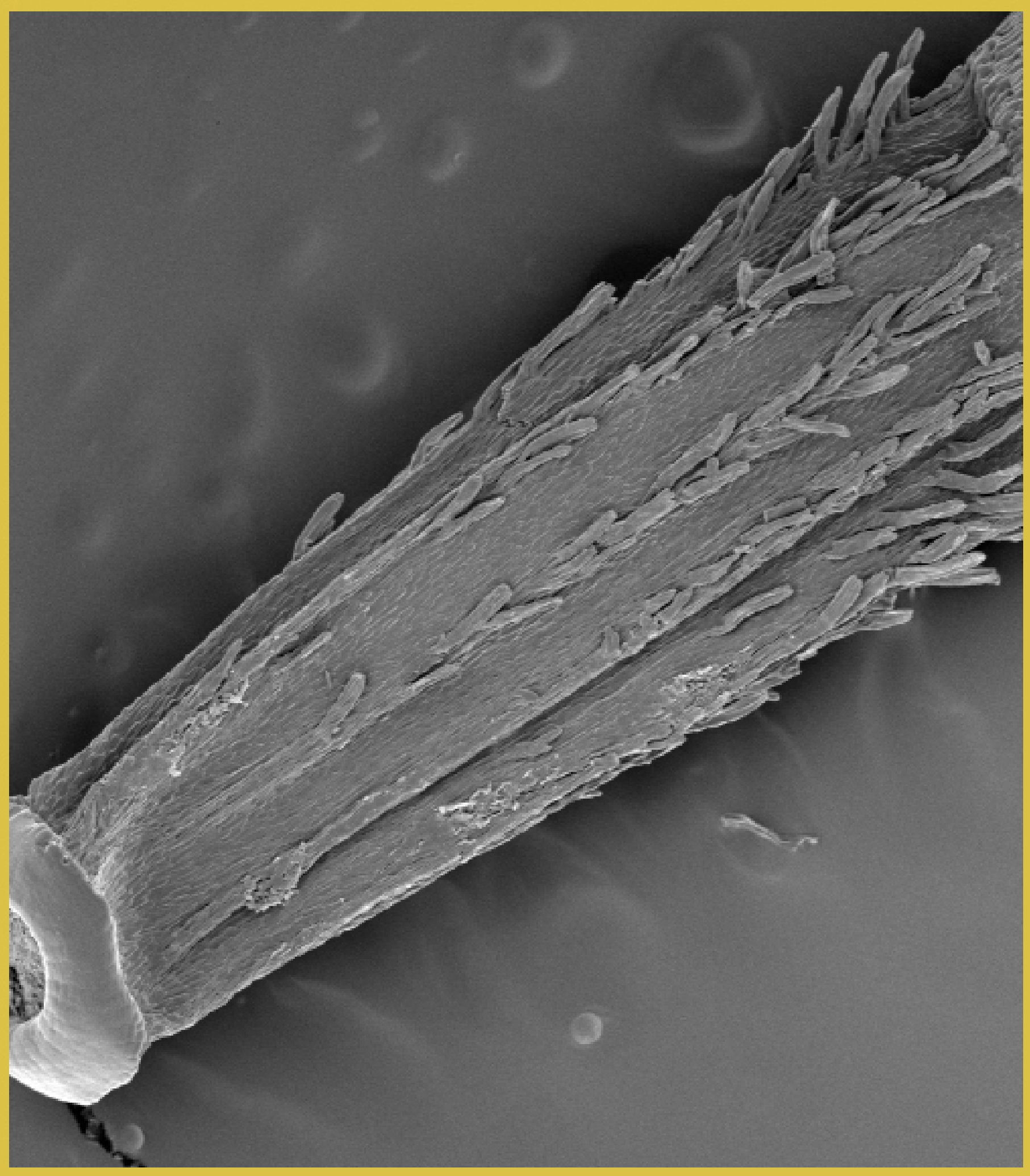
FACULTAD DE BIOLOGÍA
DEPARTAMENTO DE BOTÁNICA

Estudio biosistemático del género *Inula* L. (Asteraceae) en el Mediterráneo occidental

TESIS DOCTORAL
María Santos Vicente
Salamanca, 2013

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Estudio biosistemático del género *Inula* L. (Asteraceae) en el Mediterráneo occidental

Memoria que para optar al grado de **Doctora en Biología** por la Universidad de Salamanca presenta la licenciada **María Santos Vicente**

VºBº del Director

VºBº de la Directora

D. Enrique Rico Hernández

Dª Mª Montserrat Martínez Ortega

Salamanca, 2013

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D. ENRIQUE RICO HERNÁNDEZ, Catedrático de Universidad del Departamento de Botánica de la Universidad de Salamanca y Dª Mª MONTSERRAT MARTÍNEZ ORTEGA, Profesora Titular del Departamento de Botánica de la Universidad de Salamanca,

INFORMAN: En calidad de directores de la Memoria de Tesis Doctoral titulada "**Estudio biosistemático del género *Inula* L. (Asteraceae) en el Mediterráneo occidental**", realizada por la Licenciada en Biología Dª María Santos Vicente, consideran finalizado el trabajo y autorizan la presentación, a fin de que pueda ser juzgada por el Tribunal correspondiente.

Y para que así conste, firmamos el presente informe en Salamanca, a 11 de junio de 2013.

D. Enrique Rico Hernández		Dª Mª Montserrat Martínez Ortega

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Índice de contenidos (detallado)

CONCLUSIONES

1. El análisis filogenético basado en datos de secuenciación de la región ITS del ADN ribosómico nuclear, apoyado por caracteres morfológicos y cariológicos, pone de manifiesto que el género *Inula* L. en su circunscripción tradicional (*Inula* s.l.) es parafilético.

2. Las cuatro secciones reconocidas tradicionalmente en *Inula* s.l. (i.e., *I.* sect. *Corvisartia*, *I.* sect. *Enula*, *I.* sect. *Limbarda* e *I.* sect. *Cupularia*) se emplazan en cuatro clados diferentes, con géneros externos anidados entre ellos.

3. De acuerdo con los datos moleculares, las especies europeas y norteafricanas de *Inula* s.l. se agrupan en tres clados: clado *Corvisartia* + *Telekia*, clado *I. salicina* y clado *I. conyzae*, este último a su vez formado por tres clados (clados *I. bifrons*, *I. montana* e *I. britannica*).

4. Sobre la base de caracteres del ADN, macro- y micromorfológicos y cariológicos, se considera que el género *Corvisartia* Mérat debe ser reconocido como un género independiente. Este género incluye *Inula helenium* L. – actual tipo de *Inula* – y otras especies ajenas al área de estudio.

5. Del mismo modo, *Limbarda* Adans. también se considera un género independiente, que en el Mediterráneo Occidental sólo incluye la especie *L. crithmoides* (L.) Dumort..

6. Tanto el análisis filogenético de máxima parsimonia efectuado sobre secuencias de la región ITS del ADN ribosómico nuclear, como el análisis bayesiano de las mismas, diferencian claramente *Inula langeana* Beck e *I. conyzae* (Griess.) Meikle, especies morfológicamente bastante similares, pero que quedan emplazadas en clados diferentes. Esta separación está también apoyada por evidencias macro- y micromorfológicas.

7. La poliploidía ha jugado un papel importante en la evolución de todos los linajes que se integran en *Inula* s.s., excepto en el clado *I. salicina*. Se han hallado cinco niveles de ploidía dentro del género ($2n = 2x = 16$ a $2n = 6x = 48$) y al menos cuatro especies muestran diferentes citótipos [i.e., *I. oculus-christi* ($2x$, $4x$), *I. britannica* ($2x$, $3x$, $4x$), *I. helenioides* ($4x$, $5x$) e *I. langeana* ($4x$, $5x$, $6x$)]. Se aportan además nuevos recuentos cromosómicos para siete taxones.

8. La reconstrucción de estados de carácter ancestrales para datos cariológicos indicaría que $x = 9$ es el número básico ancestral para los representantes de Inuleae estudiados; *Corvisartia* tiene un número básico de $x = 10$, mientras que $x = 8$ se revela como una sinapomorfía clara para *Inula* s.s..

9. La reconstrucción de estados de caracter ancestrales para datos morfológicos revela que el receptáculo epaleado, las flores liguladas largas (que exceden en más de 3 mm las brácteas del involucro) y el aquenio glabro son estados plesiomórficos para *Inula* s.s..

10. Se ha estudiado por primera vez al microscopio electrónico de barrido una serie de caracteres micromorfológicos relacionados con aquenio y vilano, así como el indumento de tallos, hojas y flores liguladas; se describen tipos de pelos y patrones de indumento para todas las especies estudiadas.

11. La separación de *Corvisartia* como género independiente se apoya también en caracteres micromorfológicos. El tamaño y forma del aquenio, la inserción, longitud y número de setas del vilano, así como la presencia de papilas en las hojas, permiten diferenciar claramente *Corvisartia* de *Inula*.

12. El indumento del aquenio muestra señal filogenética, tanto la densidad como la composición del mismo. Se reconocen tres tipos de pelos en el aquenio: pelos tectores biseriados (*twin hairs*) elongados, pelos tectores biseriados (*twin hairs*) baculados y pelos glandulares biseriados. La presencia de pelos tectores biseriados elongados parece fijarse definitivamente en el clado *Inula conyzae*.

13. Los caracteres micromorfológicos son también de gran utilidad taxonómica. Por ejemplo, permite separar *Inula spiraeifolia* de *I. salicina*, con la que se ha confundido frecuentemente. *I. spiraeifolia* se caracteriza por tener lígulas con pelos glandulares biseriados y hojas esparcidamente vilosas con dos tipos de pelos (pelos tectores multicelulares y pelos glandulares uniseriados), mientras que *I. salicina* tiene lígulas glabras y hojas glabras o glabrescentes, sólo con pelos tectores multicelulares.

14. Sobre la base de todos estos caracteres (moleculares, macro- y micromorfológicos y cariológicos) se propone, por tanto, una nueva circunscripción para el género *Inula* que conlleva cambios nomenclaturales; destaca la propuesta de conservación del nombre *Inula* con un tipo conservado y la designación de un nuevo tipo para el género (*I. hirta*) a fin de evitar un número elevado de cambios nomenclaturales.

15. Se proponen cuatro combinaciones nuevas a nivel subespecífico motivadas por la separación del género *Corvisartia*: *C. helenium* subsp. *turcoracemosa* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *orgyalis* (Boiss.) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *pseudohelenium* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico y *C. helenium* subsp. *vanensis* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico.

16. Se realizan 27 nuevas lectotipificaciones, entre las que destacan las de cinco nombres aceptados: *I. helenioides* DC., *I. helvetica* Grauer, *I. langeana* Beck, *I. maletii* Maire y *I. montana* L.. Adicionalmente se llevan a cabo 4 neotipificaciones.
17. Sobre la base de un detallado estudio de caracteres macro- y micromorfológicos efectuado mediante una exhaustiva revisión de material –tanto de herbario como vivo– y de toda la literatura relevante para el grupo, y de acuerdo con los datos obtenidos a partir de los análisis filogenéticos de secuencias de ADN, se ha llevado a cabo una revisión taxonómica de los géneros *Inula* (14 especies), *Corvisartia* (1 especie) y *Limbarda* (1 especie) en el Mediterráneo occidental. Las síntesis taxonómicas resultantes incluyen las descripciones del género y especies correspondientes, revisión nomenclatural, iconografías, distribución y observaciones; en el caso de *Inula* se incluyen además dos claves de identificación: una para los taxones del Mediterráneo occidental (14) y otra para los taxones que se encuentran en la Península Ibérica e Islas Baleares (7).

CONCLUSIONS

1. The molecular phylogenetic analysis based on DNA sequences of the ITS region (rDNA) shows that the genus *Inula* L. as traditionally circumscribed (*Inula* s.l.) is paraphyletic, which is also supported by morphological and karyological data.
2. The four sections traditionally recognised in *Inula* s.l. (i.e., *I.* sect. *Corvisartia*, *I.* sect. *Enula*, *I.* sect. *Limbarda* and *I.* sect. *Cupularia*) are placed into four different clades, with external genera nested among them.
3. According to the molecular phylogenetic analysis performed, the European and North-African species of *Inula* s.l. are grouped into three clades: *Corvisartia* + *Telekia* clade, *I. salicina* clade and *I. conyzae* clade; this last clade is composed of three clades (*I. bifrons*, *I. montana* and *I. britannica* clades).
4. The genus *Corvisartia* Mérat is recovered as an independent genus based on molecular, macromorphological, micromorphological and karyological characters. This genus includes *Inula helenium* L. – the current listed type of *Inula* – and other taxa out of the study area.
5. Based on the same type of data, *Limbarda* Adans. is also recognized as an independent genus; in our study area *Limbarda* includes just one species, *L. crithmoides* (L.) Dumort..
6. Both parsimony and Bayesian analyses of the ITS region of rDNA clearly separate *I. langeana* Beck and *I. conyzae* (Griess.) Meikle; although these species are morphologically similar, both analyses place them in different clades. There are also macro- and micromorphological evidences that support this differentiation.

7. Polyploidy seems to have played an important role in the evolution of the different lineages included in *Inula* s.s., except for the case of the *I. salicina* clade. Five ploidy levels have been found in *Inula* ($2n = 2x = 16$ to $2n = 6x = 48$) and at least four species show different cytotypes [i.e., *I. oculus-christi* ($2x$, $4x$), *I. britannica* ($2x$, $3x$, $4x$), *I. helenioides* ($4x$, $5x$) and *I. langeana* ($4x$, $5x$, $6x$)]. New chromosome counts have been obtained for seven taxa.

8. The reconstruction of ancestral character states for karyological data reveals that $x = 9$ could have been the ancestral base chromosome number for the genera of Inuleae included in this study; *Corvisartia* shows $x = 10$ as base chromosome number, and $x = 8$ may represent a clear synapomorphy for *Inula* s.s..

9. The reconstruction of ancestral character states for morphological data suggests that the presence of an epaleate receptacle, of long ligulate flowers (exceeding the involucral bracts by more than 3 mm) and of a glabrous achene represent plesiomorphic character states for *Inula* s.s..
10. A set of micromorphological characters related to achene and pappus, as well as the indument of the stem, leaves and ligulate flowers have been studied here under scanning electron microscope (SEM) for the first time. Several hair types, as well as indument patterns have been described for the species studied.
11. Several micromorphological characters support additionally the splitting of *Corvisartia* as an independent genus. Size and shape of the achenes, pappus insertion, the number and length of pappus setae and a papillate leaf surface allow differentiating *Corvisartia* from *Inula*.
12. Both the composition and density of the indumentum of the achene show phylogenetic signal. Three types of achene hairs have been recognised, i.e., elongated twin hairs, baculate twin hairs and biseriate glandular hairs. The presence of elongate twin hairs seems to be definitely fixed in the *Inula conyzae* clade.
13. Several micromorphological characters are useful for taxonomic purposes. *Inula spiraeifolia* has been frequently misidentified as *I. salicina*. But *I. spiraeifolia* shows ligulate flowers with biseriate glandular hairs and villose leaf surface covered by multicellular eglandular hairs and uniseriate glandular hairs, while *I. salicina* shows glabrous ligulate flowers and a leaf surface glabrous or glabrescent, covered only by multicellular eglandular hairs.
14. A new circumscription of the genus *Inula* has been proposed based on all these characters (molecular, macromorphological, micromorphological and karyological features), which involves several nomenclatural changes. It is of particular relevance a proposal to conserve the name *Inula* with a conserved type, together with the selection of a new type for *Inula* (*I. hirta*), whose acceptance will avoid an enormous amount of nomenclatural changes.
15. Connected with the splitting of *Corvisartia* from *Inula*, four new combinations are proposed at a subspecific level: *Corvisartia*: *C. helenium* subsp. *turcoracemosa* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *orgyalis* (Boiss.) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *pseudohelenium* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico and *C. helenium* subsp. *vanensis* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico.

16. Twenty-seven new lectotypes have been designated, including five that involve accepted names, i.e., *I. helenioides* DC., *I. helvetica* Grauer, *I. langeana* Beck, *I. maletii* Maire and *I. montana* L.. Additionally, four neotypes are proposed for names in *Inula*.
17. Finally, based on a detailed study of macro- and micromorphological traits performed through an exhaustive revision of herbarium specimens and of all the relevant literature, as well as on the main results derived from the phylogenetic analyses of DNA sequence data, a complete taxonomic account has been produced for the genera *Inula* (14 species), *Corvisartia* (1 species) and *Limbarda* (1 species) in the Western Mediterranean area. The resulting taxonomic accounts include the description of the genera and species involved, a nomenclatural revision, illustrations and additional comments. In the particular case of *Inula*, two identification keys are provided: the first one includes the taxa from the Western Mediterranean (14) and the second one is focused on the taxa from the Iberian Peninsula and Balearic Islands (7).

SUMMARY

CHAPTER 1. INTRODUCTION

This Doctoral Thesis has been developed in the framework the project *Flora iberica*; specifically, it was initially focused on the production of the taxonomic accounts for *Inula* L. (Asteraceae) and related genera, which will be published in volume 16 of *Flora iberica* [Castroviejo & al. (eds.) (1986-)].

The initial proposal considered the species of *Inula* from the Iberian Peninsula and Balearic Islands as a target group. However, we decided to extend the study area, at least to the Western Mediterranean area, with the purpose of getting an improved understanding of the variability of the species.

At the moment, there is no comprehensive taxonomic contribution available for the whole genus [except for the checklists published by Anderberg (1991) and Flann (2009)]. Beck's (1882) monograph treated all European species recognised at that time. The most recent molecular studies on the tribe Inuleae Cass. (Anderberg, 2009; Englund & al., 2009) are very general and do not include a wide representation of European taxa.

Furthermore, there is no work that explores karyological, micromorphological or palynological traits in many of taxa belonging to the genus *Inula*. Additionally neither delimitation of the genus nor delimitation of the species included were completely fixed.

Our study intends to emendate the existing gaps in the knowledge of the Mediterranean taxa of the genus by analysing as many traits as possible (molecular, morphological and karyological features) in order to clarify the taxonomy of the genus and explore the phylogeny and evolution of its species.

The family Asteraceae contains the largest number of described species of any plant family, 24,000–30,000, distributed in 1,600–1,700 genera occurring on all continents except Antarctica (Funk & al., 2005; Funk & al., 2009). The origin of Asteraceae could be placed in Gondwana, as its basal group, the subfamily Barnadesioideae K. Bremer & R.K. Jansen, is restricted to South America.

The family is undoubtedly monophyletic and it is identified by a series of diagnostic characters: type of inflorescence (capitulum), anthers fused in a ring and the presence of achenes (cypselas) usually with pappus. However, the taxa belonging to the Asteraceae show great variability in other features: habit, type of capitulum, number of flowers per capitulum, chromosome number, habitat, etc.

Cassini (1819) proposed the first classifications of the family; he recognised 19–20 tribes, including the tribe *Inulées*, based on sexual characters (achene, style, stamen and corolla). In the last decade, molecular data have completely changed the phylogenetic studies. Panero & Funk (2002) published the most popular phylogeny of the family, in which 12 subfamilies are recognised; tribe Inuleae is included in the subfamily *Asteroideae* (Cass.) Lindl.

The tribe Inuleae, defined by sagittate anthers and styles with sweeping hairs, has suffered little changes along the years. However, the delimitation of the genus *Inula* is still controversial and has notably changed from Linnaeus to present. In the original description of the genus, Linnaeus (1753) included in *Inula* several species that were later transferred to other genera [e.g., *Pulicaria dysenterica* (L.) Bernh., *P. odora* (L.) Rchb., *P. vulgaris* Gaertn., or *Limbarda crithmoides* (L.) Dumort.], whereas other species originally described under *Conyza* L. [i.e., *I. bifrons* L., *I. candida* (L.) Cass. and *I. conyzae* (Griess.) DC.] are now frequently included in *Inula*.

Beck's monograph (1882) is probably the most comprehensive work on the European species of *Inula*. This author recognized 21 species (plus 14 of presumed hybrid origin) that were arranged in four sections (*I.* sect. *Corvisartia* (Mérat) DC., *I.* sect. *Enula* (Boemer) Duby, *I.* sect. *Limbarda* (Adans.) DC. and *I.* sect. *Cupularia* Gren. & Godr.), most of them nowadays recognized as independent genera.

Molecular data provides a new approach to phylogenetic studies. Anderberg and co-workers demonstrate in several works (e.g., Anderberg & al., 2005; Englund & al., 2009) that the genus *Inula*, in its traditional circumscription, is clearly paraphyletic. No new circumscription has been proposed until now, but some possible solutions have been pointed out. Above all, Anderberg's contributions are essential to understand the complexity of the tribe Inuleae and set the bases for subsequent studies, which allow evaluating the monophyly of the different groups.

According to the current knowledge of the genus *Inula* at the starting point of this Doctoral Thesis, we aimed to meet the following aims:

1. To test the monophyly of the genus *Inula* s.l. (sensu Beck, 1882) and to infer the infrageneric phylogenetic relationships from the study of European and North-African representatives of *Inula* and related genera.

2. To explore the utility of the ITS region of nuclear ribosomal DNA with the aim of solving the systematic problems of *Inula*.
3. To compare the molecular data obtained with karyological and morphological data in order to try to interpret them and test whether they reveal patterns of evolution. Based on these patterns, try to propose a new phylogenetic hypothesis.
4. To study additional macro- and micromorphological characters in detail to assess their systematic value and to find further characters that support the phylogenetic proposal.
5. To investigate whether molecular, karyological, macro- and micromorphological characters support the splitting of *Corvisartia* as an independent genus.
6. To complete the nomenclatural revision of *Inula* s.l. in accordance with the taxonomical treatment proposed; this revision is based on the critical study of as many articles and autonomous publications as possible. In relation with this revision, we also aimed to study as much type material as possible in order to fix the usage and correct application of names.
7. To produce taxonomic syntheses for the genera *Limbarda*, *Corvisartia* and *Inula* in the Western Mediterranean area, which are consistent with the phylogenetic proposal based on the critical analysis of molecular, karyological and morphological data.

CHAPTER 2. BECK REVISITED: PHYLOGENY, CHARACTER EVOLUTION IN THE EUROPEAN SPECIES OF *INULA* L. (ASTERACEAE, INULEAE) AND RESURRECTION OF THE GENUS *CORVISARTIA* MÉRAT

Introduction

The genus *Inula* L. (Asteraceae, Inuleae) as traditionally circumscribed, comprises c. 90-100 species widely distributed in Europe, Asia and Africa; c. 65 species are Eurasian and North-African perennial herbs and ca. 25 species are Central and South-African perennial herbs and shrubs (modified from Anderberg, 1991; Flann, 2009).

The delimitation of the genus is still controversial and it is clearly paraphyletic in its traditional circumscription (Anderberg & al., 2005; Englund & al., 2009).

In this chapter, we try to shed light on the taxonomic boundaries of *Inula,* discuss the advantages and disadvantages of different ways of classifying the species included in the genus and propose a rearrangement that is consistent with current knowledge of the evolutionary history of the group. Our taxonomic decisions are based on (1) a phylogenetic analysis of the ITS sequences corresponding to 32 taxa representing all Beck's sections of *Inula* and closely related genera; (2) a comprehensive survey of cytogenetic data, including a compilation of all previously published chromosome counts and the contribution of seven new ones corresponding to four relevant taxa. Additionally, a set of morphological and karyological characters – some of them traditionally considered diagnostic at generic and/or infrageneric ranks– are checked here for the first time to see whether any of them can provide additional phylogenetic information and support for molecular trees.

Material and Methods

A total of 32 taxa, 23 of them representing all four Beck's sections of *Inula* and 9 closely related genera, are included in this study. Twenty-three ITS sequences were newly generated and nine were downloaded from GenBank. Phylogenetic analysis using maximum parsimony and Bayesian inference methods were performed.

Previously published chromosome counts for genera from the Inuleae (mainly genera native to Europe) were compiled from an extensive literature survey and new reports of chromosome numbers for 16 individuals belonging to seven taxa from the genus *Inula* were also obtained.

Patterns of evolution of several characters commonly used to assess relationships among taxa within the study group (i.e., length of ligulate flowers, receptacular paleae and indument of achene) plus other relevant traits (i.e., base chromosome number) were investigated by ancestral character-state optimizations using parsimony criterion.

Results

Maximum parsimony and Bayesian inference analysis show largely congruent topologies, except for some basal nodes. Our results support the paraphyly of *Inula* in its broad traditional sense, as the four sections recognized by Beck (1882) within *Inula* are placed in four different clades with other genera (e.g., *Buphthalmum* L.) nested among them. *Inula* s.s. (i.e., *Inula* sect. *Enula*) is recovered in our analysis as a monophyletic group ("*Inula* clade"; BS = 100%, PP = 1.00). Furthermore, two major clades within this "*Inula* clade" are inferred with *I. orientalis* Lam. as a sister group; one of them (clade B; BS = 98%, PP = 1.00) grouping six species and a second weakly supported clade (clade C; BS = 62%, PP = 1.00) with eleven taxa that represent the largest part of the "*Inula* clade" [see Fig. 2.1 and 2.2].

Five base chromosome numbers have been detected within the analysed genera, ranging from $x = 5$ (*Pallenis* Cass.) to $x = 10$ (*Carpesium* L.*, Telekia* Baumg.*, Corvisartia, Duhaldea* DC. and *Buphthalmum* L.), except for $x = 7$.

Our reconstruction of ancestral character states suggests that $x = 9$ could have been the ancestral base chromosome number for the genera of Inuleae included in this study (see Fig. 2.5A). A base chromosome number of $x = 10$ is recovered as the plesiomorphic character state for the clades *Inula* and *Corvisartia* + *Telekia*, plus the genera *Carpesium*, *Duhaldea* and *Buphthalmum,* while $x = 8$ may represent one of the most clear synapomorphies for the genus *Inula* s.s..

The presence of receptacular paleae (see Fig. 2.5B) has been traditionally used as a diagnostic character within the tribe Inuleae. Along history *Inula* s.l. (i.e., including *Dittrichia*, *Limbarda* and *Corvisartia*) has been characterised by the absence of receptacular paleae, in contrast with other related genera (i.e., *Pallenis, Asteriscus* Mill.*, Telekia, Buphthalmum*). Our results show that an epaleate receptacle is the most likely plesiomorphic character state for the tribe, which further supports Anderberg's (1991) opinion based on morphological evidences. Within clade A, the species *T. speciosa* (Schreb.) Baumg. shows receptacular paleae, whereas the representatives of *Corvisartia* lack them; thus, our reconstruction of ancestral character states recovers the presence of receptacular paleae as a hypothetical autapomorphy for *T. speciosa* within clade A.

Regarding the ploidy level, the species of *Inula* included in this study show five different ploidy levels ranging from diploid to hexaploid (see Fig. 2.6). The most common one within this clade is the diploid level (2*n* = 2*x* = 16), which is present in all the species belonging to the "*I. salicina* clade", in *I. orientalis*, and in some taxa from the "*I. conyzae* clade" (*I. bifrons*, *I. montana* –both varieties–, *I. britannica* and *I. oculus-christi*). The second most common level is the tetraploid one (2*n* = 4*x* = 32), which is present in the "*I. britannica* clade" (*I. britannica*, *I. helenioides* and *I. oculus-christi*; the chromosome number of *I. auriculata* and *I. mariae* remains unknown) and in particular taxa from other groups (*I. conyzae*, *I. langeana* and *I. maletii*). In addition, the triploid level (2*n* = 3*x* = 24) has been only documented in *I. britannica*, the pentaploid level (2*n* = 5*x* = 40) in *I. langeana* and *I. helenioides*, and the hexaploid level (2*n* = 6*x* = 48) also in *I. langeana*. Therefore, as far as it is known, four taxa show several cytotypes: *I. britannica* (2*x*, 3*x*, 4*x*), *I. oculus-christi* (2*x*, 4*x*), *I. helenioides* (4*x*, 5*x*) and *I. langeana* (4*x*, 5*x*, 6*x*), all of them belonging to the "*I. conyzae* clade". Hence, it seems clear that polyploidization may have played an important role in the evolution and diversification of *Inula*.

The presence of long ligulate flowers (exceeding the involucral bracts by more than 3 mm) is the plesiomorphic character state for the "*Inula* clade" (see Fig. 2.7A). The presence of short ligulate flowers (shorter than involucral bracts or exceeding them by less than 3 mm) may represent a synapomorphy for the "*Inula bifrons* clade", while a few species such as *I. germanica* and *I. langeana* from other clades show short ligulate flowers as an autapomorphy.

With regard to the indumentum of the achene (see Fig. 2.7B), the ancestral character state seems to be a glabrous achene for the "*Inula* clade". An achene totally hairy is the plesiomorphic state for *I. montana* and *I. britannica* clades (in fact just *I. maletii* –from the "*I. montana* clade"– shows an achene only apically hairy, which represents an autapomorphy for this North African endemic species), whereas the presence of achenes only apically hairy are shared by all the species belonging to the "*I. bifrons* clade". The species included in the "*I. salicina* clade" have a glabrous achene, although this trait seems to show a low degree of lability at the population level (i.e., *I. helvetica* and *I. ensifolia* usually show glabrous achenes but individuals from some exceptional populations present achenes with sparse hairs at the apex).

Discussion

The genus *Inula* s.l. as traditionally circumscribed is clearly paraphyletic, based both on morphological and molecular data. Our knowledge on the phylogenetic relationships among the members of *Inula* s.l. is inferred from character-state changes from various types of independent datasets [plastid and nuclear DNA sequence data (Anderberg & al., 2005; Englund & al., 2009), morphology, karyology, etc.] and from an acceptable understanding of character evolution. In our opinion the paraphyly of *Inula* necessitates a taxonomic change and this could be implemented in several alternative ways, mainly regarding the circumscription of *Inula* s.s..

The available data suggest that the current circumscription of *Limbarda* and *Dittrichia* should be retained to house two of the four sections of European *Inula* recognized by Beck (1882); i.e., *I.* sect. *Limbarda* and *I.* sect. *Cupularia*.

A third section accepted by Beck, *Inula* sect. *Corvisartia*, is recovered as a monophyletic sister group of *Telekia* in our analysis. Based on plastid and nuclear DNA sequence data and on a wider sampling of the tribe (although with more limited sampling regarding European taxa), Englund & al. (2009) found that the generic type – *I. helenium*– was included in a largely heterogeneous clade (clade D in Englund & al., 2009) in a position "closer to some other genera than to the many congeners in clade E" (their clade E contains most species of *Inula*).

The fourth section accepted by Beck, *I.* sect. *Enula*, also recovered as a monophyletic group, includes most of the Eurasian species (and at least some taxa from Africa and Asia). These species show morphological and karyological traits quite distinct from those belonging to the "*Corvisartia* + *Telekia* clade".

The genus *Inula* could be circumscribed in several ways. We support the alternative of circumscribing the genus in a narrow sense (i.e., excluding the "*Corvisartia* clade", i.e., to Beck's *I.* sect. *Corvisartia*). Placing *C. helenium* and related species in a separate taxonomic entity has the advantage that the remainder of *Inula* s.s. becomes morphologically more homogeneous. The members of *I.* sect. *Corvisartia* share several morphological features that allow easy differentiation from the rest of *Inula*, such as their habit (up to 2 meters high), the presence of resin canals, larger basal leaves, diameter of the capitula up to 10 cm, outer involucral bracts ovate and a base chromosome number of $x = 10$.

This option implies that the generic name *Inula* should be restricted to the members of *I.* sect. *Corvisartia* and a high number of formal nomenclatural changes would be necessary for the species grouped in the "*Inula* clade". In order to limit the number of changes, a proposal to conserve the name *Inula* with a conserved type is necessary, as well as a new generitype for *Inula* (*I. hirta* L., Santos-Vicente & al., 2012; Chapter 5). In relation to this option and based on morphological characters, we propose that at least the following five Asian taxa should be combined under *Corvisartia* (see Appendix 2.5): *C. helenium*, *C. magnifica*, *C. racemosa* and *C. royleana* as well as *C. koeltzii,* a species probably related to the latter.

From our point of view, the generic name *Inula* should be reserved for the members of the "*Inula* clade" (= *I.* sect. *Enula*). This clade comprises all sampled

Eurasian species of *Inula* s.s., as well as two North African (*I. maletii* and *I. montana* var. *calycina*) and two Asian (*I. orientalis* and *I. auriculata*) representatives sampled in our analysis. Monophyly of this clade is well supported in our sampling both in parsimony (BS = 100%) and Bayesian analyses (PP = 1.00). In contrast with the species included in *Corvisartia*, the taxa belonging to the "*Inula* clade" share a base chromosome number of $x = 8$ as a clear synapomorphy. As an additional set of diagnostic character states, probably simplesiomorphic, these taxa share the absence of resin canals, an epaleate receptacle, outer involucral bracts linear to ovate-lanceolate, radiate heterogamous capitula of less than 10 cm in diameter, and achenes with pappus free or slightly connated at the base.

Our phylogenetic analysis recovers four major lineages that roughly correspond with the subsections and informal groups proposed by Beck (1882) and Anderberg (1991).

The first lineage, "*I. salicina* clade" (clade B), corresponds with Beck's *I.* subsect. *Longeligulatae – Leiocarpae* group and with Anderberg's "*I. salicina* group". The species belonging to this clade are characterised by presenting long ligulate flowers and glabrous achenes, as well as xeromorphic leaves. However, there are some exceptions: as autapomorphic character states *I. germanica* shows short ligulate flowers and the achenes in *I. helvetica* present only occasionally some sparse hairs at the apex. Interestingly the presence of glabrous achenes seems to be a character somehow labile in *I. helvetica* and *I. ensifolia* that usually show glabrous achenes but the individuals from some scarce populations present achenes with sparse hairs at the apex. This clade is the most stable from a karyological point of view within *Inula* s.s, since all its members are diploids with $2n = 2x = 16$. Considering that the presence of long ligulate flowers and glabrous achenes are recovered as the most probable ancestral character states for *Inula* s.s. (see Fig. 2.7), the "*I. salicina* clade" includes the probable primitive members of the genus. Most of the species included in this clade are widely distributed across most part of Europe (except for the northernmost areas) and Western Asia, but *I. salicina* reaches East Asia.

The second lineage "*I. bifrons* clade" within clade C, groups the species *I. bifrons* and *I. conyzae*. This clade corresponds with Beck's *I.* subsect. *Breviligulatae* and Anderberg's "*I. bifrons* group". It is diagnosed by two synapomorphies, i.e., short ligulate flowers and achenes only apically hairy. Both authors included in this group *I. thapsoides* (M. Bieb. ex Willd.) Spreng., a species closely related to *I. bifrons*, that shares with the former taxon the previously mentioned morphological traits as well as the chromosome number ($2n = 16$). Anderberg (1991) mentioned the presence of decurrent leaves as a diagnostic character for his "*I. bifrons* group", but this trait is not present in *I. conyzae*, which also differs from the rest of the species clustered in this clade by its chromosome number ($2n = 32$). With regard to the distribution area, *I. conyzae* is widely distributed across Europe, Asia Minor and the Caucasus region, whereas the remaining taxa from this lineage present more restricted distribution areas: *I. bifrons* in Southern Europe, *I. thapsoides* in the Caucasus region and Asia Minor.

The third lineage is the "*I. montana* clade" within clade C that groups exclusively taxa from the Western Mediterranean and most of them endemic to restricted areas (*Inula montana* var. *montana* from the Iberian Peninsula to Italy and North Africa, *I. montana* var. *calycina* exclusively present in North Africa, *I. langeana* from N and C Spain and *I. maletii* endemic to the Middle Atlas). None of these taxa was studied by Anderberg (1991) and no clear correspondence has been found between this clade and any of Beck's subsections or informal groups. Within this clade most taxa show long ligulate flowers and totally hairy achenes; however, some autapomorphic character states are observed in *I. langeana* (i.e., short ligulate flowers) and *I. maletii* (i.e., achenes only apically hairy). Polyploidy seems to have played an important role in the evolution of this clade which is highly heterogeneous regarding chromosome number. Four ploidy levels have been found within this group (see Fig. 2.6), from diploid to hexaploid level (except for triploid); a single ploidy level has been detected up to now for *I. montana* ($2x$, both varieties) and *I. maletii* ($4x$), but three ploidy levels have been found within *I. langeana* ($4x$, $5x$, $6x$).

The fourth lineage, "*I. britannica* clade" within clade C, consists of a complex of species that have been frequently misidentified (*I. helenioides*, *I. britannica*, *I. mariae* and *I. oculus-christi*). This clade corresponds with Beck's subsect. *Longeligulatae* – *Lasiocarpae* group, which roughly matches also with Anderberg's "*I. britannica* group". The species belonging to this complex are characterised by the presence of totally hairy achenes, a character state which is shared with most of the members of the "*I. montana* clade", and long ligulate flowers, a particular character state that is recovered as plesiomorphic for *Inula* s.s. in our analyses. Differentiation among the taxa included in the "*I. britannica* clade" is rather complicated and the species have been traditionally distinguished by a set of morphological characters that have proved to be very variable. However, there are other morphological features that are undoubtedly more useful to determine these taxa: the position of the outer involucral bracts (patent to erect bracts), presence or absence of pluricellular hairs in ligulate flowers, and size of the achene. With respect to chromosome number, this clade is also highly heterogeneous and obviously, polyploidyzation mechanisms may have played an important role in the evolution of the group. Four ploidy levels ranging from diploid to pentaploid have been found within it (see Fig. 2.6) and all taxa studied from a karyological point of view show, at least, two ploidy levels: *I. helenioides* ($4x$, $5x$), *I. britannica* ($2x$, $3x$, $4x$) and *I. oculus-christi* ($2x$, $4x$). Regarding distribution area, *I.*

britannica and *I. oculus-christi* are spread throughout Europe and Asia, but the remaining species show more restricted distribution areas: *I. helenioides* is endemic to Spain and France, and *I. mariae* is endemic to the Caucasus region.

Polyploidy is recognised as one of the most important forces in plant evolution and speciation. The ancestral base chromosome number suggested for the Asteraceae is x = 9 (Watanabe & al., 2007). Polyploidy is widespread within the family and occurs in most of the major clades.

According to Semple & Watanabe (2009) the ancestral base chromosome number for the tribe Inuleae seems to be x = 10; and considering only the European genera belonging to the Inuleae, we hypothesise x = 9 as the ancestral base number (see Fig. 2.5), which is the basic chromosome number in *Dittrichia*, *Jasonia*, *Limbarda, Pulicaria* and in some species of the genus *Carpesium*. Within the European representatives of Inuleae, x = 10 is retained in clades A (*Telekia* + *Corvisartia* clade) and D (*Buphthalmum* + *Duhaldea* clade), as well as in *Carpesium cernuum*. The rest of the genera included in our reconstruction of ancestral traits show a lower base chromosome number (*Pallenis* x = 5; *Asteriscus* x = 6; *Chiliadenus* and *Inula* x = 9), which agree with the hypothesis that downward dysploid events in Asteraceae are preponderant over upward events that could be more difficult to complete successfully (Semple & Watanabe, 2009).

Polyploidy is documented in three of the four major lineages of the European species of *Inula* (only the *I. salicina* group seems to be devoid of polyploid events). Within *Inula* s.s. at least four species show several cytotypes, i.e., *I. oculus-christi* (2x, 4x), *I. britannica* (2x, 3x, 4x), *I. helenioides* (4x, 5x) and *I. langeana* (4x, 5x, 6x). Increasing the number of counts would also probably increase the number of known ploidy levels and also the number of species with multiple cytotypes.

CHAPTER 3. CHROMOSOME NUMBERS IN EUROPEAN SPECIES OF *INULA* L. (ASTERACEAE).

Published in *Taxon* 58(4): 1288, E20–E21 (2009).

Some new and relevant chromosome counts for European species of *Inula* were published in *Taxon*. This short paper includes several first chromosome counts for the species, as well as first counts for accessions of a species in a particular country. We include here a brief summary of the results, which have been discussed in the previous chapter:

First chromosome count for the species:

Inula helvetica: 2*n* = 16 (Spain)

First chromosome count for an accession of the species from the referenced country:

I. bifrons: 2*n* = 16 (Spain)

I. conyzae: 2*n* = 32 (Spain)

I. ensifolia: 2*n* = 16 (Italy)

Corvisartia helenium (as *I. helenium*): 2*n* = 20 (Spain)

CHAPTER 4. TAXONOMIC RELEVANCE OF SELECTED MICROMORPHOLOGICAL CHARACTERS IN EUROPEAN AND NORTH-AFRICAN *INULA* L. (ASTERACEAE): A PHYLOGENETIC PERSPECTIVE [Caracteres micromorfológicos en taxones europeos y norteafricanos del género *Inula* L. (Asteraceae) y su importancia taxonómica: perspectiva filogenética]

The phylogenetic analysis described under Chapter 2 demonstrated that *Inula* sect. *Enula* is a monophyletic group and that four lineages can be recognised within the "*Inula* clade" (see Chapter 2 and Fig. 2.2). Some characters such as the length of ligulate flowers and achene indument show phylogenetic signal. Also a number of clear morphological synapomorphies were pointed out for most of the clades within the study group. Additionally, *Inula* sect. *Corvisartia* was split as an independent genus, *Corvisartia*, based on molecular, morphological and karyological data.

With the purpose of finding additional morphological synapomorphies for the lineages of the "*Inula* clade", as well as more characters that could contribute to support the splitting of *Corvisartia*, we aimed to study further micromorphological traits under scanning electron microscope (SEM).

In this chapter, we aim (1) to examine a set of morphological characters which have been traditionally used to define groups or to identify species (e.g., indument of achenes, stems and leaves) in the European and North African species of *Inula* and *Corvisartia* by using SEM; (2) to investigate whether these traits support the splitting of the genus *Corvisartia*; (3) to explore whether these characters show phylogenetic signal in *Inula*; and (4) to identify traits that can help to discriminate the more complex taxa in the genus *Inula*.

Material and Methods

Selected material is based on that used in the phylogenetic analysis described under Chapter 2. The dataset was composed of 17 ITS sequences: 16 from European and North African taxa of *Inula* and *Corvisaria helenium* as outgroup. A new phylogenetic analysis using maximum parsimony was performed.

The morphological study included 11 vegetative and reproductive characters: (1) size of achene, (2) shape of achene, (3) density of indument of achene, (4) composition of indument of achene, (5) base of pappus, (6) length of pappus, (7) number of setae conforming the pappus, (8) length of the denticle of setae, (9) indument of ligulate flowers, (10) indument of stem, and (11) indument of leaves.

Observations under SEM were completed with the study of additional herbarium material under stereoscopic microscope to assess the stability of traits.

Character states of the morphological traits were mapped on the strict consensus tree resulting from the maximum parsimony analysis.

Results

The topology of the strict consensus tree derived from the maximum parsimony analysis obtained in this study (see chapter 4; Fig. 4.1) is coincident with those trees presented in Chapter 2 (see Fig. 2.1 and Fig. 2.2) for *Inula* s.s.. *Corvisartia helenium* is placed as external group and two main clades are recognised with *Inula*, "*I. salicina* clade" and "*I. conyzae* clade".

The "*Inula salicina* clade" includes six species: *I. helvetica*, *I. spiraeifolia*, *I. ensifolia*, *I. hirta*, *I. salicina* and *I. germanica*. The clade receives a strong support, but interspecific relationships are not resolved.

The "*Inula conyzae* clade" includes ten species and shows little support, but three well-supported clades are recovered within this clade: "*I. bifrons* clade" (*I. bifrons* and *I. conyzae*), "*I. britannica* clade" (*I. helenioides*, *I. britannica* and *I. oculus-christi*), and "*I. montana* clade" (*I. montana* var. *montana*, *I. montana* var. *calycina*, *I. langeana* and *I. maletii*); *I. auriculata* is nested among these clades, but its phylogenetic position remains unclear.

Mature achenes of *Inula* vary between 0.5 mm (*I. britannica*) and 3 mm (*I. helenioides*, *I. montana* and *I. maletii*). Achenes are homotypic, more or less oblongoid (*I. montana* and occasionally *I. bifrons*) or cylindrical (rest of species), truncate or slightly attenuate at the apex, non angular, ribbed, with hexagonal cells at the surface, brownish, with variable indument. Mature achenes of *Corvisartia* show some differences: size varies between 3 and 5 mm and they are prismatic, truncate at the apex, with inconspicuous ribs and glabrous.

The arrangement and density of achene indument in *Inula* is variable. Those species belonging to the "*I. salicina* clade" (i.e., *I. helvetica*, *I. spiraeifolia*, *I. ensifolia*, *I. hirta*, *I. salicina* and *I. germanica*) plus *Corvisartia helenium* have glabrous achenes. *Inula maletii* and the species belonging to the "*I. bifrons* clade" (*I. bifrons* and *I. conyzae*) show achenes which are hairy only at the apex. The rest of the studied taxa show achenes totally hairy, but two variants can be distinguished according to indument density: homogeneous density ("*I. britannica* clade" and *I. auriculata*) and heterogeneous density, more concentrated to the apex ("*I. montana* clade" except *I. maletii*).

Regarding composition of the indument of the achene in *Inula,* it is formed by biseriate eglandular hairs or *twin hairs* (Hess, 1938); two types are described according to the shape of the apical cells: elongated and baculate twin hairs. Occasionally, biseriate glandular hairs are also observed. Four indument patterns were described for the achenes of *Inula* and *Corvisartia*: achene glabrous (most of "*I. salicina* clade" species plus *Corvisartia*); achenes with elongated twin hairs (rest of the species with hairy achenes); achenes with elongated twin hairs and biseriate glandular hairs (exceptionally in some populations of *I. britannica* and *I. ensifolia*); and achenes with baculate twin hairs (some populations of *I. helvetica*).

The pappus of the achenes of *Inula* is monomorphic, uniseriate, 4–8 mm in length, composed of c. 20–35 setae, persistent, whitish or brownish; setae antrorse-barbate, denticles 15–100 µm in length, free at the base (*I. montana*, *I. langeana* and *I. helenioides*) or slightly connate (rest of species). Pappus of *Corvisartia* shows some differences: 6–10 mm in length, 50–80 setae, persistent, white-brownish or gold; setae antrorse-barbate, denticles 50–80 µm in length, clearly connate at the base.

Ligulate flowers in *Inula* are glabrous (*I. hirta*, *I. salicina*, *I. spiraeifolia* and *I. bifrons*) or show variable indument composed of multicellular eglandular hairs or biseriate glandular hairs; combination of two types of hairs is only present in *I. oculus-christi* and *I. helenioides* ("*I. britannica* clade"). Ligulate flowers in *Corvisartia* show only biseriate glandular hairs.

The indument of the stem in *Inula* and leaves is usually composed of eglandular and glandular hairs; only exceptionally some specimens identified as *I. salicina* are glabrous.

Four patterns of stem indument have been observed in *Inula* and *Corvisartia*: multicellular eglandular hairs (*I. salicina*); antrorse eglandular hairs (*I. hirta*); multicellular eglandular hairs and biseriate glandular hairs (most of species of *Inula* and *Corvisartia*); and, multicellular eglandular hairs and uniseriate glandular hairs (exclusive of viscous species, i.e., *I. bifrons*, *I. maletii* and *I. spiraeifolia*).

Five patterns of leaf indument have been observed in *Inula* and *Corvisartia*: ciliate leaves with multicellular eglandular hairs (some species belonging to "*I. salicina* clade"; i.e., *I. salicina*, *I. hirta* and *I. ensifolia*); multicellular eglandular hairs and biseriate glandular hairs (most of species, with variable density); multicellular eglandular hairs and uniseriate glandular hairs (*I. bifrons* and *I. maletii*); ciliate leaves with multicellular eglandular hairs and uniseriate glandular hairs (*I. spiraeifolia*); and, multicellular eglandular hairs and papillae (exclusive of *C. helenium*).

Discussion

Morphological characters analysed in this study support the splitting of *Corvisartia* as an independent genus. The achenes of *Corvisartia* are different from those present in *Inula* both in size and shape (3–5 mm and prismatic achene in *Corvisartia* and less than 3 mm and cylindrical or oblongoid in *Inula*). Pappus in *Corvisartia* is 6–10 mm in length, clearly connate at the base (but lack of coronae characteristic from *Dittrichia*), whereas pappus in *Inula* is shorter (less than 7 mm) and free or slightly connate at the base. No relation has been found among size of achene, length of pappus and length of denticles. The presence of papillae in the leaves are also exclusive of *Corvisartia*.

The indument of achene has been traditionally used to define sections and informal groups within *Inula* s.s. (Beck, 1882). The phylogenetic significance of this trait was demonstrated in Chapter 2 (see Fig. 2.7); consequently, composition and density of achene indument were selected for a more detailed study.

Glabrous achenes are exclusive of *Corvisartia* and species of "*I. salicina* clade", was and it is considered the ancestral character state for *Inula* s.s. (see Chapter 2); on the other hand, the species of "*I. conyzae* clade" show hairy achenes. Achenes only hairy at the apex appear in "*I. bifrons* clade" (*I. bifrons* and *I. conyzae*) and *I. maletii*, which could represent an autapomorphy or symplesiomorphy shared for these three taxa. Achenes totally hairy with homogeneous density are found in "*I. britannica* clade" and *I. auriculata* (very similar to *I. oculus-christi*, whose position in "*I. conyzae* clade" is not completely resolved). Achenes totally hairy with heterogeneous density (more dense to the apex) are restricted to the "*I. montana* clade" (*I. montana* var. *montana*, *I. montana* var. *calycina* and *I. langeana*).

Twin hairs are present in most of the genera in the Asteraceae (Funk & al., 2009) and in all genera in Inuleae (Anderberg, 2009). Twin hairs have been demonstrated to show phylogenetic signal in other genera, for example, in *Filago* (Andrés-Sánchez & al., submitted). In the genus *Inula*, twin hairs are common in all lineages, but are exceptional in certain taxa belonging to the "*I. salicina* clade" (some populations of *I. ensifolia* and *I. helvetica*). Elongated twin hairs seem to be fixed in the "*I. conyzae* clade" and could represent a synapomorphy shared by the members of this clade.

Ciccarelli and co-workers (2007) pointed out that *Dittrichia* can be distinguished from *Inula* by the presence of biseriate glandular hairs in the achene; however, this type of hairs have been observed in some populations of *I. helvetica* and *I. britannica*.

The micromorphological traits referred to the pappus have not been traditionally used to define groups, although their phylogenetic relevance has been already demonstrated; e.g., setae with short denticles (< 35 µm) are present in the members of the "*I. salicina* clade", but this trait is only found in two species belonging to the "*I. conyzae* clade" (*I. auriculata* and *I. britannica*).

Anderberg (1991) described some informal groups (*tentative groups*) within *Inula* based on vegetative characters, specifically on foliar features as xeromorphy (*Inula salicina* group), leaf insertion (decurrent leaves in *I. decurrens*, *I. bifrons* and *I. thapsoides*) or sericeous indument (*I. candida* group).

Leaf xeromorphy is frequently expressed by a coriaceous consistency. In most cases, these coriaceous leaves show multicellular eglandular hairs and cilia in the margin. This pattern has been observed in the "*Inula salicina* clade" (*I. salicina*, *I. ensifolia* and *I. hirta;* partially coincident with Anderberg's *I. salicina* group). Those taxa belonging to the "*I. conyzae* clade" present multicellular eglandular hairs and biseriate glandular hairs in their leaves, with the exception of *I. bifrons* and *I. maletii*; these species, together with *I. spiraeifolia* show uniseriate glandular hairs, which are responsible of the viscosity of these plants.

However, stem indument does not seem to be relevant from a phylogenetic point of view; most of taxa have multicellular eglandular hairs and biseriate glandular hairs. As an exception, *I. hirta* shows antrorse hairs in the stem and *I. bifrons*, *I. maletii* and *I. spiraeifolia* show multicellular eglandular hairs and uniseriate glandular hairs.

Some micromorphological characters do not show phylogenetic signal, but have taxonomic relevance; these features have been used in Chapter 6 (Taxonomic accounts) to build the identification keys.

Inula spiraeifolia has been frequently confused with broad-leaf specimens of *I. salicina*. Many herbarium specimens are misidentified, which makes difficult to know the exact distribution area of *I. spiraeifolia* . The taxonomic characters commonly used to identify this species are leaf insertion and capitula arrangement (Coste, 1937; Tutin, 1976; Pignatti, 1982), a set of traits whose interpretation is occasionally complicated. Some micromorphological characters have been demonstrated to be more useful in discriminating both species: *I. spiraeifolia* shows ligulate flowers with biseriate glandular hairs and glabrescent to sparsely villose leaves with multicellular eglandular hairs and uniseriate glandular hairs; in contrast, *I. salicina* shows glabrous ligulate flowers and glabrous to glabrescent leaves only with multicellular eglandular hairs.

The taxonomic identity of *Inula langeana* has been controversial along history (see Chapter 2, section 2.4.2). This taxon has been sometimes treated as a subspecies of *I. conyzae* based on the morphological similarity that exists between both taxa. Our molecular phylogenetic analyses recovered *I. langeana* and *I. conyzae* in different lineages and the study of micromorphological characters under SEM has provided additional traits to help in identifying both species. The achenes of *I. conyzae* are only apically hairy, whereas the achenes of *I. langeana* are totally hairy but the hairs are more concentrated to the apex. There are other micromorphological traits that have demonstrated to show taxonomic relevance, but we have rejected to include them in the identification keys because their evaluation is difficult without optical instrument; e.g., *I. langeana* shows free pappus with longer setae denticles (50–90 µm), whereas *I. conyzae* shows slightly connate pappus with shorter denticles (35–65 µm).

The "*Inula britannica* clade" comprises a species complex (*I. britannica*, *I. helenioides* and *I. oculus-christi*) which have been frequently misidentified (see Notes on the correspondent taxa on Chapter 6). Some taxonomical characters have been traditional used to differentiate among the species (i.e., density of leaf indument, size and number of capitula) and its variability has been discussed by some authors (e.g., Grierson, 1975). Examinations under SEM have confirmed that the indument of the achene, stem and leaves is not useful to discriminate among these taxa. However, other micromorphological traits seem to have taxonomic value and may help to identify these three species: *I. britannica* has small achenes (0.5–1.5 mm), free pappus with c. 20 setae and ligulate flowers with biseriate glandular hairs; *I. oculus-christi* has medium-size achenes (1.5–2 mm), free pappus with c. 20 setae and ligulate flowers with multicellular eglandular hairs biseriate glandular hairs; *I. helenioides* has larger achenes (2–3 mm), slightly connate pappus with c. 30 setae and ligulate flowers with multicellular eglandular hairs biseriate glandular hairs. The combination of all these micromorphological traits, together with other features (e.g., leaf insertion, habitat and distribution area) has allowed us to identify most of the herbarium specimens revised.

CHAPTER 5. NOMENCLATUAL REVISION OF THE GENUS *INULA* L. (ASTERACEAE) AND RELATED GENERA [Revisión nomenclatural del género *Inula* L. (Asteraceae) y géneros afines] Partially published in *Taxon* 61(4): 1330– 1331 (2012).

In this chapter, a complete nomenclatural account for the accepted genera split from *Inula* s.l. (*Corvisartia*, *Limbarda* and *Inula*) is provided. Twenty-seven typifications (including five typifications of accepted names) are designated in order to determine the correct application of names, fix their usage and help defining the identity of the taxa. Sixteen names already lectotypified are also listed and annotated. In addition, four new combinations referred to *Corvisartia* are proposed.

Material and Methods

The accepted genera split from *Inula* s.l. (*Corvisartia*, *Limbarda*, *Inula*) are listed here. All the accepted species included in each genus are enumerated under the correspondent genera, mentioning the selected or listed type. Species of difficult ascription are included in the *Incertae sedis* section. Finally, some taxa mainly from Central Europe of putative hybrid origin are also included in this nomenclatural account (Putative hybrids section). In many cases, such hybrid origin has still not been confirmed. We mention here only those cases for which we have directly revised the original materials and exclusively when these materials show character states that can be considered intermediate between two well-established parental species. Notes on taxonomic and/or nomenclatural aspects suitable of discussion are added wherever it is considered appropriate. Photographs of all relevant material traced have been obtained and selected photographs are displayed.

Types for the accepted name of each taxon are cited or provided, as well as for the corresponding most widely used synonyms. In each case, the holotype or selected lectotype or neotype are mentioned. A lectotype according to Art. 9.2 of the latest edition of the ICBN (Vienna Code, McNeill & al., 2006) or a neotype (Art. 9.6 ICBN) are selected, whenever necessary. Isotypes (Art. 9.3 ICBN) and syntypes (Art. 9.4 ICBN) are mentioned only when they have been directly checked by us.

In connection with this nomenclatural account, a proposal to conserve the name *Inula* with a conserved type, together with the selection of a new type for *Inula* (*I. hirta*) in order to avoid an enormous amount of nomenclatural changes has been already published.

All relevant protologues and taxonomical literature available have been examined. Decisions are based on our taxonomic knowledge of *Inula* and related genera, as well as on a careful study of herbarium materials deposited in 50 herbaria, although we have only used material from 24 of them for typification purposes: B, B-W, BC, BM, BP, C, COI, E, FI-W, G, G-BOIS, G-DC, K, L, LINN, MA, MPU, MPU-Afrique du Nord, P-LA, S-LINN, TO, UPS-Burser, W, and WU.

Results

The genus *Corvisartia* includes a single species in Europe and North Africa: *Corvisartia helenium* (= *Inula helenium*). *Inula helenium* is the current listed lectotype of the genus *Inula* (Anderberg, 1993); therefore, we have proposed to conserve the name *Inula* with a conserved type and *I. hirta* has been proposed as new generitype for *Inula* (Santos-Vicente & al., 2012).

The European material of *C. helenium* revised by us shows little morphological variability. In contrast, Grierson (1974) has considered that it is a highly polymorphic taxon, at least in the Eastern Mediterranean area. Based on material from Turkey and considering the variation of traits such as the number and arrangement of capitula, size of involucre, shape of outer involucral bracts, and number and size of ligulate flowers, he described a number of subspecies. These subspecies, originally subordinated to *I. helenium*, must be therefore combined under *Corvisartia*: *C. helenium* subsp. *turcoracemosa* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *orgyalis* (Boiss.) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *pseudohelenium* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico and *C. helenium* subsp. *vanensis* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico.

Limbarda also includes a single species in the study area: *L. chritmoides*. Some infraspecific taxa, which might be considered within the range of variability of *L. chritmoides* have been described. Although the genus could comprise 8-9 species (see Chapter 6), we were not able to study herbarium material corresponding to all these taxa; for that reason, no new combinations are proposed in *Limbarda*.

The genus *Inula* comprises 14 accepted species in our study area. In total, 27 lectotypes are designated here, five of which correspond to accepted names: *I. helenioides*, *I. helvetica*, *I. langeana*, *I. maletii* and *I. montana*. It is worth highlighting the effort to clarify the identity and nomenclatural state of names related to *I. helenioides* and *I. langeana*, because several Spanish authors had considerably complicated the taxonomic interpretation of these taxa by describing a large number of species and infraspecific taxa.

Four taxa from which original material have been studied are included under the *Inciertae sedis* section due to the fact that their interpretation is still not clear: *I. auriculata*, *I. serpentinica*, *I. suaveolens* and *Inula thapsoides* subsp. *urumoffii*.

Afterwards, ca. 20 names are listed under the *Putative hybrids* section. It is worth emphasising the lectotypification of *I.* × *semiamplexicaulis* Reut. (*I. helvetica* × *salicina*) due to the fact that a relative large number of herbarium specimens lodged at European herbaria bear a label with this name.

Finally, we propose to conserve the name *Inula* with a conserved type in connection with the splitting of *Corvisartia* as an independent genus.

A recently published molecular phylogeny of the tribe *Inuleae* (Englund & al., 2009), as well as newly generated DNA sequence and morphological data on the tribe especially focused on the European species of *Inula* (see Chapters 2 and 4), suggest that this genus is highly paraphyletic and that *I. helenium* and its sister species (*I.* sect. *Corvisartia*) should be segregated at generic rank from the remaining species of *Inula*.

In this situation, unless this conservation proposal is accepted, application of the name *Inula* would be restricted to the *Corvisartia* species traditionally included in *Inula* sect. *Corvisartia* (i.e., *C. helenium*, *C. magnifica*, *C. racemosa*, *C. royleana* and *C. koeltzi*), and between eighty and ninety new combinations in some other genus or genera would be needed to accommodate the other species currently under *Inula*.

In order to limit the number of name changes it is necessary to propose a conserved type for the generic name under Art. 14.9 of the Vienna Code (McNeill & al., 2006). We here propose *I. hirta* L. as a conserved type for *Inula,* based on the following reasons: (1) this species is clearly part of the protologue of the genus (Linnaeus, l.c.: 883); (2) it is nomenclaturally stable (no synonyms used); (3) it is not a very variable species from the morphological point of view (no subspecies have been described within it); and (4) the type associated with this name is unambiguous (lectotype designated by Anderberg, 1998).

To summarize, the present proposal is based on the facts that: (1) *Inula* s.l. is largely paraphyletic, (2) the currently listed type of *Inula* belongs to the small independent genus *Corvisartia* and (3) most of the species currently recognized in *Inula* would remain there if our proposal is accepted. We think that nomenclatural stability would be best served through conservation of the name *Inula* with a conserved type.

CHAPTER 6. TAXONOMIC ACCOUNTS FOR THE GENERA *LIMBARDA* ADANS., *CORVISARTIA* MÉRAT AND *INULA* L. [Síntesis genéricas: géneros *Limbarda* Adans., *Corvisartia* Mérat e *Inula* L.]. *Flora iberica*, Vol. 16 (submitted).

In this chapter, a complete taxonomic account has been elaborated for the genera *Inula*, *Corvisartia* and *Limbarda* in the Western Mediterranean area (including North Africa). These three genera are composed of 75–80 species distributed in Europe, Asia and Africa, from which c. 85% belong to *Inula*. In our study area, *Inula* comprises 14 species, whereas *Corvisartia* and *Limbarda* include only one species each. Specific sections related to Iberian Peninsula have been included in order to adapt the taxonomic account to the editorial rules of *Flora iberica* [Castroviejo & al. (eds.) (1986-)], due to the fact that this chapter will be published in the forthcoming volume 16 of this work.

This taxonomic account is based on a detailed study of morphological characters, as well as on previously described results derived from the phylogenetic analyses of DNA sequence data, karyological and micromorphological data. The taxonomic treatment follows that proposed in Chapters 2 and 4 of this Doctoral Thesis.

Material and Methods

The taxonomic account is based on the detailed study of c. 5000 sheets lodged at 50 European herbaria: ABH, B, B-W, BC, BCN, BM, BOLO, BP, C, CL, COI, COI, E, FCO, FI-W, G, G-BOIS, G-DC, GDA-GDAC, GRM, HSS, JACA, JBAG-Laínz, K, L, LEB, LINN, LISU, LY, MA, MACB, MAF, MGC, MPU, MPU-Afrique du Nord, MUB-SURESTE, P-LA, PR, RNG, SALA, SANT, SEV, SEV-Histórico, SOM, S-LINN, TO, UPS-Burser, VAL, VIT, W and WU, plus F. Gómiz's (León, Spain) personal herbarium. In particular, complete collections of Iberian institutions considered "basic herbaria" for the *Flora iberica* project (i.e., BC, BCN, COI, G, GDA-GDAC, JACA, MA, MAF, MGC, SALA, SEV, VAL) were carefully examined. An special effort was made to collect and study newly discovered populations corresponding to taxa with restricted or poorly known areas.

All information obtained from the herbarium material revision performed was compiled in a database (Microsoft® Access 2002) specifically designed for that purpose. Database fields are the following: original taxon name, revised taxon name, locality, habitat, collection date, phenology, collector name, identifier name, herbarium acronym, particular interest of the herbarium sheet (e.g., typification purposes, original material for molecular or karyological analysis) and additional notes.

Considering the fact that it is difficult to display all this information in this Doctoral Thesis, an abridged list is shown in Annex I. For each taxon, three localities per country were provided except for the Iberian Peninsula, where three localities per province were supplied. Uncertain localities were revised with the help of online thesaurus and databases (see Chapter 6, section 6.5).

In addition, a comprehensive revision of all relevant taxonomic literature has been performed.

The start point was the revision of the taxonomic accounts for *Inula* and related genera published in the "basic floras" mentioned in *Flora iberica* project: Willkomm (1865), Willkomm (1893), Merino (1906), Coutinho (1939), Tutin (1976), Franco (1985), Devesa (1987), Bolòs & Vigo (1996) and Greuter (2008).

Afterwards, all taxonomic treatments published in widely known modern floras were examined; e.g., Grierson (1975), Rechinger (1980), Gorschkova (1999), Vogt (2002), Abid & Qaiser (2002). Other treatments, older but considered as reference works, were also revised; e.g., De Candolle (1836), Bentham (1873), Boissier (1875). The most comprehensive monograph on *Inula* to date was published by Beck (1882), but other relevant monographs have been studied: e.g., Borbás (1887), Avetissjan (1958).

Many literature references were consulted in specialised national and international libraries: e.g., those in Departamento de Botánica de la Universidad de Salamanca, Real Jardín Botánico de Madrid, Instituto Botánico de Barcelona, Royal Botanic Gardens, Kew (United Kingdom), Botanischer Garten und Botanisches Museum Berlin-Dahlem (Germany) and Faculty Centre of Biodiversity (former Institut für Botanik, Wien, Austria). The rest of literature was consulted in online libraries and literature databases, which have become essential resources nowadays; e.g., Biblioteca Digital del Real Jardín Botánico de Madrid, Biodiversity Heritage Library, Botanicus Digital Library, Gallica (see Chapter 6, section 6.5).

Finally, an exhaustive search was performed in online nomenclatural databases (e.g., *The International Plant Names Index* (which comprises *Index Kewensis*), *Index Nominum Genericorum*, *Index synonymique de la flora de France*, *The Global Compositae Checklist*, *Euro+Med Plantbase*) in order to compile as many original descriptions of *Inula* s.l. as possible; (see Chapter 6, section 6.5).

In total, c. 700 works (articles, protologues and autonomous works) have been studied. In order to facilitate the exploitation of the data, references were included in a database (Microsoft® Access 2002) specifically designed for that purpose. Database fields are the following: author/s, title, publication data, abbreviated title (according to

Botanico periodico huntianum or *Taxonomic literature*), volume and pages, particular interest of the work and additional notes.

At the same time, nomenclatural data referred to *Inula* s.s. were compiled. In this case, records were organised using BIBMASTER, a database application to manage biodiversity projects, developed and supported by the Coordination Unit of the Spanish GBIF national node (Pando & al., 2004). Nomenclatural module is organised into five sections: original name, bibliographic reference, accepted name, basionym and comments.

In total, 624 names related to *Inula* and related genera were compiled in order to check synonymy and priority of as many names as possible.

Nomenclatural accounts are included just before the description of taxa. Every name is accompanied by author/s, reference and publication data. First, accepted name is displayed; below, homotypic synonyms (≡), heterotypic synonyms (=), misinterpretations (–) and unrevised names (?) are listed, with the convenient annotations.

The taxonomic accounts of the genera *Limbarda*, *Corvisartia* and *Inula* include identification keys and detailed descriptions of genera and species, following the editorial criteria of *Flora iberica* [Castroviejo & al. (eds.) (1986-)].

First, a determination key is proposed in order to allow the identification of the three genera included in this study: *Limbarda*, *Corvisartia* and *Inula*. After that, three independent taxonomic accounts for each genera are developed; each generic synthesis comprises: accepted name (with reference and publication data), shortened etymology, detailed description of the genus, comments and basic literature related with the genus.

Afterwards, two identification keys are provided: the first one includes all the species of the genus represented in the study area and the second one is restricted to the species considered in *Flora iberica*. No identification keys were produced for *Limbarda* and *Corvisartia*, due to the fact that only one species belonging to each genus is known in the study area. Next to the key, an independent taxonomic account for each species is provided; each specific synthesis includes: accepted name (with reference and publication data, together with an indication on how the specific epithet should be pronounced), complete nomenclatural account, locotypic indication, available iconographies, detailed description, chromosome numbers, habitat, phenology, general distribution, distribution in Iberian Peninsula and Balearic Islands, vernacular names, observations and conservation status.

CHAPTER 7. CONCLUSIONS

1. The molecular phylogenetic analysis based on DNA sequences of the ITS region (rDNA) shows that the genus *Inula* L. as traditionally circumscribed (*Inula* s.l.) is paraphyletic, which is also supported by morphological and karyological data.
2. The four sections traditionally recognised in *Inula* s.l. (i.e., *I.* sect. *Corvisartia*, *I.* sect. *Enula*, *I.* sect. *Limbarda* and *I.* sect. *Cupularia*) are placed into four different clades, with external genera nested among them.
3. According to the molecular phylogenetic analysis performed, the European and North-African species of *Inula* s.l. are grouped into three clades: *Corvisartia* + *Telekia* clade, *I. salicina* clade and *I. conyzae* clade; this last clade is composed of three clades (*I. bifrons*, *I. montana* and *I. britannica* clades).
4. The genus *Corvisartia* Mérat is recovered as an independent genus based on molecular, macromorphological, micromorphological and karyological characters. This genus includes *Inula helenium* L. – the current listed type of *Inula* – and other taxa out of the study area.
5. Based on the same type of data, *Limbarda* Adans. is also recognized as an independent genus; in our study area *Limbarda* includes just one species, *L. crithmoides* (L.) Dumort..
6. Both parsimony and Bayesian analyses of the ITS region of rDNA clearly separate *I. langeana* Beck and *I. conyzae* (Griess.) Meikle; although these species are morphologically similar, both analyses place them in different clades. There are also macro- and micromorphological evidences that support this differentiation.
7. Polyploidy seems to have played an important role in the evolution of the different lineages included in *Inula* s.s., except for the case of the *I. salicina* clade. Five ploidy levels have been found in *Inula* ($2n = 2x = 16$ to $2n = 6x = 48$) and at least four species show different cytotypes [i.e., *I. oculus-christi* ($2x$, $4x$), *I. britannica* ($2x$, $3x$, $4x$), *I. helenioides* ($4x$, $5x$) and *I. langeana* ($4x$, $5x$, $6x$)]. New chromosome counts have been obtained for seven taxa.
8. The reconstruction of ancestral character states for karyological data reveals that $x = 9$ could have been the ancestral base chromosome number for the genera of Inuleae included in this study; *Corvisartia* shows $x = 10$ as base chromosome number, and $x = 8$ may represent a clear synapomorphy for *Inula* s.s..

9. The reconstruction of ancestral character states for morphological data suggests that the presence of an epaleate receptacle, of long ligulate flowers (exceeding the involucral bracts by more than 3 mm) and of a glabrous achene represent plesiomorphic character states for *Inula* s.s..
10. A set of micromorphological characters related to achene and pappus, as well as the indument of the stem, leaves and ligulate flowers have been studied here under scanning electron microscope (SEM) for the first time. Several hair types, as well as indument patterns have been described for the species studied.
11. Several micromorphological characters support additionally the splitting of *Corvisartia* as an independent genus. Size and shape of the achenes, pappus insertion, the number and length of pappus setae and a papillate leaf surface allow differentiating *Corvisartia* from *Inula*.
12. Both the composition and density of the indumentum of the achene show phylogenetic signal. Three types of achene hairs have been recognised, i.e., elongated twin hairs, baculate twin hairs and biseriate glandular hairs. The presence of elongate twin hairs seems to be definitely fixed in the *Inula conyzae* clade.
13. Several micromorphological characters are useful for taxonomic purposes. *Inula spiraeifolia* has been frequently misidentified as *I. salicina*. But *I. spiraeifolia* shows ligulate flowers with biseriate glandular hairs and villose leaf surface covered by multicellular eglandular hairs and uniseriate glandular hairs, while *I. salicina* shows glabrous ligulate flowers and a leaf surface glabrous or glabrescent, covered only by multicellular eglandular hairs.
14. A new circumscription of the genus *Inula* has been proposed based on all these characters (molecular, macromorphological, micromorphological and karyological features), which involves several nomenclatural changes. It is of particular relevance a proposal to conserve the name *Inula* with a conserved type, together with the selection of a new type for *Inula* (*I. hirta*), whose acceptance will avoid an enormous amount of nomenclatural changes.
15. Connected with the splitting of *Corvisartia* from *Inula*, four new combinations are proposed at a subspecific level: *Corvisartia*: *C. helenium* subsp. *turcoracemosa* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *orgyalis* (Boiss.) Santos-Vicente, M.M. Mart. Ort. & E. Rico, *C. helenium* subsp. *pseudohelenium* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico and *C. helenium* subsp. *vanensis* (Grierson) Santos-Vicente, M.M. Mart. Ort. & E. Rico.

16. Twenty-seven new lectotypes have been designated, including five that involve accepted names, i.e., *I. helenioides* DC., *I. helvetica* Grauer, *I. langeana* Beck, *I. maletii* Maire and *I. montana* L.. Additionally, four neotypes are proposed for names in *Inula*.
17. Finally, based on a detailed study of macro- and micromorphological traits performed through an exhaustive revision of herbarium specimens and of all the relevant literature, as well as on the main results derived from the phylogenetic analyses of DNA sequence data, a complete taxonomic account has been produced for the genera *Inula* (14 species), *Corvisartia* (1 species) and *Limbarda* (1 species) in the Western Mediterranean area. The resulting taxonomic accounts include the description of the genera and species involved, a nomenclatural revision, illustrations and additional comments. In the particular case of *Inula*, two identification keys are provided: the first one includes the taxa from the Western Mediterranean (14) and the second one is focused on the taxa from the Iberian Peninsula and Balearic Islands (7).

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