Transworld Research Network
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences, 2011: 199-222 ISBN: 978-81-7895-528-5
Editor: Diego Muñoz-Torrero

# 9. *Artemisia* (Asteraceae): Understanding its evolution using cytogenetic and molecular systematic tools, with emphasis on subgenus *Dracunculus*

Jaume Pellicer[1], Teresa Garnatje[2] and Joan Vallès[3]

*[1]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, United Kingdom*
*[2]Institut Botànic de Barcelona (IBB-CSIC-ICUB), Passeig del Migdia sn 08038 Barcelona, Catalonia, Spain [3]Laboratori de Botànica, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII sn 08028 Barcelona, Catalonia, Spain*

**Abstract.** The genus *Artemisia* is one of the largest of the Asteraceae family, with more than 500 species. It is widely distributed mainly across the Northern Hemisphere, being profusely represented in the Old World, with a great centre of diversification in Asia, and also reaching the New World. The evolution of this genus has been deeply studied using different approaches, and polyploidy has been found to perform an important role leading to speciation processes. Karyological, molecular cytogenetic and phylogenetic data have been compiled in the present review to provide a genomic characterization throughout some complexes within the genus.

Correspondence/Reprint request: Dr. Jaume Pellicer, Jodrell Laboratory, Royal Botanic Gardens Kew Richmond, Surrey, TW9 3AB, United Kingdom. E-mail: j.pellicer@kew.org

# 1. Introduction: A general overview on the genus *Artemisia*

The genus *Artemisia* L. is one of the widely distributed genera of the Asteraceae family, and the largest genus of the Anthemideae tribe. The number of representatives considered within the genus is variable depending on the authors consulted, and ranges from about 380 species [1] to more than 500 species [2,3]. Some of the recent revisions of the family [3,4], have considered previously recognized genera as species circumscribed within *Artemisia* [e.g. *Artanacetum* (Rzazade) Rzazade, *Oligosporus* Cass. and *Seriphidium* (Besser ex Less.) Fourr.].

The genus is distributed worldwide, mainly across the temperate zones of the Northern Hemisphere, some species reaching the Arctic, but a few species can also be found on the Southern Hemisphere [5,6]. The origin of *Artemisia*, based on fossil data, is in the semi-arid steppes of the temperate Asia at mid Cenozoic, that is, about 20 My ago [7]. In fact, Central Asia is considered its main centre of speciation and diversification, from where is expanding towards the Irano-Turanian, Mediterranean and North American regions.

From the morphological point of view, the leaves of *Artemisia* are mostly divided (with the exception of a few species, e.g. *Artemisia dracunculus* L.), alternately or sparsely distributed, and with a wide range of sizes, shapes and textures. The inflorescences, arranged in capitula are small, mainly spheroidal to ovoid, and composed of flosculose florets inserted on a receptacle protected by a bracteal involucre. Corollas are of whitish, yellow and purple color, and not too showy [5]. Fruits are pappus-lacking achenes, of small size and generally obovoid and laterally compressed. Pollen sculpture, although of limited variablility, has been used as a systematic marker [8,9]. The weak (microechinate) ornamentation of *Artemisia* contrasts with the echinate one present in most of the Anthemideae representatives [10-12].

The genus is mostly composed of perennial plants (Figs. 1b-d, f-i, m), but some of them (ca. 20 species) are known to behave either as annuals (Figs. 1e, j) or biennials (Fig. 1a) [1,2,13,14]. Within the genus there exist a certain variability of biotypes, being predominantly considered as herbs (*Artemisia annua* L., *Artemisia vulgaris* L.), subshrubs (*Artemisia changaica* Krasch., *Artemisia crithmifolia* L.) and shrubs which may develop highly lignified stems (*Artemisia tridentata* Nutt.). The ability of this genus to inhabit many different ecosystems and environmental conditions is evident, ranging from deserts and semi-deserts (steppes, tundras and slope hills), forests and deeply anthropized meadows, to humid areas, from sea level to high mountains.

**Figure 1.** Some representatives of the genus *Artemisia*: a) *Artemisia biennis* L.; b) *Artemisia desertorum* Spreng.; c) *Artemisia echegarayi* Hieron.; d) *Artemisia gmelinii* Stechm.; e) *Artemisia jacutica* Drobow; f) *Artemisia keiskeana* Miq.; g) *Artemisia mendozana* DC.; h) *Artemisia messerschmidtiana* Besser; i) *Artemisia nova* A. Nelson; j, k, l) *Artemisia palustris* L.; m) *Artemisia selengensis* Turcz. ex Besser. (Photographs: J. Pellicer).

Many of the species have traditional medicinal (e.g. *A. annua*, from which artemisinin is extracted to treat malaria), food (e.g. *A. dracunculus*, a culinary condiment, or *Artemisia absinthium* L., used to prepare liquors), soil stabilization (*Artemisia sphaerocephala* Krasch.) and ornamental (*Artemisia arborescens* L.) uses. A shared characteristic of many of the species is their intense fragrance, a result of the presence of monoterpenic and sesquiterpenic chemicals, localized in trichomes and schizogenic secretory channels [15].

Most *Artemisia* species bloom either at the end of summer or during autumn in contrast to most other Anthemideae genera which typically flower during spring and summer. Another distinctive *Artemisia* characteristic is that they are almost exclusively wind pollinated (although there are evidences of entomophily in a few species) [16,17]. This wind pollination characteristic is only shared with some closely related genera; the general case in Anthemideae is entomophily with the presence of showy capitula, attractive to insects.

## 2. Taxonomical considerations: Conflicts and reorganizations within the genus

The systematic classification of the genus *Artemisia* [see 18,19 and references therein], as well as in other Asteraceae genera, has been demonstrated to be complex and is currently under revision because of the lack of general taxonomic agreement. The species currently included in the genus were previously grouped within three independent genera (*Abrotanum*, *Absinthium* and *Artemisia*) by Tournefort [20]. Subsequently, Linné [21] organized them into a single genus (*Artemisia*), and Cassini [22] followed by Lessing [23], transferred part of the species to a newly created genus *Oligosporus*, which is currently recognized as the subgenus *Dracunculus* (Besser) Rydb. Besser [24-27] divided the genus into four groups (with the addition of *Seriphidium* Besser) which were considered as sections or subgenera (*Abrotanum* [currently *Artemisia*], *Absinthium*, *Dracunculus* and *Seriphidium*). This classification was accepted by Candolle [28] and subsequently by Rouy [29], who nevertheless carried out some rearrangements. Rydberg [30] created a new section, *Tridentatae*, to integrate some of the species previously included in *Seriphidium*, which was elevated to subgeneric rank by McArthur [31]. More recently, Ling [32,33] proposed that *Tridentatae* taxa should be subsumed into *Seriphidium* genus. Bremer and Humphries [2] accepted Ling’s proposal in their revision of the Asteracaeae. However, taxonomic consensus remains elusive as witnessed by several additional treatments aimed at clarifying the taxonomy of the genus [i.e. 1,34,35]. Some of the conflicts between traditional and molecular data [3,4,36,37] still remain unsolved.

Nevertheless, subgeneric classification of *Artemisia* on the basis of morphological characters such as floral structure has been maintained, since it fits at large scales (with some exceptions such as part of the New World species and some of representatives within the subgenera *Artemisia* and *Absinthium*) with the classifications based on the analysis of DNA sequences. The floral traits which identify each subgenus are as follows (Fig. 2):

- ***Absinthium***: Heterogamous capitula with outer florets female and central florets hermaphrodite and fertile. Hairy receptacle.
- ***Artemisia* (= *Abrotanum*)**: Heterogamous capitula with outer florets female and central florets hermaphrodite and fertile. Glabrous receptacle.
- ***Dracunculus***: Heterogamous capitula with outer florets female and central florets hermaphrodite but functionally male. Glabrous receptacle.
- ***Seriphidium* and *Tridentatae***: Homogamous capitula with all florets hermaphrodite and fertile. Glabrous receptacle.

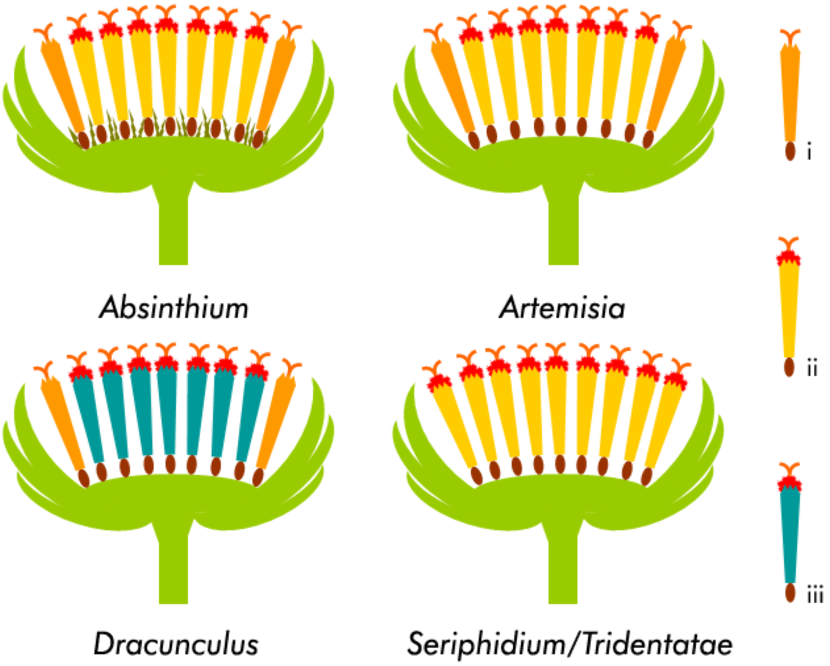

**Figure 2.** Diagrams of the flower head structure of each subgenus in *Artemisia*. i) Female florets, ii) hermaphrodite fertile florets, iii) hermaphrodite but functionally male florets.

## 3. The subgenus *Dracunculus* (Besser) Rydb.

As mentioned previously, Cassini [22] described the genus *Oligosporus*, which included the species that are currently considered as part of the subgenus *Dracunculus* (Fig. 3), to which we will devote a particular attention in this review. This group, with functionally separate sexes, that is, radial female florets and central male ones as consequence of the abortive ovaries, is composed of about 80 taxa [13,14]. Recently, Ling *et al.* [1] in their revision of the Anthemideae tribe have proposed a new classification, and as a consequence, the subgenus *Dracunculus* would be divided into two sections, *Dracunculus* Besser and *Latilobus* Y. R. Ling on the basis of their leaf morphology and indumenta.

**Figure 3.** Some representatives of *Artemisia* subgenus *Dracunculus*: a) *A. crithmifolia*; b) *A. dracunculus*; c) *Artemisia eriopoda* Bunge; d) *Artemisia ledebouriana* Besser; e) *Artemisia macilenta* (Maxim.) Krasch.; f) *Artemisia monosperma* Delile; g) *Artemisia monostachya* Maxim. ex Bunge; h) *Artemisia pycnorrhiza* Ledeb.; i) *Artemisia tomentella* Trautv. [Photographs: (a) J. Vallès, (b-i) J. Pellicer].

Previous studies based on the analysis of DNA sequences in the genus *Artemisia* [36,37] have highlighted the relationship of some closely segregated Artemisiinae (*Filifolium* Kitam., *Mausolea* Bunge ex Poljakov, *Neopallasia* Poljakov, *Turaniphytum* Poljakov) the three last ones having been previously considered within *Artemisia* as *A. eranthema* Bunge, *A. eriocarpa* Bunge and *A. pectinata* Pall., respectively. In this sense, as we discuss below, different studies have examined in depth the phylogenetic relationships within the subgenus, and to elucidate the systematics between both the New and the Old World representatives as well [36-40].

As for the whole of the genus *Artemisia*, the representatives of the subgenus *Dracunculus* are widely spread across the Northern Hemisphere, mainly in the arid zones and semiarid steppes from Europe to Asia, where the subgenus has its main hotspot, but also reaching North America. They are mostly subshrubs and herbs, basically perennial, with few annuals such as

*Artemisia demissa* Krasch., *Artemisia edgeworthii* Balakr., *Artemisia pewzowii* C. Winkl., and *Artemisia scoparia* Waldst. et Kit.

Within the representatives of the subgenus, the type species *A. dracunculus* -tarragon- (Fig. 3b) deserves special attention, since it accounts for a great economic value. This plant is popular worldwide because it is used as culinary condiment in many countries. But some other less known species also have traditional uses either to build windbreaks and soil stabilization (*A. sphaerocephala*, or *Artemisia wudanica* Liou et W. Wang) or in medicine (*Artemisia capillaris* Thunb., with detoxifying and diuretic properties; *Artemisia ordosica* Krasch., with anti-inflammatory properties).

## 4. Chromosome numbers and polyploidy

The genus *Artemisia* has been profusely studied from the karyological point of view with many papers reporting chromosome numbers in the genus over several decades covering over 50%, that is, more than 350 species [41]. The genus has two basic chromosome numbers: $x = 9$, present in all the subgenera, and the less frequent $x = 8$, reported in the subgenera *Absinthium*, *Artemisia* and *Dracunculus* [42-45]. In addition, a $2n = 14$ count has been reported, suggesting a basic number $x = 7$ [46]. Recently some authors have suggested the possibility of $x = 17$ as a basic chromosome number. Oliva and Vallès [44] reported that in the tetraploid *Artemisia umbelliformis* Lam. ($2n = 34$) an event of diploidization might have occurred with subsequent cytotype stabilization. In addition, in a recent paper [47], this new basic chromososme number has been suggested again in a group of restricted Japanese endemic species of subgenus *Artemisia* (e.g. *Artemisia momiyamae* Kitam., *Artemisia princeps* Pamp.). In the case of the $x = 8$-based species, the hypothesis of a centric Robertsonian chromosome fusion which may have caused a descending dysploidy from $x = 9$ to 8 [48] is widely accepted. Those species with $2n = 16$ frequently present a larger pair of chromosomes (Fig. 4g), sometimes with centromeric fragility, what gives support to the precedent hypothesis. Given that the findings of Oliva and Vallès [44] and Matoba *et al.* [47] are restricted to few species, and that a significantly larger pair of chromosomes is also present in the metaphase plates, other explanations instead of a $x = 17$ basic chromosome number should be taken into account. Thus, these plants might be $x = 9$-based tetraploids, but reduced from $2n = 36$ to 34 chromosomes via Robertsonian fusion, and whether this process gives rise to a new base number may be the object of further discussion according to the stability of the reported cases and new ones that might be detected.

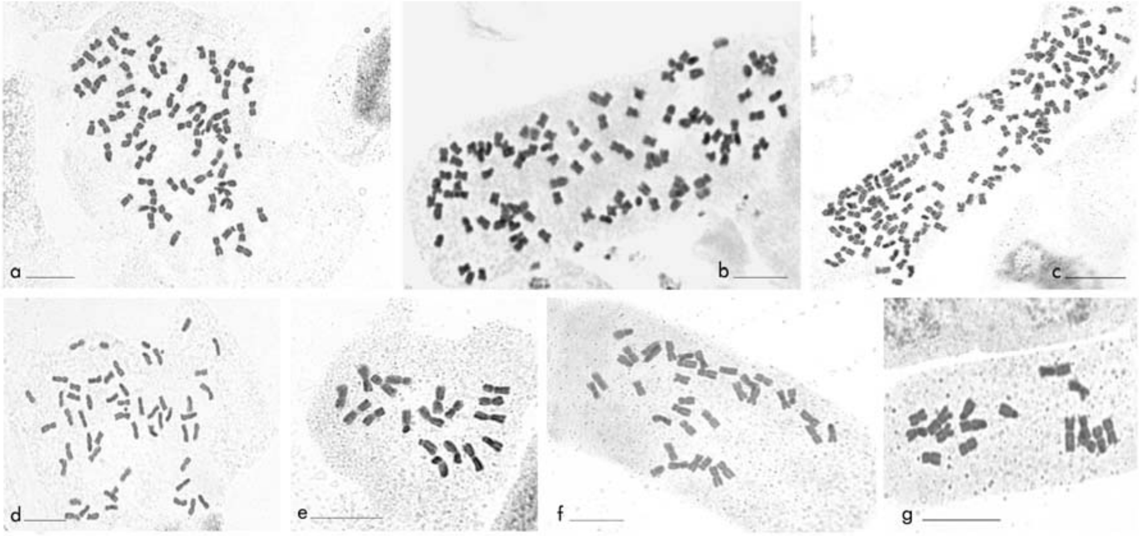

**Figure 4.** Metaphase plates of some *Artemisia* species. a) *A. echegarayi* (2*n* = 72); b) *A. macrantha* (2*n* = 108); c) *A. medioxima* (2*n* = 144); d) *A. mendozana* DC. subsp. *paramilloensis* F.A. Roig & J.A. Ambrosseti (2*n* = 54); e) *A. sphaerocephala* (2*n* = 18); f) *A. subdigitata* (2*n* = 36); g) *A. sylvatica* (2*n* = 16). Images extracted from Pellicer *et al.* [49,50] with permission of the authors. (Scale bars = 10 µm).

Both basic chromosome numbers have experienced many episodes of polyploidization. Polyploidy is an important evolutionary trait leading to plant speciation, and has special incidence in the genus *Artemisia*, being therefore, reported many times in the literature [17,20,31,45,47,49,50-59]. Ploidy levels in the genus are known up to dodecaploid and hexaidecaploid for $x = 9$ ($2n = 12x = 108$; $2n = 16x = 144$) in *Artemisia macrantha* Ledeb. and *Artemisia medioxima* Krasch., respectively [49,60, Figs. 4b-c], and hexaploid for $x = 8$ ($2n = 6x = 48$) in species such as *Artemisia verlotiorum* Lamotte or *Artemisia austriaca* Jacq. [17,61].

Some of the subgenera have been revealed to be really active in terms of polyploidy, with species accounting for large polyploid series. This is the case of the subgenus *Dracunculus*, with its type species (*A. dracunculus*) displaying ploidy levels of $2n = 2x, 4x, 6x, 8x, 10x$ [50,53,62,63]. In fact, most of the representatives of the subgenus are known to present polyploid cytotypes, and many of them, are only known at polyploid level, e.g. *A. changaica*, *A. ledebouriana* or *A. pycnorrhiza*, among others. Other examples of high impact of polyploidy (based on $x = 9$) can be found in the American endemic representatives. On the one hand, the North American taxa (subgenus *Tridentatae*) provide cytotypes from diploid to octoploid levels [45], and on the other hand, the South American endemic species might be considered as a polyploid complex with cytotypes ranging from diploid to dodecaploid levels [64].

## 5. Cytogenetic insights: Ribosomal DNA evolution

The nuclear genes encoding rRNA have been the subject of many studies in plants and in many other organisms as well. In the case of the genus *Artemisia*, the distribution and organization of these genes have been deeply studied [64-69]. Whilst in most eukaryotes, both 5S and 45S (18S-5.8S-26S) genes are usually arranged in separated tandem arrays which are transcribed by different RNA polymerases [70, Fig. 5A], there are some exceptions to this organization in some other organisms [71-74] that have evolved towards a linkage of both family genes. This is the case in the genus *Artemisia* and other related genera, where a co-localized (linked) organization of both 5S and 45S ribosomal sub-units were detected by fluorescent *in situ* hybridization [66,68,69,75, Fig. 5B]. Given the interest that such structure raised, subsequent studies were carried out in depth. Thus, this linkage was confirmed and unravelled using Southern blot hybridization and polymerase chain reactions (PCR) by Garcia *et al.* [69]. Besides, and confirming previous evidence, this feature has been recently reported beyond the *Artemisiinae* (*Artemisia* and related genera), in at least three of the major lineages within the Asteraceae [76], indicating that it could be more common than previously known in plant species.

Returning to *Artemisia*, physical mapping of ribosomal DNA using fluorescent *in situ* hybridization has been conducted with the principal objectives of better understanding the systematic and evolutionary relationships within the genus. Thus, molecular cytogenetic studies focused in different subgenera (*Artemisia*, *Dracunculus*, *Seriphidium* and *Tridentatae*) have been published [66,68,69,77], albeit some others have been restricted either to specific groups such as the annual one [75] and the South American representatives [64] or to polyploid complexes [78]. It has been reported that not all the subgenera have similar patterns of rDNA loci number and distribution, and furthermore, the changes on the number of loci in polyploid species has been revealed to follow different trends, even within groups of closely related species. As an example, we present some of the results in the subgenus *Dracunculus* [75].

In the most recent phylogenies of *Artemisia* [36-38], the subgenus *Dracunculus* appears segregated in two main clades, one that includes the type species *A. dracunculus* and its close relatives (*A. dracunculus* complex) and the main clade, which embraces most of the Eurasian taxa. Within the first group, the number of rDNA loci in diploid cytotypes is 2 (4 rDNA sites), and seems to follow almost a proportional increase of rDNA loci in parallel to ploidy levels. Thus, tetraploids have 4 rDNA loci, hexaploids 6 and the decaploid about 9-10, a trend that does not correlate with the nuclear DNA

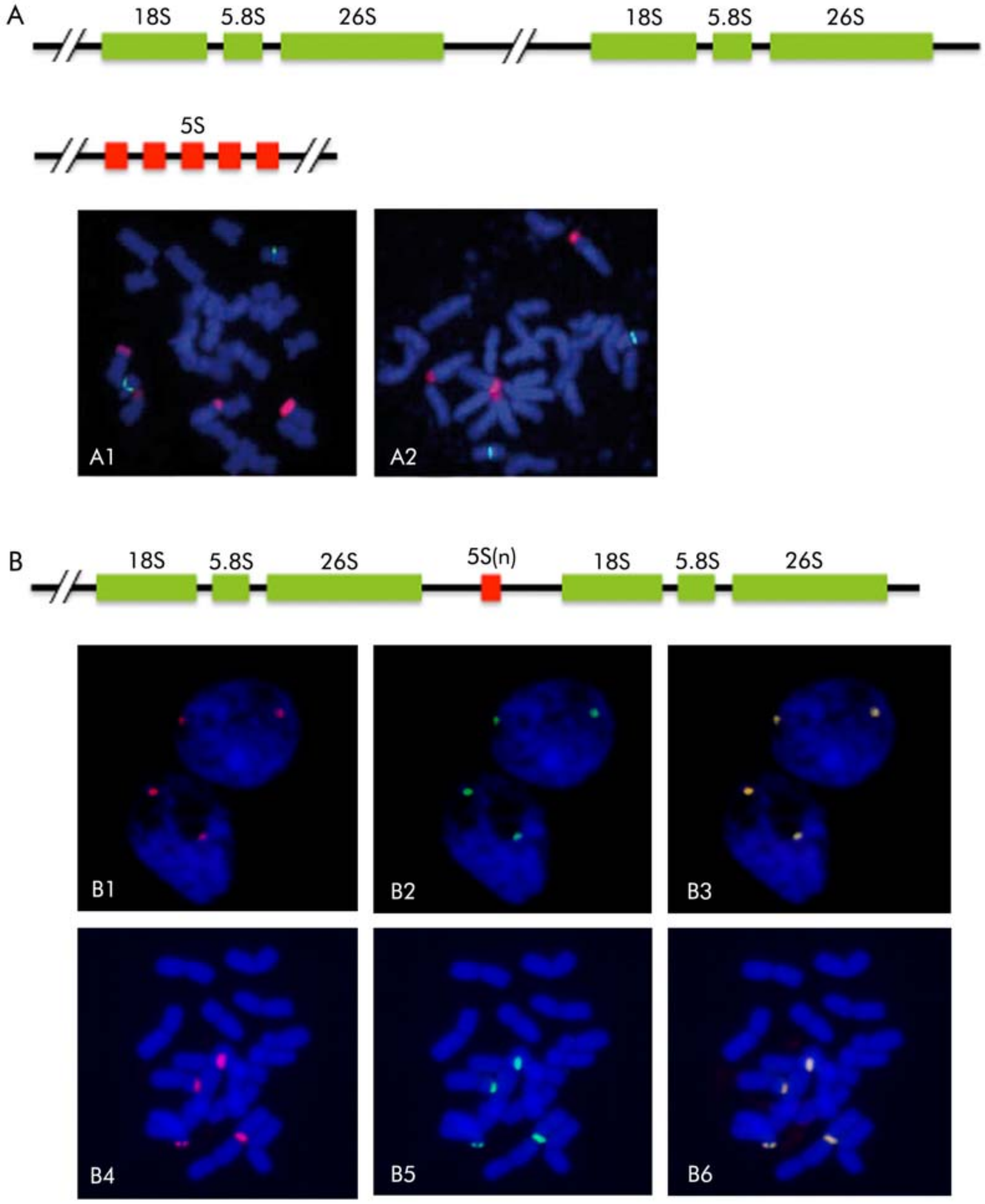

**Figure 5.** Structural organization of the rRNA genes 5S and 18S-5.8S-26S. A) Most plants and animals (e.g. A1, *Myopordon persicum* Boiss. and A2, *Myopordon aucheri* Boiss.; images extracted from Hidalgo *et al.* [79] with permission of the authors). B) Linked structure of both rRNA genes in *Artemisia* (B1-3, *Artemisia magellanica* Sch. Bip.) and relatives (B4-6 *Filifolium sibiricum* (L.) Kitam.). Note that this linked structure has been also reported in other Asteraceae lineages and other organisms.

content, as the 1C*x* values decrease in ascending in ploidy levels, and more remarkably in higher ploidy levels (Fig. 6). Contrary to that, the representatives sampled from the Eurasian clade studied (including previous results from Torrell *et al.* [66]), showed a different trend. Although the loci number was more variable between diploid cytotypes (from 3-5 rDNA loci),

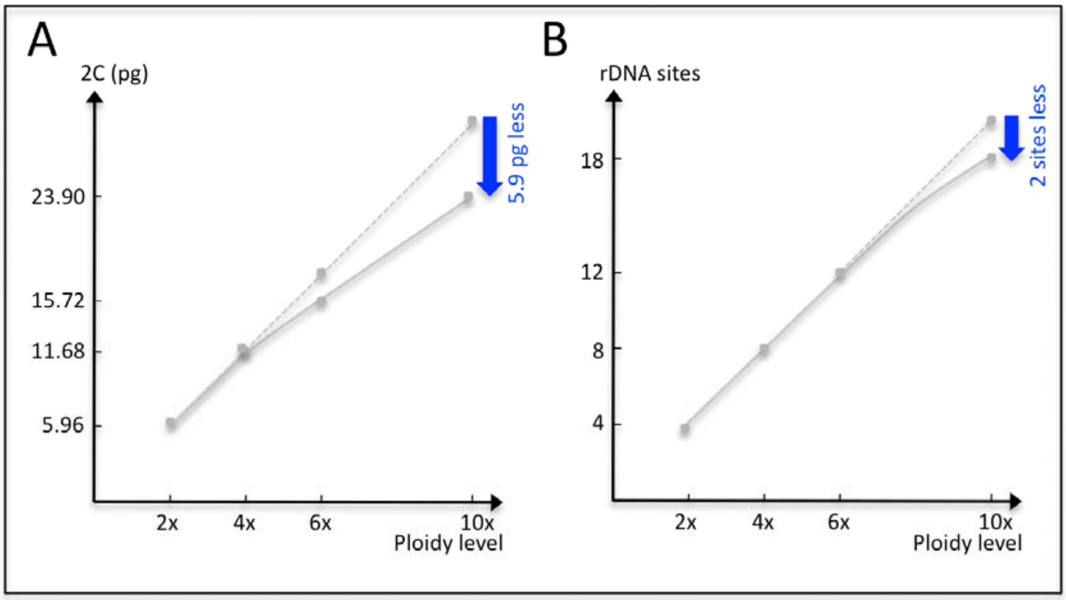

**Figure 6.** Graphical representation of the variation of genome size (A) and rDNA loci (B) in the polyploid series of the species *A. dracunculus*. Dotted lines indicate the theoretical patterns expected for a proportional increase of genome size (A) and rDNA loci (B) when ascending in ploidy levels.

this number remained the same on their related tetraploids, pointing towards a loss of rDNA loci during genome polyploidization. In the species where both 2*x* and 4*x* populations were studied, such a reduction on rDNA loci was not translated to the 1C*x* value, as their values remained about the same, indicating the genome size was doubled but not affecting the number of rDNA loci.

## 6. C-values: The genome size dynamics in *Artemisia*

Swift [80] coined the term ‘C value’ to refer to the DNA amount of the unreplicated haploid complement. Thus, the term monoploid genome size (1C*x*) is related to the nuclear DNA content in a basic chromosome set (*x*) of a somatic cell [81], and therefore, the 2C value refers to the whole genome size of a somatic cell. Genome size, estimated by means of the nuclear DNA content, has been revealed variable across plant groups, becoming an important character in biodiversity. As presently understood, differences among angiosperms are about 2400-fold, from the minute genome of *Genlisea margaretae* Hutch. (Lentibulariaceae) with 2C = 0.128 pg [82] to the recently discovered record holder *Paris japonica* (Franch. & Sav.) Franch. (Melanthiaceae), with a 2C = 304.46 pg [83]. The study of genome size has applications in different fields such as ecology, systematics and evolution [84,85]. Many correlations between this parameter and the biology (e.g. life cycle, guard cell sizes or polyploidy) and the ecology (e.g. plant distribution, environmental stress or insularity) of the species have been reported [e.g. 85-90].

The genus *Artemisia*, and more in depth some of its subgenera, have been studied from this viewpoint, and many studies concerning genome size values and its evolutionary implications have been published [49,90-93]. To provide a framework about the dynamics of this parameter within the genus we present some of the results from Pellicer *et al.* [94]. The range of 2C values within the genus varies about 7.5-fold, a range that increases to ca. 9.2-fold when the annual taxa are taken into account [91,92]. At the present time, the species *A. scoparia* (2C = 3.54, [92]) is known to have the smallest genome size within the genus, although such a value could be even slightly smaller in the closely related *A. capillaris* (mean 2C = 3.37, [75]). At the upper end of the range, the hexaidecaploid (16*x*) populations of the species *A. medioxima* reported from Russia, are the largest in the genus with a mean 2C = 31.01 pg. Nonetheless, the latter species, which is the highest polyploid in the genus, becomes the one with the smallest 1C*x* value (1.93 pg) together with *A. capillaris* (1.68 pg), while the annual diploid *Artemisia leucodes* Schrenk, with 1C*x* = 7.7 pg [93], accounts for the largest one.

As a result of the analysis of genome size evolution in *Artemisia* within a phylogenetic context [94], the authors concluded that at diploid level, although the distribution of nuclear DNA amounts found was fairly consistent with the phylogenetic lineages, the differences were not big enough to discriminate among them. In this sense, those subgenera whose phylogenetic resolution and the traditional classification matched (*Dracunculus*, *Seriphidium* and *Tridentatae*) showed narrower genome size ranges than those with a more conflictive evolutionary history, such as the subgenus *Artemisia* and *Absinthium*, both appearing segregated in independent clades (Fig. 7).

At the beginning of this section, we mentioned that genome size can be influenced by several biological factors. One of them is polyploidy, quite frequent in plants [e.g. 94-96], and which is considered one of the major forces inducing genome size changes. Polyploidy is directly linked with different chromosomal and genomic reorganizations which may imply either an elimination of redundant [97,98] and non-random [99-101] sequences, or contrarily, the accumulation of repetitive sequences, often related to retrotransposons [102].

Within the genus, the nuclear DNA content increases with ascending ploidy levels, but not proportionally (Fig. 8), and the differences between the observed and the expected values for a theoretical proportional increase become more evident in high polyploids (with some exceptions, see [94]). This genome downsizing in polyploids can be also detected at the 1C*x* values, although the changes at this level are less evident. Seeing such a tendency and the quantitative consequences on the genome size in polyploid species, the authors proposed the existence of an upper limit for the nuclear DNA content in the genus. In order to check if this assumption was potentially feasible or not, different mathematical functions were tested to finally reach the conclusion that as the coefficient of correlation for a given Michaelis-Menten model was highly significant ($P<0.01$) it could be theoretically assumed that genome size in polyploids increases approaching asymptotically to a maximum.

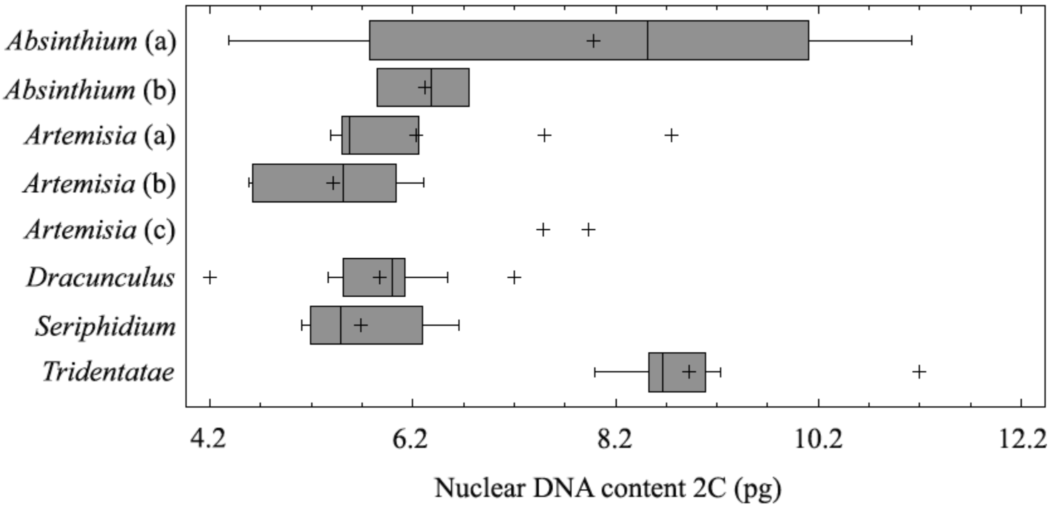

**Figure 7.** Distribution of genome size (2C values of diploid representatives) in *Artemisia*. Note that subgenera *Absinthium* and *Artemisia* are split in two and three lineages respectively, according to the phylogenetic frame depicted in Pellicer *et al.* [94]. Image used with permission of the authors.

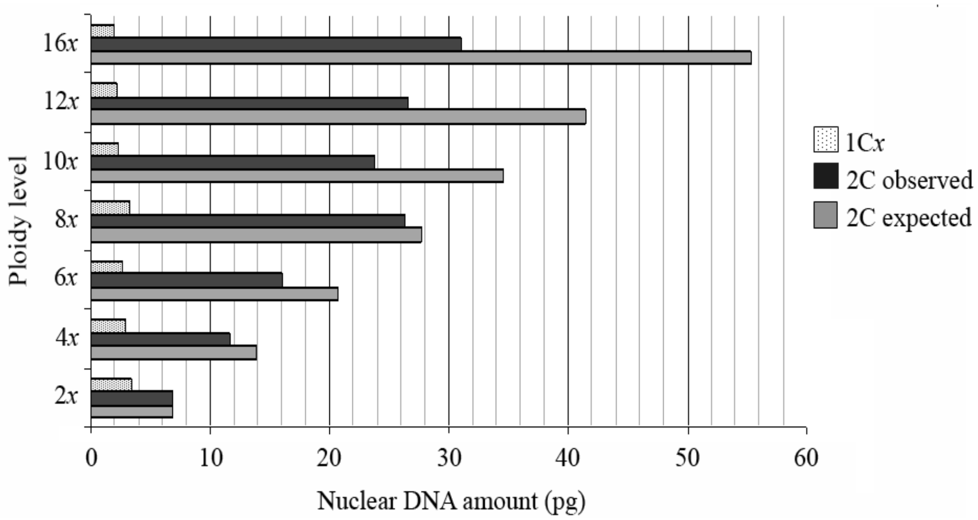

**Figure 8.** Evolution of DNA content at different ploidy levels in the genus *Artemisia* (from Pellicer *et al.* [94] with permission of the authors).

## 7. The phylogenetic relationships in *Artemisia* and allied genera

Since late 1990s and up to present, several approaches to unravel the phylogenetic relationships within the genus *Artemisia* and the subtribe *Artemisiinae* as well have been published [36-39,103-106], but some groups still lack a complete evolutionary knowledge and are being currently investigated, i.e. subgenera *Dracunculus* [38] and *Tridentatae* [39]. The systematics of the genus is challenging to interpret and has engendered controversy among investigators. In some cases processes such as hybridization and polyploidization complicate the relationships between the species at molecular level. In addition, several small segregate genera (i.e. *Crossostephium* Less., *Filifolium*, *Mausolea* or *Picrothamnus* Nutt. among others), previously recognized under *Artemisia*, appear scattered along the phylogenies (Fig. 9), increasing the need for an in depth research into the group.

One of the first attempts to investigate from a broad point of view the phylogenetic relationships within the genus was carried out by Torrell *et al.* [104]. The phylogenetic analyses, based on nuclear DNA sequences (ITS), confirmed the monophyly of the genus including the formerly segregated *Seriphidium* and *Oligosporus*, the independence of the subgenus *Seriphidium* and *Tridentatae*, and also pointed out the conflictive circumscription of the subgenus *Artemisia* which appeared scattered along the phylogeny. These previous findings were supported and deeply investigated with the addition of larger datasets by Watson *et al.* [105] and Vallès *et al.* [40], who suggested the inclusion within the genus of the segregated genera *Artemisiastrum* Rydb., *Crossostephium*, *Filifolium*, *Mausolea*, *Neopallasia*, *Picrothamnus*, *Sphaeromeria* Nutt. and *Turaniphytum*. However, some new conflicts arose; as Watson *et al.* [105] maintained the generic status of the *Seriphidium* (even with no phylogenetic support) and expanded it to the subgenus *Tridentatae*, while Vallès *et al.* [40] decided to keep both *Seriphidium* and *Tridentatae* as independent from each other and at subgeneric level within *Artemisia*.

The addition of an extra nuclear DNA region (ETS) to the formerly studied ITS phylogenies revealed new linkages which were previously unknown to date [36,37]. Sanz *et al.* [36] found the *Kaschgaria* Poljakov group as sister to the remaining *Artemisia*, a point that has not been further confirmed due to the lack of statistical support in the node splitting the *Artemisia* and *Kaschgaria* lineages [37,94, Fig. 9]. Besides, Sanz *et al.* [36] considered two major lineages within *Artemisia*, the subgenus *Dracunculus* clade, including the segregated *Filifolium*, *Mausolea*, *Neopallasia* and *Turaniphytum*, and a second lineage embracing the remaining representatives of the genus (*Absinthium*, *Artemisia*, *Seriphidium* and *Tridentatae*) plus the segregated *Sphaeromeria* and *Picrothamnus* within the North American endemic clade.

Similar results were reported by Tkach *et al.* [37], although the simplicity of the phylogenetic structure proposed by Sanz *et al.* [36] needed amplification and additional study. In fact, as the data sample was enlarged, new conflicts with the traditional classification rose up, some of them which had been evident since early studies of the genus. Thus, the split of the subgenus *Artemisia* into independent lineages became more evident along the phylogeny, and also some of the representatives of the subgenus *Absinthium* (considered by the authors as a section of the former) were placed at undetermined position (Fig. 9). In this sense, it is interesting to remark that the *A. vulgaris* complex was linked to a group of North American (plus few Arctic) taxa currently recognized to belong to the subgenus *Artemisia*. This fact would give support to the hypothesis of McArthur and Plummer [107], of a migration via Bering strait of representatives of the subgenus *Artemisia* from the Old to the New World, but failing to confirm that those would be the ancestors of the subgenus *Tridentatae*.

As discussed in the previous paragraphs, the North American endemic *Tridentatae* and the subgenus *Dracunculus* are being currently investigated, and both have been the subject of research projects [75,106] to clarify, among other issues, their phylogenetic relationships. On the one hand, the *Tridentatae* has puzzled researchers in many fields for some time because of its geographic circumscription. First approaches to study the molecular systematics of the subgenus were carried out by Kornkven [109]. At that point, conflicts between the nuclear (ITS) and the chloroplast DNA surfaced [103,110], making a proper understanding of the evolution of the group difficult. Many of the species are landscape dominant, so authors pointed out the possibility that interspecific gene flow and subsequent chloroplast capture might have favoured the existence of such conflicts [103]. In addition to the recent taxonomical re-evaluation published by Shultz [35], new phylogenetic frameworks have been constructed and a broader circumscription of the subgenus *Tridentatae* including other North American endemic previously recognized as non-*Tridentatae* have been proposed [39].

On the other hand, phylogenetic studies including a representative sample of the subgenus *Dracunculus* are still very scarce. Previous studies have reported the existence of two major lineages (Fig. 9), one including the *A. dracunculus* complex (the type species plus some other allied taxa) and the main lineage, which includes the vast majority of the species within the subgenus, mostly distributed across Eurasia. Besides, the inclusion of the segregated *Mausolea eriocarpa*, *Neopallasia pectinata*, and *Turaniphytum eranthemum* has been confirmed by nuclear and chloroplastic DNA sequences [36, 38] and therefore, taxonomic rearrangements have been proposed to return this species to *Artemisia*. In the case of *Filifolium sibiricum*, while its

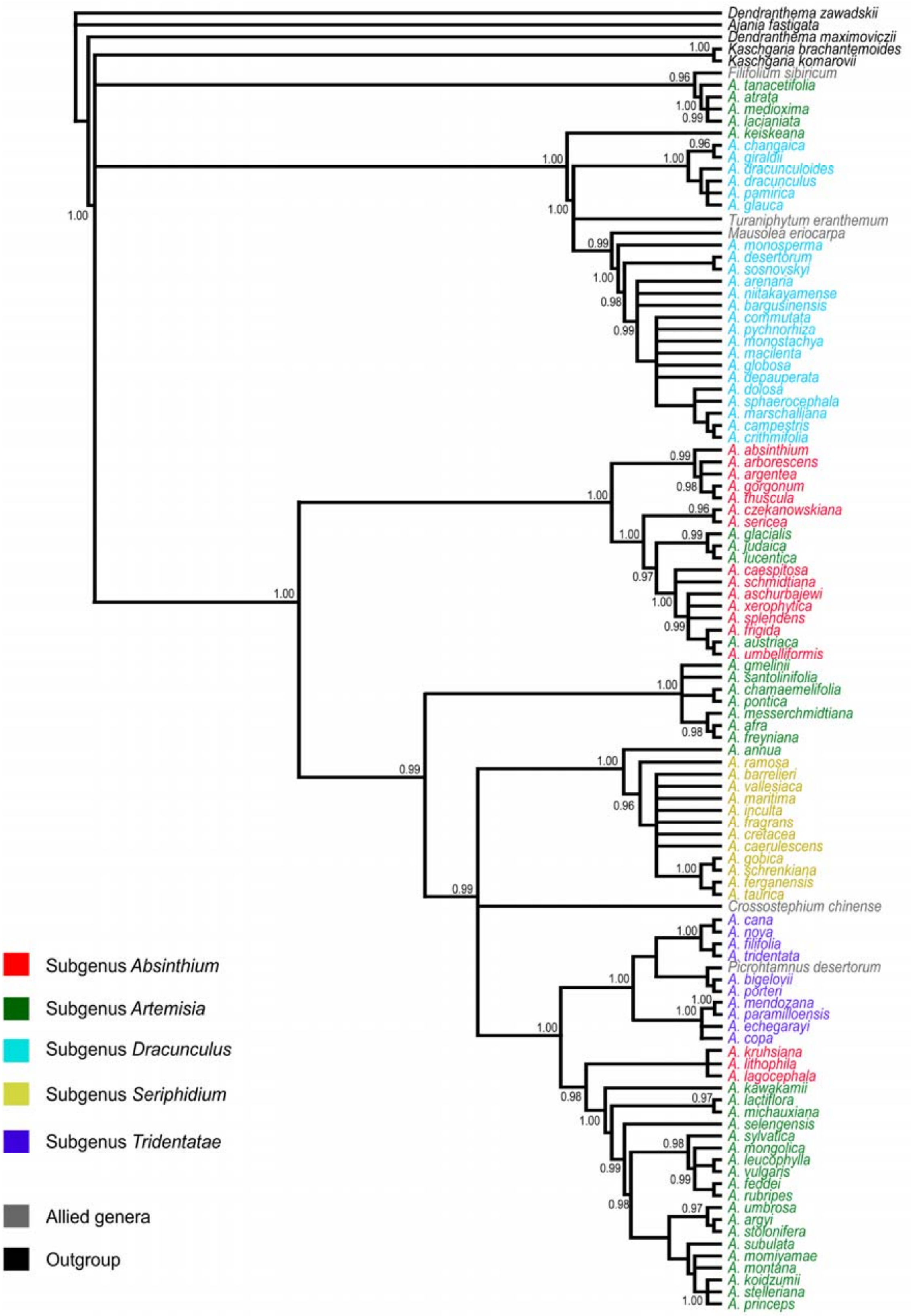

**Figure 9.** Phylogeny of *Artemisia* resulting from Bayesian analysis adapted from Pellicer *et al.* [94] with minor modifications (only PP values ≥ 0.95 are indicated on branches).

inclusion within the genus *Artemisia* is confirmed, its specific placement is not yet resolved. Vallès *et al.* [40] and Sanz *et al.* [36] reported this species as sister to the subgenus *Dracunculus*, but recently Pellicer *et al.* [94] have found that previous findings could be influenced by sample size, as their study embeds the species in one of the subgenus *Artemisia* clades next to the *Dracunculus* lineages but with not enough statistic power to be considered as sister group.

The *A. dracunculus* complex, including ca. 10 species, is characterized by the synapomorphy of simple linear-lanceolate leaves, with the exception of *Artemisia jordanica* Danin, which is the unique case of simple leaves beyond the complex [111]. The group is characterized, as previously discussed, by a great incidence of polyploidy. Although most of the representatives have been described at diploid (*Artemisia giraldii* Pamp., *A. glauca* Pall., *A. pamirica* C. Winkl.) or tetraploid levels (e.g. *A. changaica*, *A. glauca*, *A. subdigitata*), a complete polyploid series of $2n = 18, 36, 56, 72, 90$ has been reported in the type species (*A. dracunculus*). The Eurasian clade embeds the majority of the species of the subgenus, which is characterized by a low sequence divergence among species, and therefore, by a lack of enough statistical node support in many subclades, making it sometimes difficult to find convincing explanations for the evolutionary relationships within the group. Among the North American endemic complex, some of the representatives that have been traditionally included in the subgenus *Dracunculus* (*Artemisia filifolia* Torr., *Artemisia pedatifida* Nutt. and *Artemisia porteri* Cronquist, respectively, *Picrothamnus desertorum* [= *Artemisia spinescens* D.C. Eaton], but the molecular evidence has highlighted a strong relationship to the *Tridentatae*. Thus, the recent taxonomic treatments of the group have proposed the inclusion of these species within the subgenus *Tridentatae* [35], and the current phylogenetic studies also have pointed out some additional taxonomic rearrangements needed [39].

## 8. The pollen grain: A useful systematic marker

The pollen types in the Asteraceae have been revealed quite variable in terms of shapes and structures [112]. Within the *Artemisiinae* tribe, two basic pollen types have been described on the basis of the exine ornamentation [113]: the *Artemisia*-type, with spinules (microechinate), and the *Anthemis*-type, with patent spines (echinate) (Fig. 10). In this sense, the exine ultrastructure has been studied for both pollen types for some time, but the first study describing this spineless form in the genus *Artemisia* and relatives was Wodehouse [114], being later confirmed by several studies with samples across the globe [e.g. 8-11,17,115-118]. In addition, morphometrical comparisons carried out between the *Anthemis* and *Artemisia* pollen types have highlighted that differences are not only based on the spines size, but also on the pollen size, shape, and the spine density [12].

The segregation of both pollen types throughout the *Artemisiinae* has been also the focus of several studies [10-12,36,40,118]. These studies have revealed that each pollen-type defines one of the two main groups within the subtribe: the *Dendranthema* and relatives clade, characterized by the *Anthemis*-type pollen, and the *Artemisia* and allies clade, by the *Artemisia*-type one. Although some exceptions to the rule are presented in these studies the hypothesis of possible pollen-type reversions along the *Artemisiinae* via species hybridization may be important and require further study.

Returning back to the genus *Artemisia*, the number of studies dealing with pollen morphology and its systematic implications has recently notably increased [8-10,119-121]. From the morphologic point of view, these studies consider the pollen of *Artemisia* as 3-zonocolporate, isopolar, radially symmetrical, prolate to perprolate and, as described above, microechinate.

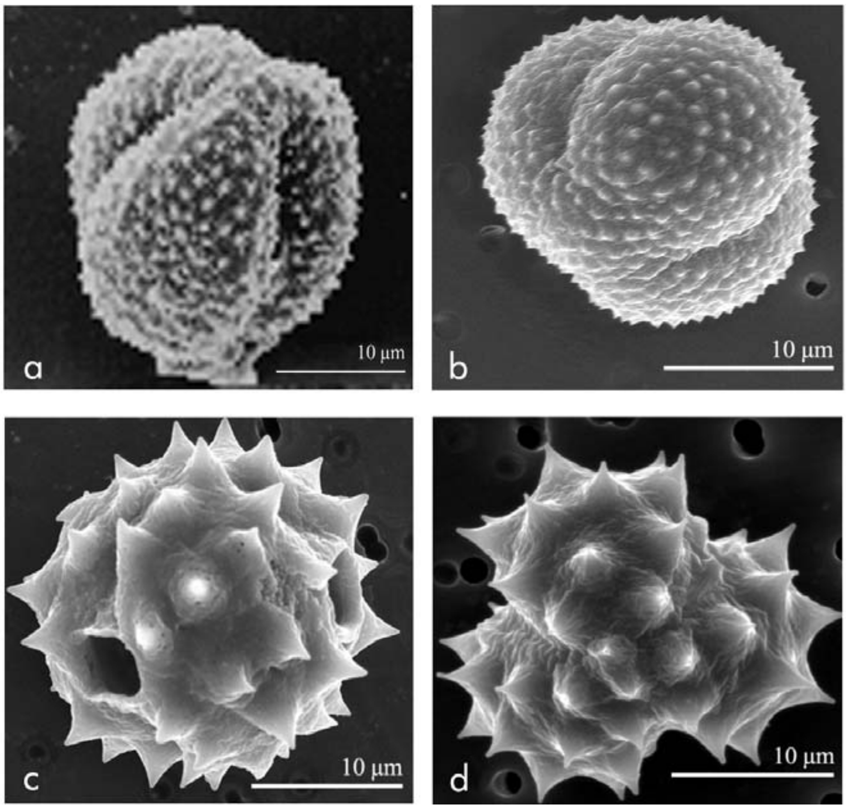

**Figure 10.** Some examples of pollen types. *Artemisia*-type: a) *Artemisia incana* Druce and b) *Crossostephium chinense* (L.) Makino. *Anthemis*-type: c) *Ajania nubigena* (Wall.) C. Shih and d) *Cancrinia discoidea* (Ledeb.) Poljakov ex Tzvelev. Images extracted from Martín *et al.* [10] and Pellicer *et al.* [12] with permission of the authors.

The study of Jiang *et al.* [8] further investigated in detail the *Artemisia* pollen traits and divided it in two main groups, the *Myriantha*-type and the *Mongolica*-type, the latter being subdivided in four subtypes (*Sacrorum, Oligocarpa, Lavandulaefolia,* and *Anomala*). The authors based this classification on the evolution and distribution of the species, since the *Myriantha* (primitive) type is widely found in plants inhabiting in high latitudes whereas the subtypes of *Mongolica* (derived) seem to be related with low latitude distributions. However, while the former (*Artemisia* and *Anthemis*) pollen types might be used as a systematic marker at subtribal level, many of the authors have concluded that the differences within *Artemisia* are few, and have poor diagnostic potential to be useful distinguishing between species [8,9,120].

## 9. Conclusions and future prospects

This review has considered a significant number (around 40, see the literature cited, particularly [75,108] and references therein) of studies and research approaches that have been devoted to elucidate, from different points of view, the evolutionary history of the genus. *Artemisia* is a giant within the Anthemideae, and that is why such a big volume of work has been and it is currently being carried out on the genus. The combination of results from an important variety of disciplines such as, karyology (chromosome counts, karyotype elaboration and fluorochrome banding), molecular cytogenetics (*in situ* hybridization of different DNA probes), molecular systematics (DNA sequencing programs and population genetics), taxonomy (including micro- and macromorphologic approaches) is essential to achieve a level of knowledge which is allowing researchers to propose and test robust hypotheses about the evolution of the genus.

Even so more multidisciplinary approaches covering specific groups and/or complexes within the genus which are currently lacking for a complete evaluation are needed. These will complement the present knowledge of the genus and also may suggest that further taxonomic reconsiderations are needed not only in *Artemisia* but also within the *Artemisiinae*.

## Acknowledgements

The authors acknowledge all researchers involved in *Artemisia* and/or related genera studies for their substantial contributions and improvements to the knowledge of this group of plants from all perspectives, and all our colleagues who have kindly given their permission to use graphics and captions already published. Dr. Oriane Hidalgo (Ohio University) is acknowledged for her suggestions and assistance on the draft of this paper. The authors address also their thanks to Prof. E. Durant McArthur (Rocky Mountain Research Station), who reviewed and improved a first version of the manuscript. This work has been supported by different projects from the DGICYT (Spanish government, CGL2007-64839-C02-01 and 02/BOS, CGL2010-22234-C02-01 and 02/BOS) and the Generalitat de Catalunya (Catalan government, 2005SGR00344, 2009SGR439).

## References

1. Ling, Y.R., Humphries, C.J., and Shultz, L. 2006, Flora of China, Vol. 20 (Asteraceae), Editorial Committee (Eds.), Science Press and Missouri Botanical Garden Press. Beiging, - St. Louis. http://flora.huh.harvard.edu/china/mss/volume20/index.htm
2. Bremer, K., and Humphries C.J. 1993, *Bull. Nat. Hist. Mus. London (Bot.)*, 23, 71.
3. Kubitzki, K. 2007. The families and genera of vascular plants. Vol VIII Flowering plants. Eudicots. Asterales. Kadereit J.W. and Jeffrey C. (Eds.) Springer-Verlag: Berlin Heidelberg, 358.
4. Funk, V.A., Susanna, A., Stuessy, T., and Bayer, R. (Eds.) 2009, Systematics, Evolution and Biogeography of the Compositae. International, Washington D.C., 631
5. Bremer, K. 1994, Asteraceae: cladistics and classification. Timber Press: Portland.
6. Ling, Y.R. 1994, *Compositae Newslett.*, 25, 39.
7. Wang, W.M. 2004, *Bot. J. Linn. Soc.*, 145, 331.
8. Jiang, L., Wang, Q., Ye, L.Z., and Ling, Y.R. 2005, *Wuhan Univ. J. Nat. Sci.*, 10, 448.
9. Hayat, M.Q., Ashraf, M., Khan M.A., Yasmin, G., Shaheen N., and Jabeen, S. 2010, *Plant J. Bot.*, 42, 751.
10. Martín, J., Torrell, M., and Vallès, J. 2001, *Plant Biol.*, 3, 372.
11. Martín, J., Torrell, M., Korobkov, A.A., and Vallès, J., 2003, *Plant Biol.*, 5, 85.
12. Pellicer, J., Hidalgo, O., Garcia, S., Garnatje, T., Korobkov, A.A., Vallès, J., and Martín, J. 2009, *Bot. J. Linn. Soc.*, 161, 171.
13. Poljakov, P.P. 1961, *Trudy Instituta Bot. Akad. Nauk Kazaksh. S.S.R.*, 11, 134.
14. Poljakov P. P. 1961, Rod 1550. Polyn-*Artemisia* L., Shishkin B.K., and Bobrov E.G. (Eds.) Flora SSSR, Vol. 25. Leningrad.
15. Greger, H. 1977, Anthemideae - Chemical Review, Heywood, V.H., Harborne, J.B., and Turner, B.L. (Eds.), The biology and chemistry of the Compositae, Vol. 2. Academic Press, London, 899.
16. Garnock-Jones, P.J. 1986, *Bot. J. Linn. Soc.*, 92, 285.
17. Vallès, J., Suárez, M., and Seoane, J.A. 1987, *Acta Salmanticensia*, *Ciencias* 66, 167.
18. Vallès, J., and McArthur, E.D. 2001, *Artemisia* systematics and phylogeny: cytogenetic and molecular insights, McArthur, E.D., Fairbanks, D.J. (Eds.)

Shrubland Ecosystem Genetics and Biodiversity. Department of Agriculture Forest Service, Rocky Mountain Research Station: Utah, 67.

19. Vallès, J., and Garnatje, T. 2005, *Artemisia* and its allies: genome organization and evolution and their biosystematic, taxonomic and phylogenetic implications in the Artemisiinae and related subtribes (Asteraceae, Anthemideae). In: Sharma AK., Sharma A., (Eds.). Plant genome: biodiversity and evolution, 255. Vol. 1B: Phanerogams (Higher groups). Enfield, M/S Science Publishers.
20. Tournefort, J.P. 1700, Institutiones rei herbariae. Paris.
21. Linné, C. 1735, Systema naturae. Leiden.
22. Cassini, A.H.G. 1817, *Bull. Sci. Soc. Philom. Paris*, 3, 31.
23. Lessing, C.F. 1832, Synopsis generum Compositarum. Berlin.
24. Besser, W.S.J.G. 1829, *Bull. Soc. Imp. Naturalistes Moscou*, 1, 219.
25. Besser, W.S.J.G. 1832, *Bull. Soc. Imp. Naturalistes Moscou*, 3, 5.
26. Besser, W.S.J.G. 1834, *Bull. Soc. Imp. Naturalistes Moscou*, 7, 5.
27. Besser, W.S.J.G. 1836, *Bull. Soc. Imp. Naturalistes Moscou*, 8, 3.
28. Candolle, A.P. 1837, Prodromus systematis naturalis regni vegetabilis. Paris.
29. Rouy, G.C.C. 1903, Flore de France 8. Paris.
30. Rydberg, P.A. 1916, *North American Flora*, 34, 244.
31. McArthur, E.D., Pope, C.L., and Freeman, D.C. 1981, *Amer. J. Bot.*, 68, 589.
32. Ling, Y.R. 1991, *Bull. Bot. Res., Harbin*, 12, 1.
33. Ling, Y.R. 1995, The New World *Seriphidium* (Besser) Fourr., Hind, D.J.N., Jeffrey, C., and Pope, G.V. (Eds.). Advances in Compositae systematics. Royal Botanic Gardens, Kew, 283.
34. Shultz, L.M. 2006, "*Artemisia*", Flora of North America vol. 21: Asterales. Editorial Committee, (Eds.) Oxford University Press, New York.
35. Shultz, L.M. 2009, *Syst. Bot. Monographs*, 89, 1.
36. Sanz, M., Vilatersana, R., Hidalgo, O., Garcia-Jacas, N., Susanna, A., Schneeiwess, G.M., and Vallès, J. 2008, *Taxon*, 57, 66.
37. Tkach, V.N., Hoffmann, M.H., Röser, M., Korobkov, A.A., and Von Hagen, K.B. 2008, *Evolution*, 62, 184.
38. Pellicer, J.P., Vallès, J., Korobkov, A.A., and Garnatje, T. 2011, *Taxon*, 60, 691.
39. Garcia, S., McArthur, E.D., Pellicer, J., Sanderson, S.C., Vallès, J., and Garnatje, T. 2010, *Amer. J. Bot.*, 98, 638.
40. Vallès, J., Torrell, M., Garnatje, T., Garcia-Jacas, N., Vilatersana R., and Susanna, A. 2003, *Pl. Biol.*, 5, 274.
41. Watanabe, K. 2004, *Compositae Newslett.*, 41, 64.
42. Solbrig O.T. 1977, Chromosomal cytology and evolution in the family Compositae. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.). The biology and chemistry of the Compositae, Vol. I. London, New York, San Francisco: Academic Press, 269.
43. Schweizer, D., and Ehrendorfer, F. 1983, *Biol. Zentralbl.*, 102, 637.
44. Oliva, M., and Vallès, J. 1994, *Canad. J. Bot.*, 72, 1126.
45. McArthur, E.D., and Sanderson, S.C., 1999, *Amer. J. Bot.*, 86, 1754.
46. Wiens, D., and Richter, J.A., 1996, *Amer. J. Bot.*, 53, 981.
47. Matoba, H., Nagano, K., and Hoshi, Y. 2007, *Cytologia*, 72, 181.
48. Vallès, J., and Siljak-Yakovlev, S. 1997, *Canad. J. Bot.*, 75, 595.

49. Pellicer, J., Garcia, S., Garnatje, T., Hidalgo, O., Korobkov, A.A., Dariimaa, S., and Vallès, J. 2007, *Bot. J. Linn. Soc.*, 153, 301.
50. Pellicer, J., Garcia, S., Garnatje, T., Dariimaa, Sh., Korobkov, A.A., and Vallès, J. 2007, *Chromosome Bot.*, 2, 45.
51. Ehrendorfer, F. 1964, *Oesterr. Bot. Z.*, 111, 84.
52. Estes, J.R. 1969, *Brittonia*, 21, 29.
53. Kawatani, T., and Ohno, T. 1964, *Bull. Natl. Inst. Hyg. Sci. Tokyo*, 82, 183.
54. Korobkov, A.A. 1972, *Bot. Zhurn.*, 57, 1316.
55. Persson, K. 1974, *Opera Bot. (Lund)*, 35, 1.
56. McArthur, E.D., and Pope, C.L. 1979, *Great Basin Naturalist*, 39, 419.
57. Garcia, S., Garnatje, T., Dariimaa, Sh., Tsooj, Sh., and Vallès, J. 2006, *Bot. J. Linn. Soc.*, 150, 203.
58. Sánchez-Jiménez, I., Pellicer, J., Hidalgo, O., Garcia, S., Garnatje. T., and Vallès, J. 2009, *Folia Geobot.*, 44, 307.
59. Zhang, M.J., and He, X.L. 2010, *Pl. Syst. Evol.*, 210, 109.
60. Malakhova, L.A. 1990, *Byull. Glavn. Bot. Sada*, 155, 60.
61. Torrell, M., Vallès, J., Garcia-Jacas, N., Mozzafarian, V., and Gabrielian, E. 2001, *Bot. J. Linn. Soc.*, 135, 51.
62. Krogulevich, R.E. 1978, Kariologicheskii analiz vidov flory vostochnogo Sayana. In: Malyshev L.I., Peshkova G.A., (Eds.). Flora Pribaikalya. Novosibirsk: Nauka, 19.
63. Kreitschitz, A., and Vallès, J. 2003, *Folia Geobot.*, 38, 333.
64. Pellicer, J., Garnatje, T., Molero, J., Pustahija, F., Siljak-Yakovlev, S., and Vallès, J. 2010, *Austr. J. Bot.*, 58, 605.
65. Torrell, M., Cerbah, M., Siljak-Yakovlev, S., and Vallès, J. 2001, *Bocconea*, 13, 623.
66. Torrell, M., Cerbah, M., Siljak-Yakovlev, S., and Vallès, J. 2003, *Plant Syst. Evol.*, 239, 141.
67. Hoshi, Y., Matoba, H., and Kondo, K. 2006, *Caryologia*, 59, 312.
68. Garcia, S., Garnatje, T., Hidalgo, O., McArthur, E.D., Siljak-Yakovlev, S., and Vallès, J. 2007, *Pl. Syst. Evol.*, 267, 79.
69. Garcia, S., Lim, K.Y., Chester, M., Garnatje, T., Pellicer, J., Vallès, J., Leitch, A.R., and Kovarik, A. 2009, *Chromosoma*, 118, 85.
70. Grummt, I., and Pikaard, C.S. 2003, *Nat. Rev. Mol. Cell. Biol.*, 4, 641.
71. Goyon, C., Rossignol, J.L., and Faugeron, G. 1996, *Nucleic Acids Res.*, 24, 3348.
72. Vahidi, H., Curran, J., Nelson, D.W., Webster J.M., McClure, M.A., and Honda, B.M. 1988, *J. Mol. Evol.*, 27, 222.
73. Belkhiri, A., Buchko, J., and Klassen, G.R. 1992, *Mol. Biol. Evol.*, 110, 86
74. Vitturi, R., Colomba, M.S., Pirrone, A.M., and Mandrioll, M. 2002, *J. Hered.*, 93, 279.
75. Pellicer, J. 2009, Ph.D. dissertation, University of Barcelona, Barcelona, Spain.
76. Garcia, S., Panero, J.L., Siroky, J., and Kovarik, A. 2010, *BMC Plant Biol.*, 10, 176.
77. Pellicer, J., Garcia, S., Garnatje, T., Hidalgo, O., Siljak-Yakovlev, S., and Vallès, J. 2008, *Collect. Bot. (Barcelona)*, 27, 19.
78. Pellicer, J., Garnatje, T., Hidalgo, O., Tagashira, N., Vallès, J. and Kondo, K. 2010, *Plant Biosyst.*, 144, 841.

79. Hidalgo, O., Garcia-Jacas, N., Garnatje, T., Romashchenko, K., Susanna, A., and Siljak-Yakovlev, S. 2008, *Taxon*, 57, 769.
80. Swift, H. 1950, *Proc. Nat. Acad. Sci. U.S.A.*, 36, 643.
81. Greilhuber, J., Dolezel, J., Lysak, M.A., and Bennett, M.D. 2005, *Ann. Bot.*, 95, 255.
82. Greilhuber, J., Borsch, T., Müller, K., Worberg, A., Porembski, S., and Barthlott, W. 2006, *Pl. Biol.*, 8, 770.
83. Pellicer, J., Fay, M.F., and Leitch, I.J. 2010, *Bot. J. Linn. Soc.*, 164, 10.
84. Bennett, M.D., and Leitch, I.J. 2005, *Ann. Bot.*, 95, 45.
85. Bennett M.D., and Leitch, I.J. 2005, *Ann. Bot.*, 95, 1.
86. Vinogradov, A.E. 2003, *Trends Genet.*, 19, 609.
87. Albach, D.A., and Greilhuber, J. 2004, *Ann. Bot.*, 94, 897.
88. Garnatje, T., Vallès, J., Garcia, S., Hidalgo, O., Sanz, M., Canela, M.Á., and Siljak-Yakovlev, S. 2004, *Biol. Cell*, 96, 117.
89. Price, H.J., Dillon, S.L., Hodnett, G., Rooney, W.L., Ross, L., and Johnston, J.S. 2005, *Ann. Bot.*, 95, 219.
90. Beaulieu, J.M., Leitch., I.J., Patel, S., Pendharkar, A., and Knight, C.A. 2008, *New Phytol.*, 179, 975.
91. Garcia, S., Canela, M.Á., Garnatje, T., McArthur, E.D., Pellicer, J., Sanderson, S.C., and Vallès, J. 2008, *Biol. J. Linn. Soc.*, 94, 631.
92. Torrell, M., and Vallès, J. 2001, *Genome*, 44, 231.
93. Garcia, S., Sanz, M., Garnatje, T., Kreitschitz, A., McArthur, E.D., and Vallès, J. 2004, *Genome*, 47, 1004.
94. Pellicer, J., Garcia, S., Canela, M.A., Garnatje, T., Korobkov, J.D., and Vallès, J. 2010, *Pl. Biol.*, 12, 820.
95. Soltis, D.E., and Soltis, P.S. 2000, *Proc. Nat. Acad. Sci. U.S.A.*, 97, 7051.
96. Wendel, J.F. 2000, *Pl. Mol. Biol.*, 42: 225.
97. Cui, L., Wall, P.K., Leebens-Mack, J.H., Lindsay, B.G., Soltis, D.E., Doyle, J.J., Soltis, P.S., Carlson, J.E., Arumuganathan, K., Barakat, A., Albert, V.A., Ma, H., and de Pamphilis, C.W. 2006, *Genome Res.*, 16, 738.
98. Petrov, D.A. 2001, *Genetica*, 114, 81.
99. Bennetzen, J.L., Ma, J., and Devos, K.M. 2005, *Ann. Bot.*, 95, 127.
100. Feldman, M., Liu, B., Segal, G., Abbo, S., Levy, A.A., and Vega, J.M. 1997, *Genetics*, 147, 1381.
101. Ozkan, H., Levy, A.A., and Feldman, M. 2001, *Plant Cell*, 13, 1735.
102. Parisod, C., Alix, K., Just, J., Petit, M., Sarilar, V., Mhiri, C., Ainouche, M., Chalboub, B., and Grandbastien M.A. 2010, *New Phytol.*, 186, 37.
103. Kornkven, A.B., Watson, L.E., and Estes, J.R. 1998, *Amer. J. Bot.*, 85, 1787.
104. Torrell, M., Garcia-Jacas, N., Susanna, A., and Vallès, J. 1999, *Taxon*, 48, 721.
105. Watson, L.E., Bates, P.L., Evans, T.M., Unwin, M.M., and Estes, J.R. 2002, *BMC Evol. Biol.*, 2, 17.
106. D’andrea, S., Caramiello, R., Ghignone, S., and Siniscalco, C. 2003, *Plant Biosyst.*, 137, 121.
107. McArthur, E.D., and Plummer, A.P. 1978, *Great Basin Naturalist Mem.*, 2, 229.
108. Garcia, S. 2007, Ph.D. dissertation, University of Barcelona, Barcelona, Spain.

109. Kornkven, A.B. 1997, Ph.D. Thesis dissertation, University of Oklahoma, Norman, U.S.A.
110. Kornkven, A.B., Watson, L.E., and Estes, J.R. 1999, *Syst. Bot.*, 24, 69.
111. Danin, A. 1999, *Willdenowia*, 29, 177.
112. Jeffrey, C. 2007, Introduction with keys to tribes. In: Kadereit, J.W., Jeffrey, C. (Eds.). Flowering plants vol. VIII. Eudicots. Asterales.: Springer-Verlag Press: Berlin Heidelberg, 61.
113. Stix, E. 1960, *Grana Palynol.*, 2, 41.
114. Wodehouse, R.P. 1926, *Bull. Torr. Club*, 53, 479.
115. Monoszon, M.X. 1948, Morfologiya pyltsy polynei. In: Sukachev, V.N. (Ed.). Trudy konferentsii po sporovo-pyltsemu analizu. Izdatelstvo Moskovskogo Universiteta: Moskva, 37.
116. Straka, H. 1952, *Svensk Bot. Tidsskr.*, 46, 204.
117. Grigoreva, V.V., Korobkov, A.A., and Tokarev, P.I. 2009, *Bot. Zhur.*, 94, 328.
118. Chen, S.G., and Zhang, J.T. 1991, *Acta Phytotax. Sin.*, 29, 246.
119. Hayat, M.Q., Ashraf, M., Khan M.A., Yasmin, G., Shaheen, N., and Jabeen, S. 2009, *Afr. J. Biotechnol.*, 8, 6568.
120. Ghahreman, A., Noorbakhsh, S.N., Mehdigholi, K. and Attar, F. 2007. *Iran J. Bot.*, 13, 21.
121. Sun, H.Z., He, L.X., Chen, T.S., and Zhang, X.J. 2007, *Progr. Nat. Sci.*, 17, 35.