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EVOLUCIÓ, FILOGÈNIA I SISTEMÀTICA DEL COMPLEX *ARCTIUM-COUSINIA*

Memòria presentada per Sara López Viñallonga per a optar al títol de Doctor per la Universitat de Barcelona

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7. 3. Chromosome counts in the genera *Cousinia*, *Olgaea* and *Syreitschikovia* (Compositae)

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RESUM. Les escasses dades cariològiques disponibles tant per al complex *Arctium-Cousinia* com pel grup *Onopordum*, el qual mostra paral·lelismes amb el complex *Arctium-Cousinia*, han motivat aquest treball en que s'aporten recomptes cromosòmics amb l'objectiu de contribuir a entendre l'evolució cariològica d'aquests taxons. Es presenten 20 recomptes per al gènere *Cousinia* s. l. 13 dels quals són dades noves i els set restants complementen, confirmen o qüestionen recomptes anteriors. Tres de les 13 seccions presents a aquest estudi han estat comptades aquí per primera vegada. Els recomptes de les set espècies del llinatge Arctioide, $2n = 36$, mostren una gran uniformitat cariològica. En canvi, al grup Cousinioide els tretze recomptes que s'aporten coincideixen amb els tres principals nombres cromosòmics de la seva característica sèrie disploide $2n = 22$, 24 i 26. Tots aquests resultats confirmen la idea de que, malgrat que el grup Arctioide i Cousinioide conformen un grup natural, han seguit camins evolutius diferents. Finalment es presenten nous recomptes per als gèneres *Olgaea* i *Syreitschikovia*, $2n = 26$ i 24 respectivament, que mai havien estat comptats. Aquestes dades confirmen l'existència de dos llinatges també al grup *Onopordum*; els taxons colonitzadors ($x = 17$) i els perennes ($x = 12$, 13). No s'han trobat evidències de poliploïdia recent a cap dels grups estudiats.

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Chromosome counts in the genera *Cousinia*, *Olgaea* and *Syreitschikovia* (Compositae)

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Abstract

The scarce karyological data available for both the *Arctium–Cousinia* complex and the *Onopordum* group has led us to provide more data essential to understand the karyological evolution of these taxa. Chromosome counts were made on somatic metaphases using the squash technique. The root tips were pretreated with 8–hydroxyquinoleine solution at 4ºC, fixed in fresh Carnoy I solution overnight at – 20ºC, hydrolysed with 5N HCl for 50 min at room temperature, stained with 1% acetic orcein and squashed in 45% acetic acid. We report twenty chromosome counts for the genus *Cousinia* s.l. Thirteen of them are new data and the remaining seven provide confirmation of scarce or uncertain previous data. Three of the thirteen sections present are counted for the first time. Our counts of the seven Arctioid species show karyological uniformity with $2n = 36$. In the Cousinioid group, our thirteen counts agree with the three major numbers of its characteristic dysploidy series $2n = 22$, 24 and 26. We also report two new chromosome counts for the genera *Olgaea* and *Syreitschikovia*, $2n = 26$ and 24 respectively, never counted before. Our results confirm the idea that, although the Arctioid and Cousinioid clades form a monophyletic group, they have followed different evolutionary paths. In the *Onopordum* group, our results confirm the existence of two lines; the colonizing taxa ($n = 17$) and the perennial genera ($n = 12$, 13). The evidence for recent polyploidization is absent in both *Arctium–Cousinia* complex and the *Onopordum* group.

Key words: dysploidy; karyology; polyploidy; Arctioid clade; Cousinioid clade.

Introduction

Our contribution deals with two of the informal (not taxonomic) groups defined in the latest survey of the large tribe Cardueae Cass. (Compositae): The *Arctium–Cousinia* complex and the *Onopordum* group. According to its most recent circumscription (Susanna and Garcia–Jacas 2007), the *Arctium–Cousinia* complex is a monophyletic group composed of four genera. In the last work focused on this complex (López–Vinyallonga et al. 2009), all the analyses performed, based on DNA sequences (nrDNA ITS and cpDNA *rps4–trnT–trnL*), consistently divided the complex in two major lineages: the Arctioid and the Cousinioid clades. This division agrees with pollen morphology and chromosome number.

The Arctioid group comprises the genera *Arctium* L. with 11 species, *Hypacanthium* Juz. with two species, the monotypic *Schmalhausenia* C. Winkl. and 24 species of *Cousinia* belonging to subgenera *Cynaroides* and *Hypacanthodes*. As stated by Knapp (1987), most species of *Cousinia* subg. *Cynaroides* and *Hypacanthodes* grow only in the mountainous terrain of the Pamir–Alai range and in the western Tien Shan in Central Asia. The two species of *Hypacanthium* are endemic to the western Tien Shan, and the monotypic *Schmalhausenia* is endemic to the subalpine and alpine zone in the northern Tien Shan. *Arctium* s.str. is Eurosiberian in distribution (Duistermaat 1996). The pollen type of the Arctioid species, named *Arctiastrum*, is orbicular and spiny. According to Tscherneva (1985) and Susanna et al. (2003a), the Arctioid group, is karyologically uniform since all the studied species have n = 18, despite being formed by four different genera and despite some morphological incongruences. This high number, the highest in all the Cardueae, suggests that the Arctioid group constitute an old polyploid complex (Tscherneva 1985) or paleopolyploid following the nomenclature in Wagner (1980) and Ramsey and Schemske (2002).

The Cousinioid group comprises only *Cousinia* subgenus *Cousinia* with ca. 500 species (Mehregan and Kadereit 2008; López–Vinyallonga et al. 2009). According to Rechinger (1986) and Knapp (1987), this genus is distributed in the Turkestan mountain region (Tien Shan and Pamir–Alai) and in the Irano–Turanian region. Its pollen, named *Cousinia* pollen type, is oblong and smooth. This group shows a dysploid series of x =

9, 10, 11, 12 and 13. Like other groups of tribe Cardueae dysploidy in *Cousinia* is probably descending as is generally accepted in the tribe. Frankton and Moore (1961), Fernández Casas and Fernández Morales (1979), Siljak–Yakolev (1986) and Garcia Jacas and Susanna (1992) pointed out that high basic numbers should be regarded as more primitive than the reduced ones. This was considered a general trend by Stebbins (1950, 1971) and Grant (1981).

In the literature there are only 149 species for which chromosome numbers are reported among the ca. 500 species belonging to the *Arctium–Cousinia* complex. This number represents only 55% of the species of the subg. *Cynaroides*, 30% of the subg. *Hypacanthodes* and 22.5% of the subg. *Cousinia*. Besides, some of these counts are unconfirmed. These percentages are very low, which demonstrates that this huge complex is not enough karyologically studied.

As regards to the second group of our present study, the *Onopordum* complex (Cardueae–Carduinae) is formed by two well–defined groups. The first group is formed by a single large genus of widespread biennials, *Onopordum* L. (60 species), native to the Irano–Turanian and Mediterranean regions and introduced as noxious weeds in Australia, California and South America (Susanna and Garcia–Jacas 2007). The second one is constituted by seven small genera of perennial herbs with a narrow Central and East Asian distribution: *Alfredia* Cass. (four species), *Ancathia* DC. (one species), *Lamyropappus* Knorring & Tamamsch. (one species), *Olgaea* Iljin (16 species), *Syreitschikovia* Pavlov (two species), *Synurus* Iljin (four species) and *Xanthopappus* C. Winkl. (one species). Previous counts on this group have reported $2n = 34$ in *Onopordum* and $2n = 26$ in *Ancathia* and *Synurus*. In *Alfredia*, two different numbers have been reported, $2n = 24$ and $2n = 26$. There are no counts in the literature for the rest of the genera of this complex.

The scarce karyological data available for both the *Arctium–Cousinia* complex and the *Onopordum* group has led us to provide more data, which are essential to understand the karyological evolution of these taxa.

Material and methods

Chromosome counts were made on somatic metaphases using the squash technique. Root tip meristems from germinating achenes, either collected in the field or from wild plants cultivated in pots in the Botanical Institute of Barcelona, were used. Voucher specimens are deposited in the herbarium of the Botanical Institute of Barcelona (BC).

The root tips were pretreated with 0.002M 8–hydroxyquinoline solution at 4ºC for 8 h. After a distilled water wash, the material was fixed in fresh Carnoy I solution (3 : 1 v/v absolute ethanol : glacial acetic acid) overnight at – 20ºC, and stored in 70% ethanol at – 20ºC. This material was hydrolysed with 5N HCl for 50 min at room temperature, washed with distilled water and stained with 1% acetic orcein and squashed in 45% acetic acid. For all the counts, at least five plates from five to ten different individuals were examined. Preparations were made permanent by freezing with CO_2, ethanol–dehydrating and mounting in Canada balsam. Metaphase plates were photographed using an Olympus 3030 digital camera mounted on an Olympus microscope U–TV1 X. The chromosome preparations are preserved in the Botanical Institute of Barcelona.

In this work, we follow the sectional classification proposed by Tscherneva (1962; 1988) for the species distributed in Central Asia and the one from Rechinger (1972) for the species distributed in the Irano–Turanian region.

Results and discussion

The Arctium–Cousinia complex

Cousinia subgenus Cousinia

<u>Cousinia sect. Alpinae Bunge</u>

Cousinia serawschanica C. Winkl.
Tadjikistan: Kishlak Magian settlement, 39º13’17’’ N, 67º39’24’’ E, 2200 m, 18 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2526* (BC). 2*n* = 24 (Fig. 1a).

According to our data, this is the first chromosome count for this species which agrees with the chromosome number established for *C.* sect. *Alpinae* in previous reports from Central Asia by Tscherneva (1985) and Susanna et al. (2003b). Our count confirms x = 12 as a basic chromosome number for this section. There is a single previous count by Podlech and Bader (1974) of x = 13 in a species of *C.* sect. *Alpinae* from Afghanistan, so this section has two different chromosome numbers.

Cousinia sect. Carduncellus (Juz.) Rech. f.

Cousinia ferruginea Kult.
Tadjikistan: mountains above Kara–Chuira, 39º05'55'' N, 71º20'43'' E, 3700 m, 25 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2560* (BC); Gergatal mountains, Surjov, 39º13'14'' N, 71º10'11'' E, 25 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2563* (BC). 2n = 26 (Fig. 1b).
According to the available data, this is the first chromosome count for this species which is consistent with the number given for *C.* sect. *Carduncellus* x = 13.

Cousinia princeps Franch.
Tadjikistan: Zimargh, 39º08'04'' N, 68º41'36'' E, 3400 m, 14 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2493* (BC). 2n = 26 (Fig. 1c).
According to our data, this is the first chromosome count for this species which agrees with the number established for *C.* sect. *Carduncellus* x = 13.

Our two counts confirm x = 13 as a basic chromosome number for *C.* sect. *Carduncellus* in accordance with five previous reports by Tscherneva (1985) and Susanna et al. (2003b). There are some different and conflicting previous counts for this section. The report of 2n = 18 by Chuksanova in Fedorov (1969) for *C. tianschanica* was in conflict with 2n = 26 by Tscherneva (1985) and Susanna et al. (2003b). Podlech and Bader (1974) reported 2n = 24 for *C. buphthalmoides* but according to Tscherneva (1985) this species has 2n = 26. There is one more count for this section by Chuksanova in Fedorov (1969): 2n = 36 for *C. glaucifolia*, a species considered a synonym of *C. outichaschensis* which disagrees with 2n = 26 reported by Tscherneva (1985). With all these previous reports, we think that x = 13 is confirmed as the basic chromosome number for *C.* sect. *Carduncellus* and $x = 9$, $x = 12$ and $x = 18$ must be discarded. The most likely explanation for the wrong previous counts are putative mistakes in the determination of the species counted.

Cousinia sect. Coronophora (Juz.) Rech. f.

Cousinia radians Bunge
Tadjikistan: Kondara river canyon, Vorzovski Rayon Nature Reserve, 38º48'34'' N, 68º48'45'' E, 11 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2452* (BC). $2n = 26$ (Fig. 1d).
Our report agrees with one by Tscherneva (1985) although disagrees with the report of $2n = 18$ by Chuksanova in Fedorov (1969).

Our report, together with the previous counts of *C. coronata* (Aryavand 1976, Tscherneva 1985, Susanna et al. 2003b) and *C. mulgediifolia* (Tscherneva 1985), confirm $x = 13$ as the basic chromosome number for *C.* sect. *Coronophora.*

Cousinia sect. Cousinia

Cousinia aleppica Boiss.
Turkey: Gaziantep, 4 Aug 2002, *K. Ertuğrul, N. Garcia–Jacas, A. Susanna 2317 & T. Uysal* (BC). $2n = 26$ (Fig. 1e).
According to our data, this is the first chromosome count for this species which agrees with one of the numbers established for *C.* sect. *Cousinia*, $x = 13$.

Cousinia congesta Bunge
Uzbekistan: between Samarkand and Kitov, Takhta–Karachi pass, 1600 m, 7 Nov 1999, *L. Kapustina, F. Khassanov, A. Susanna 2059 & J. Vallès* (BC). $2n = 24$ (Fig. 1f).
Our count agrees with previous reports from Iran (Aryavand 1975; Ghaffari et al. 2006) but not with the $2n = 26$ reported by Chuksanova in Fedorov (1969) and Susanna et al. (2003b) based on seed material of the same population. After a careful revision of the preparations used for this doubtful count, preserved in the Botanical Institute of

Barcelona, we think that some chromosomes might get broken and therefore the number of chromosomes for this species was overestimated.

There are three reported basic chromosome numbers for *C.* sect. *Cousinia*: one $x = 9$ by Chuksanova in Fedorov (1969), eight $x = 12$ by Aryavand (1975), Tscherneva (1985), Susanna et al. (2003b) and Ghaffari et al. (2000, 2006) altogether with the provided in the present work and finally two $x = 13$ by Poddubnaja–Arnoldi (1931) plus the reported here. In agreement with all these data, $x = 12$ and $x = 13$ are confirmed as basic chromosome numbers for *C.* sect. *Cousinia* and $x = 9$ needs confirmation.

<u>Cousinia sect. Decumbentes Rech. f.</u>

Cousinia decumbens Rech. f.

Iran: Kuh–e–Shavar, 3400 m, 24 Aug 2005, *K. Romashchenko & A. Susanna 2622* (BC). $2n = 26$ (Fig. 1g).

According to our data, this is the first chromosome count for this species and for *C.* sect. *Decumbentes* which basic chrosomome number is $x = 13$.

<u>Cousinia sect. Eriocousinia Tscherneva</u>

Cousinia franchetii C. Winkl.

Tadjikistan: Zimargh, 39º08'29'' N, 68º42'09'' E, 3400 m, 13 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2498* (BC). $2n = 26$ (Fig. 1h).

Our count confirms that by Tscherneva (1985) from Tadjikistan and agrees with one of the given basic chromosome numbers of *C.* sect. *Eriocousinia*, $x = 13$.

Cousinia libanotica DC

Lebanon: Jabal el Mekmel, 19 Sept 2005, *M. Bou Dagher Kharrat, O. Hidalgo & K. Romashchenko 408* (BC). $2n = 24$ (Fig. 1i).

According to our data, this is the first chromosome count for this species, which agrees with one of the established chromosome numbers of *C.* sect. *Eriocousinia*, $x = 12$.

In accordance with previous authors, *C.* sect. *Eriocousinia* has three basic chromosome numbers, x = 11, 12 and 13 reported by Susanna et al. (2003b), Ghaffari et al. (2006) and Tscherneva (1985) respectively, and the two latest are confirmed in this work.

Cousinia sect. Homalochaete C. Winkl.

Cousinia coerulea Kult.

Tadjikistan: Vorzov canyon, 38º57'52'' N, 68º46'12'' E, 12 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2459* (BC). 2n = 24 (Fig. 1j).

According to our data, this is the first chromosome count for this species and for *C.* sect. *Homalochaete*, the basic chrosomome number of which is x = 12.

Cousinia sect. Jurineopsis (Juz.) Tschern.

Cousinia submutica Franch.

Tadjikistan: Voru, 39º13'39'' N, 67º59'07'' E, 2000–2300 m, 16 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2515* (BC). 2n = 26 (Fig. 1k).

According to the available data, this is the first report for this species and for *C.* sect. *Jurineopsis*, the basic chrosomome number of which is x = 13.

Cousinia sect. Microcarpae Bunge

Cousinia pulchella Bunge

Tadjikistan: Guissar–Darvaz Mt., Takob area, Rog, 38°51'11'' N, 68°59'50'' E, 2442 m, 26 Aug 2007, *I. Kudratov, K. Romashchenko 614 & A. Susanna* (BC). 2n = 22 (Fig. 1l).

According to our data, this is the first chromosome count for this species.

Cousinia sewerzowii Regel

Kazakhstan: Aksu Dzabagly Nature Reserve, 1800 m, 29 Aug 2000, *A. Ivashchenko, A. Susanna 2178 & J. Vallès* (BC); Aksu Dzabagly Nature Reserve, Chimkentskaya, Tiulkubas, Mashat canyon, 31 Aug 2000, *A. Ivashchenko, A. Susanna 2207 & J. Vallès* (BC). 2n = 22 (Fig. 2a).

Our count confirms the previous one from Kirgizstan by Tscherneva (1985).

There are three chromosome numbers for this section; x = 11 by Aryavand (1976), Tscherneva (1985) and Susanna et al. (2003b) and the present work. The number x = 12 was reported for two species, *C. centauroides* Fisch. & Mey. ex Bunge and *C. integrifolia* Franch., by Tscherneva (1985) and for one, *C. arachnoidea* Fisch. & C. A. Mey., by Susanna et al. (2003b). Finally, x = 13 was reported by Koul (1964), Podlech and Dieterle (1969), Ghaffari (1984), Tscherneva (1985), Susanna et al. (2003b) and Ghaffari et al. (2006). In agreement with all these data, three basic chromosome numbers are confirmed for *C.* sect. *Microcarpae; x* = 11, 12 and 13.

Cousinia subgenus Cynaroides Tscherneva

<u>Cousinia sect. Chrysis Juz.</u>

Cousinia aurea C. Winkl.
Tadjikistan: Schtut, Penjikent road, 39º24'42'' N, 68º02'34'' E, 16 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2514* (BC). 2n = 36 (Fig. 2b).
Our count confirms previous reports by Tscherneva (1985) and Chuksanova in Fedorov (1969) and agrees with the number given for C. subg. *Cynaroides* and all the Arctioid species of the *Arctium–Cousinia* complex.

Cousinia karatavica Regel and Schmalh.
Kazakhstan: Dzhambulskaya oblast, Karatau mountains, Kuyuk pass, 35 km from Dzhambul on the road to Tashkent, 1000 m, 28 Aug 2000, *A. Ivashchenko, A. Susanna 2162 & J. Vallès* (BC); Dzhambulsky reg., between Ajsha–Bibi and Shakbak–Ata, Kuik pass, 42°45'57'' N, 70°59'29'' E, 758 m, 22 Aug 2007, *K. Romashchenko 607* (BC). 2n = 36 (Fig. 2c).
Our counts agree with the unique number established for C. subg. *Cynaroides* and confirms previous reports by Tscherneva (1985) and Susanna et al. (2003b) while is in conflict with the previous 2n = 26 by Chuksanova in Fedorov (1969).

Cousinia refracta (Bornm.) Juz.

Tadjikistan: Dushanbe: Guissar–Darvaz region, Kondara river canyon, Vorzovski Rayon Nature Reserve, 38º48’43’’ N, 68º48’13’’ E, 11 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2456* (BC). 2*n* = 36 (Fig. 2d).

According to our data, this is the first chromosome count for this species which agrees with the number given for *C.* subg. *Cynaroides*.

Cousinia sect. Ctenarctium Juz.

Cousinia anomala Franch.

Tadjikistan: Khujand (Leninabad), Zeravshan reg., v. Rebat, Ispena, 39°22’18’’ N, 68°12’13’’ E, 1795 m, 31 Aug 2007, *I. Kudratov, K. Romashchenko 627 & A. Susanna* (BC). 2*n* = 36 (Fig. 2e).

According to the available data, this is the first chromosome count for this species which is consistent with the chromosome number established for C. subg. *Cynaroides*.

Cousinia sect. Pseudarctium Juz.

Cousinia tomentella C. Winkl.

Tadjikistan: Dushanbe: Guissar–Darvaz, Kondara river canyon, Vorzovski Rayon Nature Reserve, 38°48’35’’ N, 68°48’30’’ E, 1299 m, 28 Aug 2007, *I. Kudratov, K. Romashchenko 616 & A. Susanna* (BC). 2*n* = 36 (Fig. 2f)

This is the first report for this species, according to our data, which agrees with the number given for C. subg. *Cynaroides*.

Cousinia subgenus Hypacanthodes Tscherneva

Cousinia sect. Lacerae Tscherneva

Cousinia fedtschenkoana Bornm.

Tadjikistan: Guissar–Darvaz reg., Sioma river head, 38°56’18’’ N, 68°42’41’’ E, 2696 m, 02 Sept 2007, *I. Kudratov, K. Romashchenko 632 & A. Susanna* (BC). 2*n* = 36 (Fig. 2g)

According to the available data, this is the first chromosome count for this species which coincides with the number established for C. subg. *Hypacanthodes*.

Cousinia macilenta C. Winkl.
Tadjikistan: Kishlak Magian settlement,, 39º12'50'' N, 67º39'18'' E, 2200 m, 18 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2530* (BC). 2*n* = 36 (Fig. 2h)
According to our data, this is the first chromosome count for this species which agrees with the chromosome number stated for C. subg. *Hypacanthodes*.

These two counts are the first reports for *C.* sect. *Lacerae*, whose basic chrosomome number is *x* = 18 as expected, as it belongs to *C.* subg. *Hypacanthodes*.

Our results regarding the *Arctium–Cousinia* complex confirm the idea that, even though the Arctioid and Cousinioid clades form a monophyletic group, they have followed strongly different chromosomal and pollen type evolutionary paths (Susanna et al. 2003a). As expected, all the counts for the Arctioid species are 2*n* = 36, previously stated as the somatic chromosome number for *Arctium* by Moore and Frankton (1974).

The Cousinioid group is an acute contrast to the sole 2*n* = 36 of the Arctioid group. On the basis of our results and the above cited previous works, somatic chromosome numbers in the Cousinioid lineage are 2*n* = 26, 24, 22, 20 and 18 in a dysploid series ranging from *x* = 13 to 9. Our molecular results do not provide data on whether dysploidy is ascendant or descendent in the Cousinioid group (López–Vinyallonga et al. 2009), but descending dysploidy predominates in other groups of the Cardueae (reviewed in the introduction). In subtribe Centaureinae, basal groups have chromosome numbers ranging from *x* = 16 to 13 whereas the complex of genera with derived features have *x* = 12 to 7 (Garcia–Jacas et al. 2001).

Ecological considerations support the descending trend. Selvi and Bigazzi (2002) suggested that in *Nonea* Med. (Boraginaceae) descending dysploidy was correlated to short life cycle as an adaptation to arid habitats. Watanabe et al. (1999) also found a relationship between low chromosome numbers, annual habit and dry habitats in *Pogonolepis* Steetz, *Sondottia* P. S. Short and *Trichantodium* Sond. & F. Muell.

(Asteraceae–Gnaphalieae). It seems possible that descending dysploidy is related to the adaptation to more extreme habitats in the Cousinioid clade. The species of the Arctioid group, which do not exhibit dysploidy, are found in mesophylous mountain areas. Instead, the Cousinioid species, where a dysploid series is found, grow mainly in arid zones (López–Vinyallonga et al. 2009).

The *Arctium–Cousinia* complex has six basic chromosome numbers, but not all of them are represented equally. On the basis of all the counts published by now, together with the reports presented here, we have verified that x = 12, 13 and 18 are the most common ones (with the relative abundances of 38.9%, 40.9% and 16.1% respectively) while x = 9, 10 and 11 are hardly found in the complex (with the relative abundances of 4.7%, 1.3% and 4.7% respectively).

We have found little correspondence between phylogeny and karyology by mapping the chromosome counts for the 63 species present in the Bayesian phylogenetic tree by López–Vinyallonga et al. (2009) for which chromosome number have been reported (Fig. 3). Only the species with $2n$ = 36, all belonging to the Arctioid group, merge in the same clade. The species from *Cousinia* s. str. appear scattered through the tree, and do not group according either to sectional classification or to chromosome numbers. Moreover, even some sections of the Cousinioid clade have more than one basic chromosome number; e.g., *C.* sect. *Alpinae*, *Cousinia*, *Eriocousinia* and *Microcarpae*, showing dysploidy at the sectional level too. The karyological data have failed in providing any help to resolve the problem of the evolution in this complex, where molecular reconstruction has also failed.

Regarding polyploidy, we have not found any confirmed case in the entire *Arctium–Cousinia* complex. This is unfrequent in the Cardueae, a group with many pioneer polyploid colonizers (e.g., *Carthamus*, Vilatersana et al. 2000). Hybrid polyploids should be evident in crosses involving species with different basic chromosome numbers, but they have not been detected. Either hybrids are scarce or they must occur only between species with the same chromosome number and they should be homoploid, as recently pointed out in the related genus *Centaurea* (Garcia–Jacas et al. 2009).

The Onopordum group

Olgaea Iljin

Olgaea pectinata Iljin

Kazakhstan: Chimkentskaya oblast, Boranchi–Asu mountain pass, near Il Tal village, 30 Aug 2000, *A. Ivashchenko, A. Susanna 2187 & J. Vallès* (BC). 2n = 26 (Fig. 2i)

According to the available data, this is the first count for this species and for the genus *Olgaea*, the basic chrosomome number of which is x = 13.

Syreitschikovia Pavlov

Syreitschikovia spinulosa (Franch.) Pavlov

Kazakhstan: Chimkentskaya oblast, Lengerskii rayon, Aksu Dzabagly Nature Reserve, Darbassa canyon, 1840 m, 31 Aug 2000, *A. Ivashchenko, A. Susanna 2200 & J. Vallès* (BC). 2n = 24 (Fig. 2j)

According to our data this is the first report for this species and for the genus *Syreitschikovia*, the basic chrosomome number of which is x = 12.

In the *Onopordum* group, our results confirm the existence of two well–separated lines. Colonizing taxa of the *Onopordum* group have x = 17 (Watanabe, http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e). On the contrary, species from the perennial, Middle–Asian genera *Olgaea* and *Syreschikovia* have x = 13 or x = 12 (counted here for the first time). This is a fine parallelism with the *Arctium–Cousinia* complex: biennial, widespread *Onopordum* with x = 17 is comparable to *Arctium*, both in life cycle and chromosome number. The rest of the perennial genera of this group which have been counted (*Alfredia, Ancathia, Olgaea, Synurus* and *Syreitschikovia*) have x = 13 and x = 12, therefore is comparable with the *Cousinia* clade. Curiously, polyploidy is unknow in both cases.

Concluding remarks

It is possible that hybridization is much more frequent but undetected because of lack of sufficiently detailed taxonomic knowledge and the existence of several morphologically very similar species. However, the complete absence of polyploid chromosome numbers in *Arctium–Cousinia* complex among the 149 species analyzed (Watanabe, http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e) implies that polyploid hybrid speciation played no role in the evolution of this complex (López–Vinyallonga et al. 2009).

Acknowledgements

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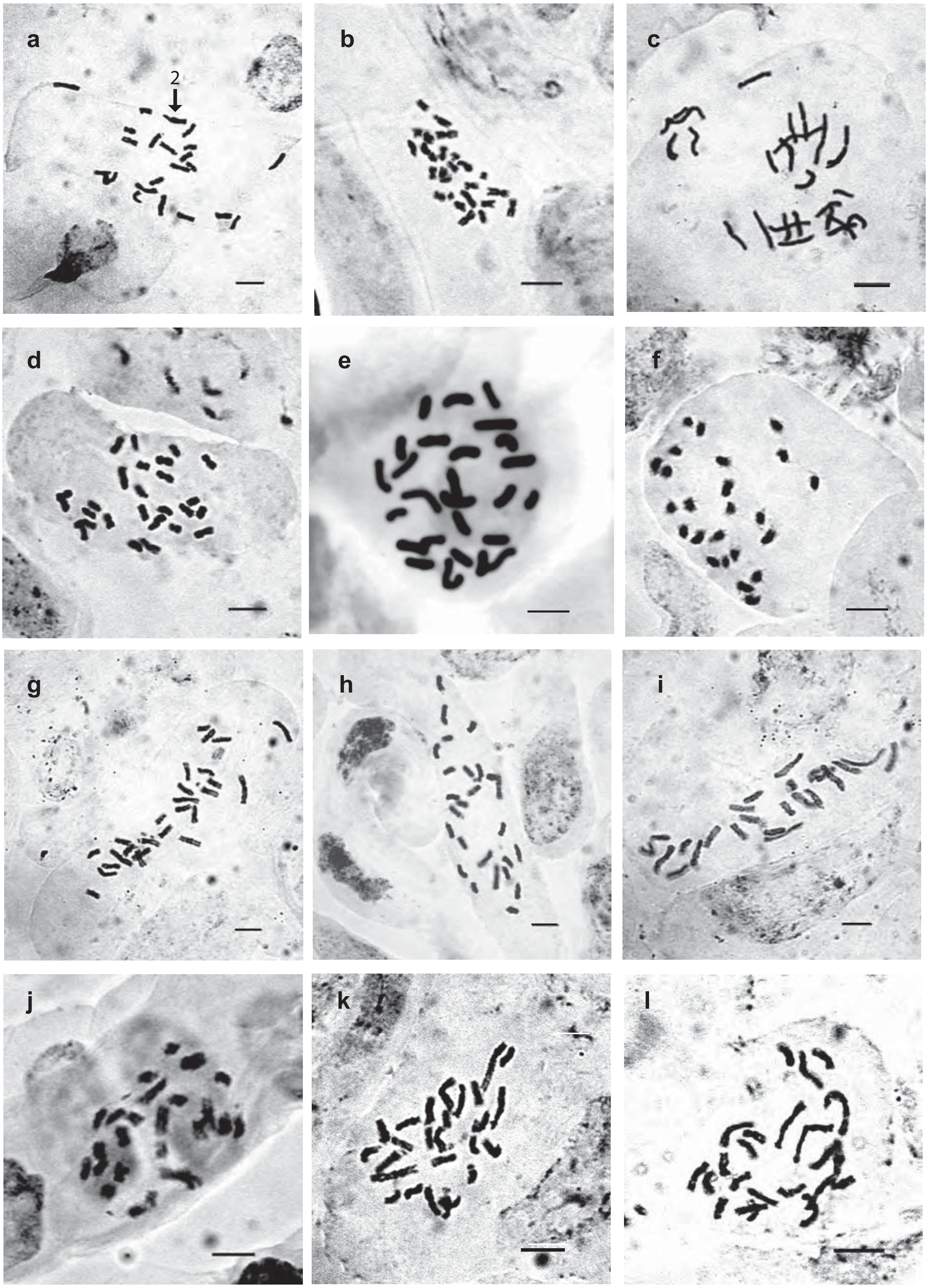
Figure captions

Fig. 1 Somatic metaphases of *Cousinia* spp. Scale bars = 10 μm. Fig. a *Cousinia serawschanica* (2*n* = 24). Fig. b *C. ferruginea* (2*n* = 26). Fig. c *C. princeps* (2*n* = 26). Fig. d *C. radians* (2*n* = 26). Fig. e *C. aleppica* (2*n* = 26). Fig. f *C. congesta* (2*n* = 24). Fig. g *C. decumbens* (2*n* = 26). Fig. h *C. franchetii* (2*n* = 26). Fig. i *C. libanotica* (2*n* = 24). Fig. j *C. coerulea* (2*n* = 24). Fig. k *C. submutica* (2*n* = 26). Fig. l *C. pulchella* (2*n* = 22). Numbers in arrows indicate overlapping chromosomes

Fig. 2 Somatic metaphases of *Cousinia*, *Olgaea* and *Syreitschikovia* spp. Scale bars = 10 μm. Fig. a *Cousinia sewerzowii* (2*n* = 22). Fig. b *C. aurea* (2*n* = 36). Fig. c *C. karatavica* (2*n* = 36). Fig. d *C. refracta* (2*n* = 36). Fig. e *C. anomala* (2*n* = 36). Fig. f *C. tomentella* (2*n* = 36). Fig. g *C. fedtschenkoana* (2*n* = 36). Fig. h *C. macilenta* (2*n* = 36). Fig. i *Olgaea pectinata* (2*n* = 26). Fig. j *Syreitschikovia spinulosa* (2*n* = 24). Numbers in arrows indicate overlapping chromosomes

Fig. 3 50% majority–rule consensus tree obtained from the Bayesian analysis of the regions ITS and *rps4–trnT–trnL* after López–Vinyallonga et al. (2009). Numbers above branches are posterior probabilities. The chromosome numbers are mapped in the branches of the tree with the following patterns: thin black line with grey squares, *x* = 18; thick solid black, *x*= 13; dashed black, *x* = 12; thick solid grey, *x* = 11; thin black line with thick dashed black, *x* = 10; thin dashed black, *x* = 9; thin solid black, no data available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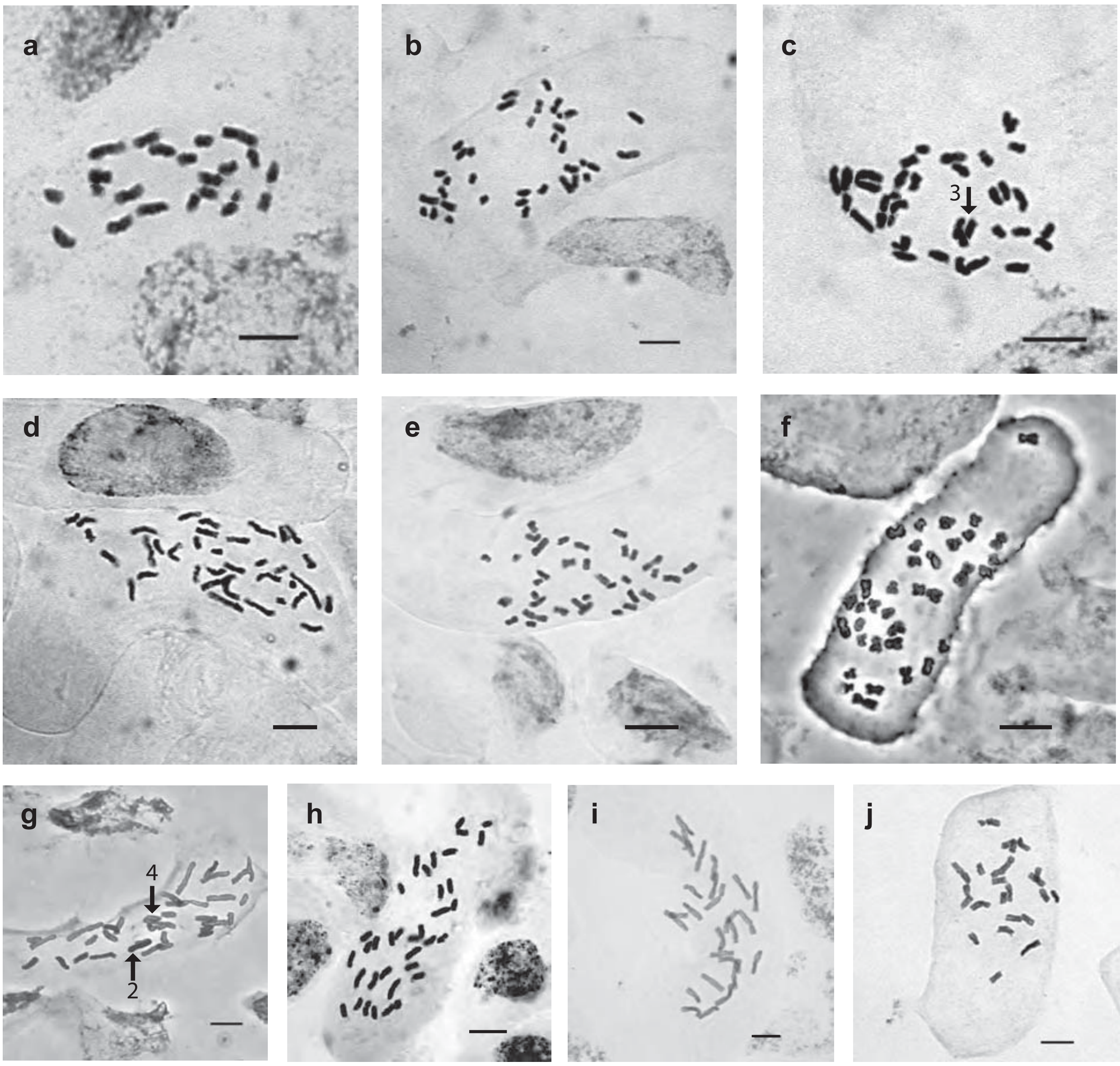
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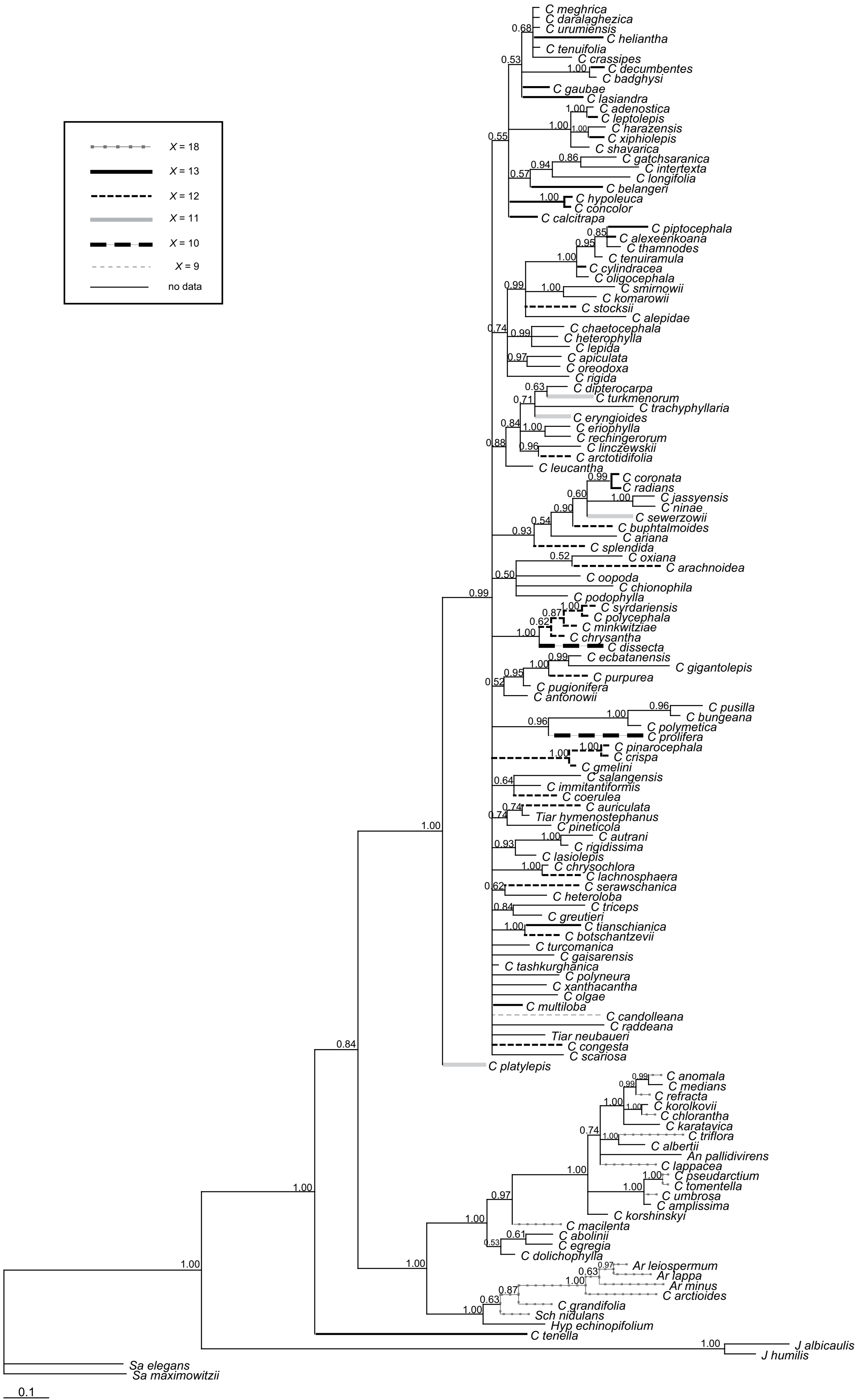
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X = 11
X = 10
X = 9
no data
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C daralaghezica
C urumiensis
C heliantha
C tenuifolia
C crassipes
C decumbentes
C badghysi
C gaubae
C lasiandra
C adenostica
C leptolepis
C harazensis
C xiphiolepis
C shavarica
C gatchsaranica
C intertexta
C longifolia
C belangeri
C hypoleuca
C concolor
C calcitrapa
C piptocephala
C alexeenkoana
C thamnodes
C tenuiramula
C cylindracea
C oligocephala
C smirnowii
C komarowii
C stocksii
C alepidae
C chaetocephala
C heterophylla
C lepida
C apiculata
C oreodoxa
C rigida
C dipterocarpa
C turkmenorum
C trachyphyllaria
C eryngioides
C eriophylla
C rechingerorum
C linczewskii
C arctotidifolia
C leucantha
C coronata
C radians
C jassyensis
C ninae
C sewerzowii
C buphtalmoides
C ariana
C splendida
C oxiana
C arachnoidea
C oopoda
C chionophila
C podophylla
C syrdariensis
C polycephala
C minkwitziae
C chrysantha
C dissecta
C ecbatanensis
C gigantolepis
C purpurea
C pugionifera
C antonowii
C pusilla
C bungeana
C polymetica
C prolifera
C pinarocephala
C crispa
C gmelini
C salangensis
C immitantiformis
C coerulea
C auriculata
Tiar hymenostephanus
C pineticola
C autrani
C rigidissima
C lasiolepis
C chrysochlora
C lachnosphaera
C serawschanica
C heteroloba
C triceps
C greutieri
C tianschianica
C botschantzevii
C turcomanica
C gaisarensis
C tashkurghanica
C polyneura
C xanthacantha
C olgae
C multiloba
C candolleana
C raddeana
Tiar neubaueri
C congesta
C scariosa
C platylepis
C anomala
C medians
C refracta
C korolkovii
C chlorantha
C karatavica
C triflora
C albertii
An pallidivirens
C lappacea
C pseudarctium
C tomentella
C umbrosa
C amplissima
C korshinskyi
C macilenta
C abolinii
C egregia
C dolichophylla
Ar leiospermum
Ar lappa
Ar minus
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C grandifolia
Sch nidulans
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C tenella
J albicaulis
J humilis
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Sa maximowitzii
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