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Chromosome Numbers 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. We report 20 chromosome number records for the genus *Cousinia* s.l. from the area of Near East and Central Asia. Thirteen of them are first chromosome counts for the studied species, and the remaining seven records provide confirmation of scarce or uncertain previous data. We also present the first chromosome counts for three of the 13 sections of this genus. Our records for Arctioid species show karyological uniformity with $2n=36$. In the Cousinioid group, 13 records agree with the three major numbers of its characteristic dysploidy series $2n=22$, 24 and 26. We report first chromosome counts for the genera *Olgaea* and *Syreitschikovia* from Kazakhstan, being $2n=26$ and 24, respectively. Our results confirm a hypothesis that the Arctioid and Cousinioid clades, although forming a monophyletic group, have followed different evolutionary paths. In the *Onopordum* group, our results confirm the existence of two lineages; the colonizing biennial taxa are characterized by $n=17$, while the perennial genera have $n=12$, 13. The evidence for recent polyploidization is absent in both the *Arctium–Cousinia* complex and the *Onopordum* group.

Keywords Arctioid clade · Central Asia · Cousinioid clade · Dysploidy · Karyology · Near East · Polyploidy

Introduction

Our contribution deals with two informal groups defined in the latest survey of the large tribe Cardueae Cass. (Compositae): the *Arctium–Cousinia* complex and the *Onopordum* group. According to the most recent circumscription (Susanna and

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Garcia-Jacas 2007), the *Arctium–Cousinia* complex is a monophyletic group composed of four genera. Based on recent DNA sequence data (nrDNA ITS and cpDNA *rps4–trnT–trnL*; López–Vinyallonga et al. 2009), this complex comprises two major lineages: the Arctioid and the Cousinioid clades. This division agrees with pollen morphology and chromosome numbers.

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* Cass. classified to subgenera *Cynaroides* and *Hypacanthodes*. As stated by Knapp (1987), most species of *Cousinia* subgen. *Cynaroides* and subgen. *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 because all the studied species have $n=18$, despite comprising four different genera and some morphological incongruence. This high number, the highest in all Cardueae, suggests that the Arctioid group constitutes an old polyploid complex (Tscherneva 1985) or palaeopolyploid following the nomenclature by Wagner (1980) and Ramsey and Schemske (2002).

The Cousinioid group comprises *Cousinia* subgen. *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. Several authors (Frankton and Moore 1961; Fernández Casas and Fernández Morales 1979; Siljak-Yakolev 1986; Garcia Jacas and Susanna 1992) pointed out that higher basic chromosome numbers should be regarded as more primitive than the lower ones. This was considered a general trend by Stebbins (1950, 1971) and Grant (1981).

Chromosome number records have been published only for 149 species of ca. 500 species belonging to the *Arctium–Cousinia* complex. This number represents only 55% of the species of the *Cousinia* subgen. *Cynaroides*, 30% of the subgen. *Hypacanthodes,* and 22.5% of the subgen. *Cousinia*. Some of these counts, however, have not been confirmed. These percentages are rather low, which demonstrates that this complex has been karyologically poorly studied.

The *Onopordum* complex (Cardueae–Carduinae) is formed by two well-defined groups. The first group is represented 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 comprises 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).

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Previous chromosome number records in 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 chromosome counts 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 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 0.002 M 8-hydroxyquinoline solution at 4°C 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 −20°C, and stored in 70% ethanol at −20°C. This material was hydrolyzed with 5 M 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 counts, at least five plates from 5–10 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 classification by Rechinger (1972) for the species distributed in the Irano-Turanian region.

Results and Discussion

The Arctium–Cousinia *Complex*

Cousinia subgenus *Cousinia*

Cousinia sect. *Alpinae* Bunge

Cousinia serawschanica C. Winkl.

Tadjikistan: Kishlak Magian settlement, 39°13′17″ N, 67°39′24″ E, 2,200 m, 18 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2526* (BC). $2n=24$ (Fig. 1a).

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

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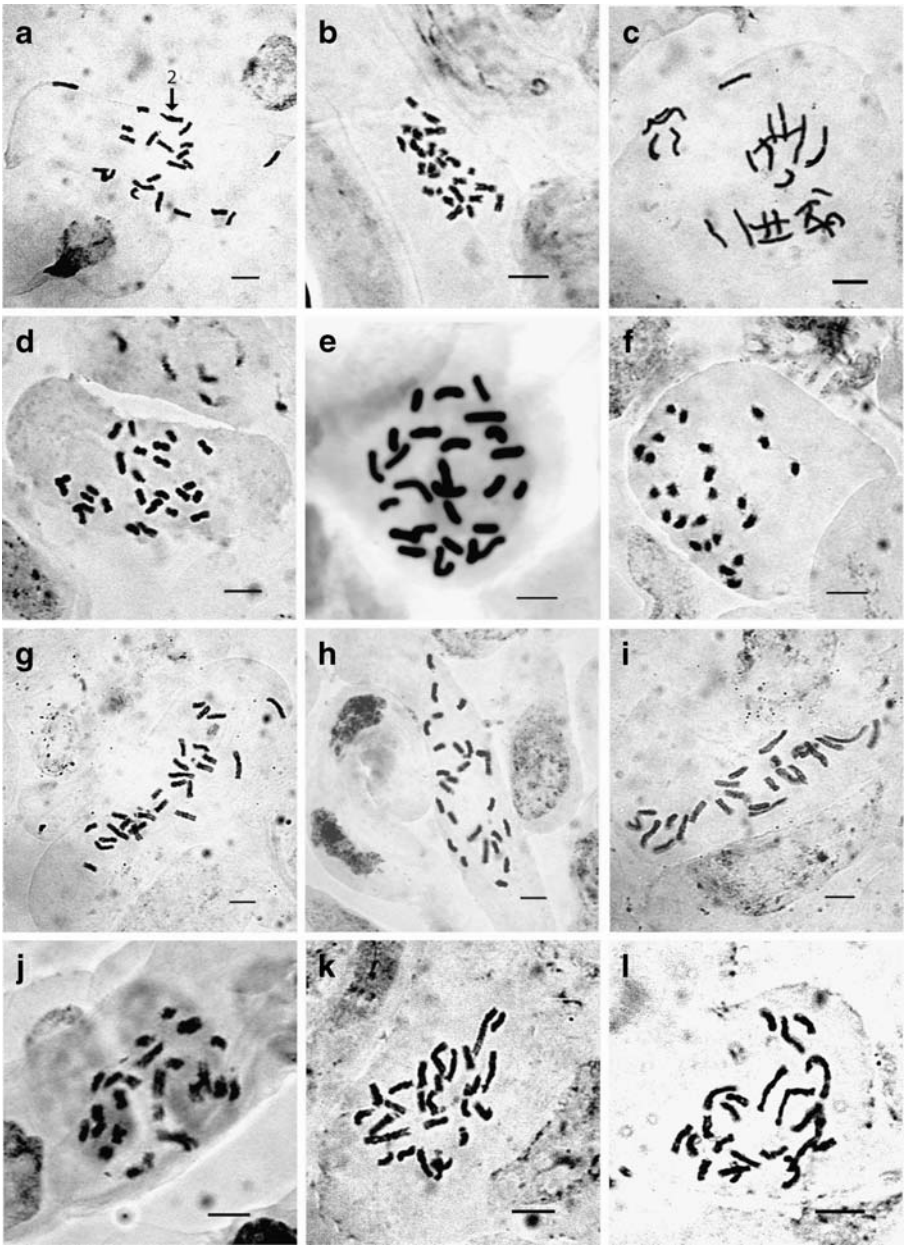

Fig. 1 Somatic metaphases of *Cousinia* species. Scale bars – 10 μm. **a** *Cousinia serawschanica* (2*n*=24); **b** *C. ferruginea* (2*n*=26); **c** *C. princeps* (2*n*=26); **d** *C. radians* (2*n*=26); **e** *C. aleppica* (2*n*=26); **f** *C. congesta* (2*n*=24); **g** *C. decumbens* (2*n*=26); **h**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

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

Cousinia ferruginea Kult.

Tadjikistan: mountains above Kara–Chuira, 39°05′55″ N, 71°20′43″ E, 3,700 m, 25 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2560* (BC); Gergatal mountains, Surjov, 39°13 14″ N, 71°10 11″ E, 25 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2563* (BC). $2n=26$ (Fig. 1b).

According to the available data, these are the first chromosome counts for this species, being consistent with the number $x=13$ given for *Cousinia* sect. *Carduncellus*.

Cousinia princeps Franch.

Tadjikistan: Zimargh, 39°08 04″ N, 68°41 36″ E, 3,400 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, consistent with the number $x=13$ reported for *Cousinia* sect. *Carduncellus*.

Our records confirm $x=13$ as a basic chromosome number for *Cousinia* sect. *Carduncellus,* in accordance with five reports by Tscherneva (1985) and Susanna et al. (2003b). There are, however, some conflicts in previous records 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 another chromosome number 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). Considering all these previous reports, we think that $x=13$ can be confirmed as the basic chromosome number for *C.* sect. *Carduncellus*, while $x=9$, $x=12$ and $x=18$ should be discarded. The most likely explanation for the wrong previous counts is misidentification of the analyzed species.

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

Cousinia radians Bunge

Tadjikistan: Kondara river canyon, Vorzovski Rayon Nature Reserve, 38°48 34″ N, 68°48 45″ E, 11 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2452* (BC). $2n=26$ (Fig. 1d).

Our report agrees with the one by Tscherneva (1985), although it disagrees with the report of $2n=18$ by Chuksanova in Fedorov (1969). Considering also previous counts for *C. coronata* (Aryavand 1976; Tscherneva 1985; Susanna et al. 2003b) and *C. mulgediifolia* (Tscherneva 1985), the number $x=13$ is confirmed here as the basic chromosome number for *Cousinia* 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).

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According to our data, this is the first chromosome count for this species. It agrees with one of the numbers reported for *Cousinia* sect. *Cousinia*, $x=13$.

Cousinia congesta Bunge

Uzbekistan: between Samarkand and Kitov, Takhta–Karachi pass, 1,600 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 number $2n=26$ 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 latter count, preserved in the Botanical Institute of Barcelona, we think that some chromosomes might have got broken, and therefore the number of chromosomes for this species was overestimated.

There are three basic chromosome numbers reported for *Cousinia* sect. *Cousinia*: one record indicated $x=9$ (Chuksanova in Fedorov 1969), eight records were of $x=12$ (Aryavand 1975; Tscherneva 1985; Susanna et al. 2003b; Ghaffari et al. 2000, 2006) together with our present data, and finally two records were of $x=13$ (Poddubnaja-Arnoldi 1931) plus the record given here. In agreement with all these data, $x=12$ and $x=13$ are confirmed as the basic chromosome numbers for *C.* sect. *Cousinia*, while the number $x=9$ needs confirmation.

Cousinia sect. *Decumbentes* Rech. f.

Cousinia decumbens Rech. f.

Iran: Kuh-e-Shavar, 3,400 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 *Cousinia* sect. *Decumbentes*, indicating the basic chrosomome number $x=13$.

Cousinia sect. *Eriocousinia* Tscherneva

Cousinia franchetii C. Winkl.

Tadjikistan: Zimargh, 39°08 29″ N, 68°42 09″ E, 3,400 m, 13 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2498* (BC). $2n=26$ (Fig. 1h).

This count confirms the record by Tscherneva (1985) from Tadjikistan, and agrees with one of the reported basic chromosome numbers of *Cousinia* 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 reported chromosome numbers of *Cousinia* sect. *Eriocousinia*, $x=12$.

In accordance with previous authors, *C.* sect. *Eriocousinia* has three basic chromosome numbers, $x=11$, 12 and 13, as reported by Susanna et al. (2003b),

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Ghaffari et al. (2006) and Tscherneva (1985), respectively. The two latest counts are confirmed here.

Cousinia sect. *Homalochaete* C. Winkl.

Cousinia coerulea Kult.

Tadjikistan: Vorzov canyon, 38°57 52″ N, 68°46 12″ E, 12 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2459* (BC). 2*n*=24 (Fig. 1j).

According to our data, this is the first chromosome count for this species as well as for *Cousinia* sect. *Homalochaete*, indicating the basic chrosomome number *x*=12.

Cousinia sect. *Jurineopsis* (Juz.) Tschern.

Cousinia submutica Franch.

Tadjikistan: Voru, 39°13′39″ N, 67°59′07″ E, 2,000–2,300 m, 16 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2515* (BC). 2*n*=26 (Fig. 1k).

According to the available data, this is the first report for this species and for *Cousinia* sect. *Jurineopsis*, indicating the basic chrosomome number *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). 2*n*=22 (Fig. 1l).

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

Cousinia sewerzowii Regel

Kazakhstan: Aksu Dzabagly Nature Reserve, 1,800 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). 2*n*=22 (Fig. 2a).

This count confirms the previous records from Kirgizstan by Tscherneva (1985).

There are three basic chromosome numbers given for this section. The number *x*=11 was reported by Aryavand (1976), Tscherneva (1985) and Susanna et al. (2003b), and is presented also here. The number *x*=12 was reported for two species, *C. centauroides* Fisch. & Mey. ex Bunge and *C. integrifolia* Franch., by Tscherneva (1985), and for *C. arachnoidea* Fisch. & C. A. Mey. by Susanna et al. (2003b). Finally, the number *x*=13 was reported by Koul (1964), Podlech and Dieterle (1969), Ghaffari (1984), Tscherneva (1985), Susanna et al. (2003b) and Ghaffari et al. (2006).

Cousinia subgenus *Cynaroides* Tscherneva

Cousinia sect. *Chrysis* Juz.

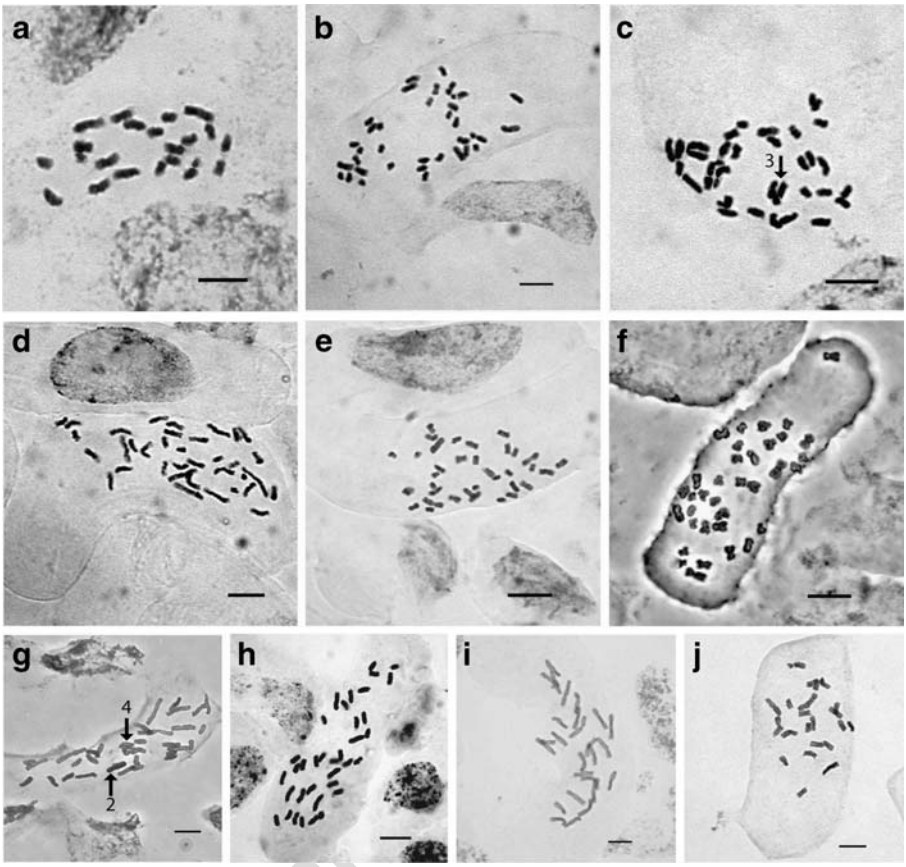

Fig. 2 Somatic metaphases of *Cousinia*, *Olgaea* and *Syreitschikovia* species. Scale bars – 10 μm. **a** *Cousinia sewerzowii* (2*n*=22); **b** *C. aurea* (2*n*=36); **c** *C. karatavica* (2*n*=36); **d** *C. refracta* (2*n*=36); **e** *C. anomala* (2*n*=36); **f** *C. tomentella* (2*n*=36); **g**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

Cousinia aurea C. Winkl.

Tadjikistan: Schtut, Penjikent road, 39°24′42″ N, 68°02′34″ E, 16 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2514* (BC). 2*n*=36 (Fig. 2b).

This count confirms previous reports by Tscherneva (1985) and Chuksanova in Fedorov (1969), and agrees with the number given for *Cousinia* subgen. *Cynaroides* and all Arctioid species from 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, 1,000 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). 2*n*=36 (Fig. 2c).

This count agrees with the unique number given for *Cousinia* subgen. *Cynaroides*, and confirms previous reports by Tscherneva (1985) and Susanna et

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al. (2003b). On the other hand, it is in conflict with the previous record of $2n=26$ by Chuksanova in Fedorov (1969).

Cousinia refracta (Bornm.) Juz.

Tadjikistan: Dushanbe: Guissar-Darvaz region, Kondara river canyon, Vorzovski Rayon Nature Reserve, 38°48 43″ N, 68°48 13″ E, 11 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2456* (BC). $2n=36$ (Fig. 2d).

According to our data, this is the first chromosome count for this species. It agrees with the number given for *Cousinia* subgen. *Cynaroides*.

Cousinia sect. *Ctenarctium* Juz.

Cousinia anomala Franch.

Tadjikistan: Khujand (Leninabad), Zeravshan reg., v. Rebat, Ispena, 39°22 18″ N, 68°12 13″ E, 1,795 m, 31 Aug 2007, *I. Kudratov, K. Romashchenko 627 & A. Susanna* (BC). $2n=36$ (Fig. 2e).

According to the available data, this is the first chromosome count for this species. It is consistent with the chromosome number given for *Cousinia* subgen. *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, 1,299 m, 28 Aug 2007, *I. Kudratov, K. Romashchenko 616 & A. Susanna* (BC). $2n=36$ (Fig. 2f)

This is the first report for this species, according to our data, and it agrees with the number given for *Cousinia* subgen. *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, 2,696 m, 2 Sept 2007, *I. Kudratov, K. Romashchenko 632 & A. Susanna* (BC). $2n=36$ (Fig. 2g)

According to the available data, this is the first chromosome count for this species. It coincides with the number given for *Cousinia* subgen. *Hypacanthodes*.

Cousinia macilenta C. Winkl.

Tadjikistan: Kishlak Magian settlement, 39°12 50″ N, 67°39 18″ E, 2,200 m, 18 Aug 2004, *I. Kudratov, K. Romashchenko & A. Susanna 2530* (BC). $2n=36$ (Fig. 2h)

According to our data, this is the first chromosome count for this species. It agrees with the chromosome number stated for *Cousinia* subgen. *Hypacanthodes*.

These two counts are the first reports for *Cousinia* sect. *Lacerae*. They indicate the basic chromosome number of $x=18$, which is expected in *C.* subgen. *Hypacanthodes*.

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Our results regarding the *Arctium–Cousinia* complex confirm a hypothesis that the Arctioid and Cousinioid clades, even though forming a monophyletic group, have strongly followed different chromosomal and pollen type evolutionary paths (Susanna et al. 2003a). As expected, all counts for the Arctioid species are $2n=36$, previously stated as the somatic chromosome number for *Arctium* by Moore and Frankton (1974). The Cousinioid group is an acute contrast to the sole $2n=36$ of the Arctioid group. On the basis of our results and the above cited previous works, somatic chromosome numbers in the Cousinioid lineage are $2n=26$, 24, 22, 20 and 18 in a dysploid series ranging from $x=13$ to 9. Previous molecular studies have not clarified whether dysploidy is ascending or descending in the Cousinioid group (López–Vinyallonga et al. 2009), but descending dysploidy predominates in other groups of the Cardueae (see Introduction). In the 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 they are not represented equally. On the basis of published counts, together with the reports presented here, we have verified that $x=12$, 13 and 18 are the most common numbers (with the relative abundances of 38.9%, 40.9% and 16.1%, respectively), while the numbers $x=9$, 10 and 11 are rare 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 number data for the 63 species present in the Bayesian phylogenetic tree by López-Vinyallonga et al. (2009) for which chromosome numbers have been reported (Fig. 3). Only the species with $2n=36$, all belonging to the Arctioid group, were found in the same clade. The species from *Cousinia* s. str. appeared scattered on the tree, and do not group according to either the sectional classification or chromosome numbers. Moreover, some sections of the Cousinioid clade have even more than one basic chromosome number; e.g., *C Cousinia* sect. *Alpinae*, *Cousinia*, *Eriocousinia* and *Microcarpae*, showing dysploidy at the sectional level, too. The karyological data have failed in providing more insights into the evolution of this complex, in which molecular reconstructions have neither been conclusive.

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 on the tree using different line patterns for the given branches ▶

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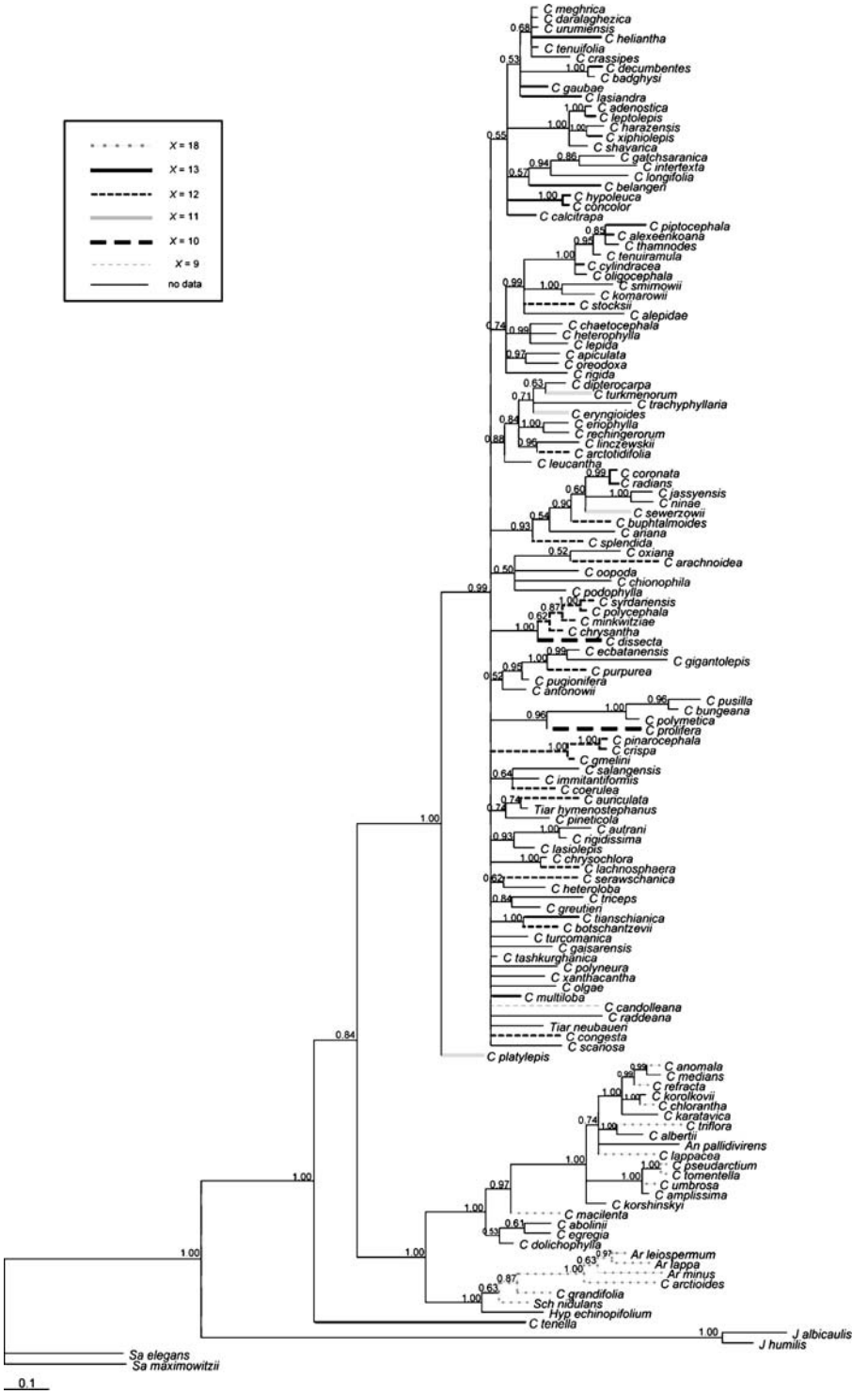

There are no confirmed reports of polyploidy within the entire *Arctium–Cousinia* complex. This is rather infrequent in the Cardueae, a group with many pioneer polyploid colonizers (e.g., *Carthamus*; Vilatersana et al. 2000). Allopolyploids or hybrids should be evident in crosses involving species with different basic chromosome numbers, but they have not been detected. Either they are scarce or hybridization is homoploid, occurring only between species with the same chromosome number, 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 as well as for the genus *Olgaea*, indicating the basic chromosome number $x=13$.

Syreitschikovia Pavlov

Syreitschikovia spinulosa (Franch.) Pavlov

Kazakhstan: Chimkentskaya oblast, Lengerskii rayon, Aksu Dzabagly Nature Reserve, Darbassa canyon, 1,840 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*, indicating the basic chromosome number $x=12$.

In the *Onopordum* group, our results confirm the existence of two well-separated lineages. The colonizing biennial taxa of the *Onopordum* group have $x=17$ (Watanabe 2002), while the species from the perennial, Middle-Asian genera *Olgaea* and *Syreschikovia* have $x=13$ or $x=12$ (counted here for the first time). There is a parallelism with the *Arctium–Cousinia* complex: biennial, widespread *Onopordum* with $x=17$ is comparable to *Arctium*, both in life cycle and chromosome number. The perennial genera of this group, which have been studied (*Alfredia, Ancathia, Olgaea, Synurus* and *Syreitschikovia*), have $x=13$ and $x=12$, and are comparable with the *Cousinia* clade. Curiously, polyploidy is unknown in both cases.

Concluding Remarks

It is possible that hybridization is much more frequent but remains undetected in the studied taxa, due to insufficient taxonomic knowledge and the existence of several morphologically very similar species. However, the complete absence of polyploid chromosome numbers among the 149 species analyzed of the *Arctium–Cousinia* complex (Watanabe 2002) implies that (allo-)polyploid speciation did not play a role in the evolution of this complex (López-Vinyallonga et al. 2009).

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