Running Title: Karyology in *Cousinia*

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New chromosome counts in the genus *Cousinia* and the related genus *Schmalhausenia* (Asteraceae, Cardueae).

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Twenty chromosome counts of genus *Cousinia* and the monotypic genus *Schmalhausenia* from Armenia, Iran, Kazakhstan and Uzbekistan are reported; 12 of them are new, and eight are confirmation of scarce or disputable previous data. Correlation between karyological data, pollen type and molecular phylogeny is very good, and on this basis two main groups can be defined. The first group is the Arctioid group, which comprises the genera *Arctium* and *Schmalhausenia* together with a small part of the genus *Cousinia*, with x = 18. The second group is the genus *Cousinia sensu stricto*, with a dysploid series ranging from x = 13 to x = 11. Some considerations on the chromosomal evolution in the group are also made.

ADDITIONAL KEY WORDS: Carduinae, Compositae, dysploidy, karyology.

INTRODUCTION

The genera *Arctium* L., *Cousinia* Cass., *Hypacanthium* Juz. and *Schmalhausenia* C. Winkl. form the *Arctium* group of the tribe Cardueae-Carduinae (Häffner, 2000; Susanna & Garcia-Jacas, in press). Häffner (2000) adds the genera *Lipskyella* Juz. and *Tiarocarpus* Rech. f., merged into *Cousinia* by Susanna & Garcia-Jacas (in press). This complex is a very well defined group on the basis of some important morphologic characters never found in combination elsewhere in the tribe: the receptacle has strongly twisted scales; the achenes are always tigrine (with a pattern of wavy fringes), very often winged, and without nectary; and the pappus is formed by free deciduous bristles (Susanna & Garcia-Jacas, in press).

The largest of the genera of the group and indeed one of the largest of the family Asteraceae is *Cousinia*, formed of ca. 600 species (Mabberley, 1990). *Cousinia* is centred in the Iran and Turkestan mountain regions, with an astonishing number of endemics. The latest revision classified *Cousinia* in three subgenera (*Cynaroides*, *Hypacanthodes* and *Cousinia*) and 50 sections (Tscherneva, 1988a,b). The next genus, *Arctium*, comprises only 11 species, most of them with a sub cosmopolitan distribution. According to the latest revision of the genus by Duistermaat (1996), the genus has four sections: *Arctium* sect. *Arctium* (with all the species classically included in *Arctium*), and sections *Lappaceum*, *Nanarctium* and *Pseudarctium*, first described as sections of the genus *Cousinia* by Tscherneva (1988a,b). The rest of the genera of the *Arctium* group recognized by Susanna & Garcia-Jacas (in press) are

very small: *Schmalhausenia* is monotypic, and *Hypacanthium* has only three species. Both genera are narrow endemics of the mountains of Central Asia (Tien-Shan and Pamir) and were first described as species of *Cousinia*.

Many of the problems in the *Arctium* group arise from the conflicting boundaries between the genera *Arctium* and *Cousinia*, which have been signalled from old (Boissier, 1875; Kuntze, 1891; revised in Duistermaat, 1996). All recent studies have confirmed this connection: morphological surveys by Dittrich, 1977; Duistermaat, 1996, 1997; Petit, Mathez & Qaid, 1996; Petit, 1997; Häffner, 2000; or molecular analyses by Häffner & Hellwig, 1999; Garcia-Jacas *et al.*, 2002, are all coincident. However, despite the difficulties in establishing a natural classification of the genera of the *Arctium* group, there are some useful characters that can be used for establishing a natural generic delineation: morphology, pollen types and karyology.

Morphology: Morphological characters that are useful for the genus delineation in the *Arctium* group are revised in Duistermaat (1996, 1997) and Susanna *et al.* (in press): they are mainly leaves, bracts and floral morphology.

Pollen: Schtepa (1966, 1976) and Kuprianova & Tscherneva (1982) noted that *Cousinia* subgenera *Cynaroides* and *Hypacanthodes* have pollen similar to *Arctium*, which they named *Arctiastrum* pollen type, while *Cousinia* subg. *Cousinia* has a different pollen type, named *Cousinia* pollen type. *Schmalhausenia* has *Arctiastrum* pollen type (Susanna, unpublished data). The pollen type of *Hypacanthium* is unknown.

Chromosome numbers: according to literature (cf. Duistermaat, 1996), *Arctium* has always x = 18 and 2*n* = 36. The same number is shared by all the studied species of *Cousinia* from the subgenera *Cynaroides* and *Hypacanthodes*

(Tscherneva, 1985). The genus *Schmalhausenia* also has 2*n* = 36 (published in this paper). Note that the distribution of this number within the group coincides exactly with that of the *Arctiastrum* pollen type. To the contrary, *Cousinia* subgenus *Cousinia*, with *Cousinia* pollen type, has 2*n* = 22, 24 and 26 (Moore, 1973, 1977; Goldblatt, 1981, 1988; Goldblatt & Johnson, 1990, 1991; Ghaffari & Djavadi, 1998; Ghaffari, Attar & Ghahreman, 2000); other old counts of 2*n* = 18 and 20 have never been confirmed and seem very doubtful.

Once outlined the potential of karyology in the classification of the *Arctium* group, our goals in this paper is contributing to the general knowledge of chromosome numbers in the *Arctium* group especially in *Cousinia*, with very scarce counts for such a large genus, and confirming the relationships between systematics, pollen and karyology.

MATERIAL AND METHODS

Chromosome counts were made on somatic metaphases using the squash technique. Root meristems from germinating seeds collected in the wild or from wild plants cultivated in pots were used.

Samples were pretreated with 8-hydroxyquinoline at 4°C for 8 hours. The material was fixed with Carnoy for 24h at low temperatures. Before staining, the material was hydrolyzed with 5N HCl for 1 h at room temperature. It was stained with 1% acetic orcein and mounted in 45% acetic acid. For all the counts, a minimum of five plates from different individuals was examined. Preparations were made permanent by freezing with CO_2, dehydrating in ethanol and mounting in Canada

balsam. Digital photographs were taken using an Olympus 3030 camera mounted on an Olympus microscope U-TV1 X. The preparations and the herbarium vouchers are preserved in the Botanical Institute of Barcelona (BC).

The existence of previous chromosome counts for the studied species was checked in the indexes of plant chromosome numbers by Fedorov (1969), Moore (1971, 1972, 1973, 1974, 1977), Goldblatt (1981, 1984, 1985, 1988), and Goldblatt & Johnson (1990, 1991, 1994, 1996, 1999).

RESULTS

Cousinia Cass.

Cousinia subgenus *Cousinia*

Cousinia sect. *Alpinae* Bunge

Cousinia chrysantha Kult.

Kazakhstan, Shimkiendsky ob.: Aksu Dzabagly reservation, Darbassa canyon, *Ivaschenko, Susanna 2198 & Vallès*, 31.viii.2000 (BC). 2*n* = 24 (Fig. 1).

According to our data, this is the first chromosome count for this species. Our result coincides with that of Tscherneva (1985) in two species from the same section, also

from Central Asia (Uzbekistan and Tajikistan). Instead, Podlech & Bader (1974) found *x* = 13 in a species of this section from Afghanistan. The presence of two different chromosome numbers in sect. *Alpinae* needs confirmation.

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

Cousinia tianshanica Kult.

Kazakhstan, Shimkiendsky ob.: Aksu Dzabagly reservation, Aksu canyon, *Ivaschenko, Susanna 2191 & Vallès*, 30-VIII-2000 (BC). 2*n* = 26 (Fig. 2).

Our count agrees with Tscherneva (1985), but disagrees with Chuskanova (see Fedorov, 1969), who reported 2*n* = 18;. The basic chromosome number x = 13 for sect. *Carduncellus* is confirmed by five more counts by Tscherneva (1985). However, there is a count by Podlech & Bader (1974) of *Cousinia buphtalmoides* Regel with the disaccording result of 2*n* = 24. Nevertheless, Tscherneva (1985) counted 2*n* = 26 for this species, and it seems obvious that sect. *Carduncellus* has x = 13.

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

Cousinia coronata Franch.

Uzbekistan: road Tashkent-Samarkanda, 10 km from the cross to Jizzak, *Kapustina, Khassanov, Susanna 2039 & Vallès*, 2.xi.1999 (BC). 2*n* = 26 (Fig. 3).

There is only a previous count for this species by Aryavand (1976), on material from the Moscow Botanical Garden, with the same result. The other two studied species of this section have *x* = 13 too (Tscherneva, 1985), even though Chuskanova counted 2*n* = 18 in *Cousinia radians* Bunge (see Fedorov, 1969).

Cousinia sect. *Cousinia*

Cousinia congesta Bunge

Uzbekistan: between Samarkanda and Kitov, Takhta-Karachi pass, *Kapustina, Khassanov, Susanna 2059 & Vallès*, 7.xi.1999 (BC). 2*n* = 26 (Fig. 4).

Our count agrees with a previous one by Chuskanova (cf. Fedorov, 1969). Instead, Aryavand (1975) found x = 12 on Iranian material, while Chuskanova's was from Turkmenistan.

Cousinia minkwitziae Bornm.

Kazakhstan, Dzambulsky ob.: Talaski Alatau, 6 km W from Il Tai, *Ivaschenko, Susanna 2183 & Vallès*, 29.viii.2000 (BC). 2*n* = 24 (Fig. 5).

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

Cousinia polycephala Rupr.

Kazakhstan, Dzambulsky ob.: 30 km from the Kurdai pass, *Ivaschenko, Susanna 2161 & Vallès*, 27.viii.2000 (BC). 2*n* = 24 (Fig. 6).

The only previous count for this species by Tscherneva (1985) coincides with ours.

Cousinia syrdariensis Kult.

Kazakhstan, Dzambulsky ob.: 30 km from the Kurdai pass, *Ivaschenko, Susanna 2159 & Vallès*, 27.viii.2000 (BC). 2*n* = 24 (Fig. 7).

As far as we know, this is the first report for this species.

There are very few counts for sect. *Cousinia* in the literature. However, most of them are coincident with ours in pointing out *x* = 12 as the base chromosome number of this section. However, there is the exception of our count for *Cousinia congesta* with *x* = 13. Maybe the placement of this species in a different section looks more adequate: in the revision by Tscherneva (1988a,b), this species was placed in sect. *Cousinia* series *Congestae* (Bunge) Tscherneva. Instead, Rechinger (1972) keeps an independent sect. *Congestae* Bunge. The chromosome number strongly favours a different section.

Cousinia sect. *Cynaroideae* Bunge

Cousinia canescens DC.

Iran, Azarbaijan-e-Sharghi: 25 Km from Ahad on the road to Kaleibar, *Garcia-Jacas, Mozaffarian, Susanna 1668 & Vallès*, 6.viii.1996 (BC). 2*n* = 24 (Fig. 8).

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

Cousinia purpurea C. A. Mey.

Armenia, Ararat: near village Shahap, near the road, *K. Tamanian*, 13.vii.1995 (ERE). 2*n* = 24 (Fig. 9).

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

All previous counts in species from sect. *Cynaroideae* resulted in the same chromosome number *x* = 12 (Ghaffari, 1984, 1986; Ghaffari *et al*., 2000; Alfzal Rafi, 1980; Tscherneva, 1985).

Cousinia sect. *Eriocousinia* Tscherneva

Cousinia caespitosa C. Winkl.

Kazakhstan, Dzambulsky ob.: Aksu Dzabagly reservation, 2000-2500 m, *Ivaschenko, Susanna 2170 & Vallès*, 29.viii.2000 (BC). 2*n* = 22 (Fig. 10).

As far as we know, this is the first report for this species. To the contrary, *Cousinia franchetii* C. Winkl. from the same section has 2*n* = 26 (Tscherneva, 1985). To date, sect. *Eriocousina* has two base chromosome numbers: *x* = 11 and 13.

Cousinia sect. *Leiocaules* Bunge

Cousinia astracanica (Spreng.) Tamamsch.

Kazakhstan, Almatinsky ob.: Sogeti mt., *Ivaschenko, Susanna 2104 & Vallès*, 22.viii.2000 (BC). 2*n* = 24 (Fig. 11).

According to our data, this is the first chromosome count for this species. All the other counts within sect. *Leiocaules* coincide in *x* = 12 (Tscherneva, 1985) with the only exception of *Cousinia glandulosa* Kult., which would had 2*n* = 26 according to Chuskanova (see Fedorov, 1969).

Cousinia sect. *Microcarpae* Bunge

Cousinia arachnoidea Fisch. & C. A. Mey.

Kazakhstan, Almatinsky ob.: Malai Sary pass, *Ivaschenko, Susanna 2140 & Vallès*, 25.viii.2000 (BC). 2*n* = 24 (Fig. 12).

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

Cousinia microcarpa Boiss.

Kazakhstan, Dzambulsky ob.: 30 km from the Kurdai pass, *Ivaschenko, Susanna 2160 & Vallès*, 27.viii.2000 (BC). 2*n* = 26 (Fig. 13).

Our count agrees with previous ones by Ghaffari (1984) on Iranian material, Tscherneva (1985) on material from Tajikistan and Koul (Fedorov, 1969) and

Podlech & Dieterle (1969) on material from Afghanistan. There is a disaccording count on material from India by Mehra & Remanandan (1969) with *n* = 10.

Cousinia platylepis Schrenk

Kazakhstan, Dzambulsky ob.: 30 km from the Kurdai pass, *Ivaschenko, Susanna 2158 & Vallès*, 27.viii.2000 (BC). 2*n* = 22 (Fig. 14).

As far as we know, this is the first report for this species.

Our results indicate three different counts for sect. Microcarpae: *x* = 11, 12, 13. Previous chromosome counts for this sections resulted in *x* = 13 in *Cousinia microcarpa* and *x* = 11 in *C. sewerzowii* Regel (Tscherneva, 1985) and *C. leiocephala* (Regel) Juz. (Aryavand, 1976)

Cousinia sect. *Tenellae* Bunge

Cousinia tenella Fisch. & C. A. Mey.

Iran, Azarbaijan-e-Gharbi: 30 km from Tabriz to Ahar, 1600 m, *Garcia-Jacas, Mozaffarian, Susanna 1657 & Vallès* (BC). 2*n* = 26 (Fig. 15).

This is one of the rare annual species of the genus. Our count agrees with a previous one by Aryavand (1975) on materials from the Botanical Garden of Tashkent (probably from Turkmenistan).

Subgenus *Cynaroides* Tscherneva

Cousinia sect. *Chrysis* Juz.

Cousinia karatavica Regel & Schmalh.

Kazakhstan, Dzambulsky ob.: Karatau mt., Kuyuk pass, *Ivaschenko, Susanna 2162 & Vallès*, 28.viii.2000 (BC). 2*n* = 36 (Fig. 16).

Our result coincides with that of Tscherneva (1985), but Chuskanova found 2*n* = 26 (Fedorov, 1969). *Cousinia aurea* C. Winkl., also from sect. *Chrysis*, has 2*n* = 36 (Tscherneva, 1985).

Cousinia sect. *Lappaceae* Bunge

Cousinia lappacea Bunge

Kazakhstan, Dzambulsky ob.: Kurdai pass, *Ivaschenko, Susanna 2150 & Vallès*, 27.viii.2000 (BC). 2*n* = 36 (Fig. 17).

According to our data, this is the first chromosome count for this species. This is a monotypic section, but all the species counted in subg. *Cynaroides* have 2*n* = 36.

Cousinia sect. *Oligantha* Juz.

Cousinia triflora Schrenk

Kazakhstan, Jambulska ob.: 20 km from the Kurdai pass, *Ivaschenko, Susanna 2157 & Vallès*, 27.viii.2000 (BC). 2*n* = 36 (Fig. 18).

According to our data, this is the first chromosome count for this species. This is the same case as the previous section: a monotypic section and a count that coincides with all the counts in subg. *Cynaroides*.

Cousinia sect. *Pseudarctium* Juz.

Cousinia umbrosa Bunge

Kazakhstan, Almatinsky ob.: Alatau mt. above Almaty, *Ivaschenko, Susanna 2100 & Vallès*, 21.viii.2000 (BC). 2*n* = 36 (Fig. 19).

A previous count by Tscherneva (1985) agrees with ours. The only other count of a species of this section, *Cousinia pseudarctium* Bornm., was also 2*n* = 36 (Tscherneva, 1985).

Schmalhausenia

Schmalhausenia nidulans (Regel) Petr.

Kazakhstan, Almatinsky ob.: Alatau mt. above Almaty, *Ivaschenko, Susanna 2088 & Vallès*, 21.viii.2000 (BC). 2*n* = 36 (Fig. 20).

According to our data, this is the first chromosome count for this species. This count coincides with all the previous ones from subgenera *Cynaroides* and *Hypacanthodes* of the genus *Cousinia*, and with all the counts in the genus *Arctium*.

DISCUSSION AND CONCLUSIONS

Our results confirm the presence of four different chromosome numbers in the *Arctium* group. The chromosome number 2*n* = 36, which we have found in the genus *Cousinia* subgenera *Cynaroides* and *Hypacanthoides* and in the genus *Schmalhausenia*, is also characteristic of the genus *Arctium*. In *Cousinia* subg. *Cousinia*, we have three different chromosome numbers: 2*n* = 22, 24 and 26. In our opinion, the base numbers 2*n* = 18 and 20 reported in very old studies should be disregarded.

Regarding the attempts of a natural delineation of the genera of the complex, karyology agrees very closely with the division suggested by pollen types and DNA sequence analyses, both nuclear (ITS) and chloroplastic (*mat*K). The species with 2*n* = 36 share the same pollen type, the *Arctiastrum* type, orbicular and spiny, that we find in the genus *Arctium* too. The species with 2*n* = 22, 24 and 26 have the smooth, oblong *Cousinia* pollen type of the genus *Cousinia sensu stricto*. The analyses based

on DNA sequences consistently grouped together the species with 2*n* = 36 in a monophyletic clade, the Arctioid clade. On the other hand, the species with 2*n* = 22, 24 and 26, were placed in a different monophyletic group, the *Cousinia* clade (Susanna *et al.*, in press). These results confirm the relevance of karyologic data within the *Arctium* group and especially in the genus *Cousinia*.

Notwithstanding the good expectatives, we have still the inconvenient of a limited sampling of the genus *Cousinia*. Subgenus *Cynaroides* is well represented in our study, with four out of seven sections included in this paper; two more sections are known from literature, and with six out of seven karyologically studied sections we are sure that the chromosome number of subg. *Cynaroides* is constant: *x* = 18. Instead, our sampling of *Cousinia* subg. *Cousinia* is very limited, if we compare our sectinal sampling with the classification by Tscherneva (1988a,b). The counts gathered from the literature are also scarce for the size of the genus. Owing to this low level of karyologic knowledge of the genus *Cousinia* s. str. as a whole, no authoritarian conclusions can be drawn yet. However, some general patterns emerge.

On the basis of our own results and those by other authors, at least some sections are karyologically well known: sect. *Cynaroideae*, sect. *Carduncellus*, sect. *Leiocaules* and a section not included in our sampling, sect. *Stenocephalae* Bunge from Iran (Ghaffari and Djavadi, 1998). These sections have only one chromosome number: sect. *Cynaroideae* has *x* = 12, sect. *Carduncellus* has *x* = 13, sect. *Leiocaules* has *x* = 12 and sect. *Stenocephalae* has *x* = 13. However, many other sections have more than one chromosome number. In one case, we have found even three different numbers for a section (sect. *Microcarpae* has *x* = 11, 12 and 13).

Thanks to our previous molecular study of the genus *Cousinia*, we can advance a hypothesis, even though tentative, on the sense of this remarkable dysploidy. The phylogeny suggested by the DNA sequences of the ITS and *mat*K region shows a close relationship between the species with $x = 11$ and those with $x = 13$ (Susanna *et al.*, in press). Instead, the species with $x = 12$ form a different group. This contradicts the logical order of descending dysploidy, which must be from $x = 13$ to $x = 12$ and then to $x = 11$. The most conservative hypothesis is that the number $x = 12$ of the species included in the molecular study derive from a group with $x = 13$ not represented in the analysis. On the other hand, the number $x = 11$ derives from a group with $x = 13$, to whom it is united in our phylogram, through a group with $x = 12$ that was not included in our partial sampling. This hypothesis implies that descending dysploidy is frequent in the group, and also that it has occurred more that once in the subgenus *Cousinia*; both implications were proven in the related subtribe Centaureinae (Garcia-Jacas *et al.*, 2001). Anyway, at our present level of knowledge, this is only speculative and will need to be confirmed with more extensive surveys, both karyological and molecular, involving a larger representation of *Cousinia*. These new studies would complement the urgent and deep revision of present sectional classification of subgenus *Cousinia*, which, both on karyologic and molecular grounds, seems to have serious flaws.

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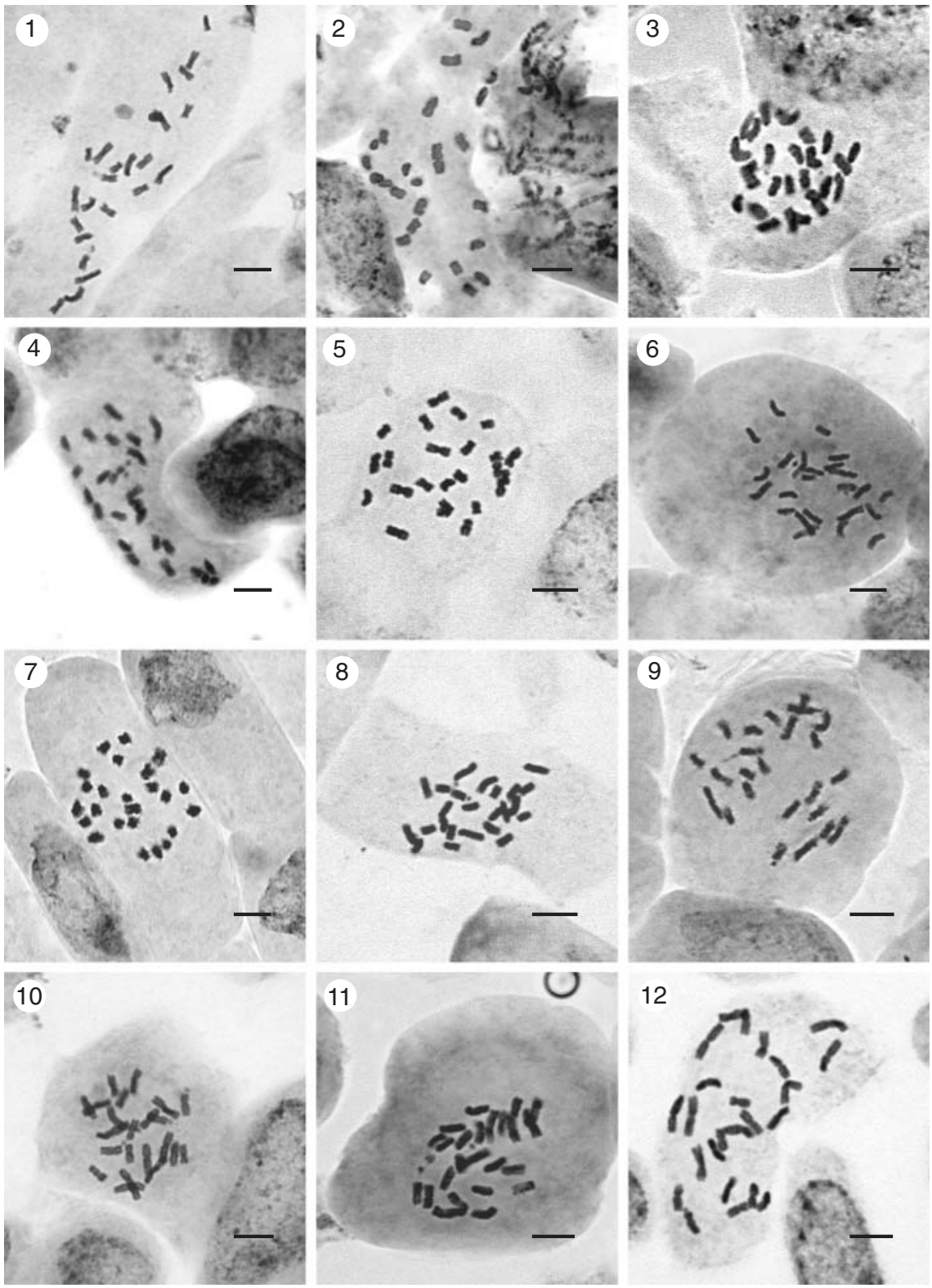
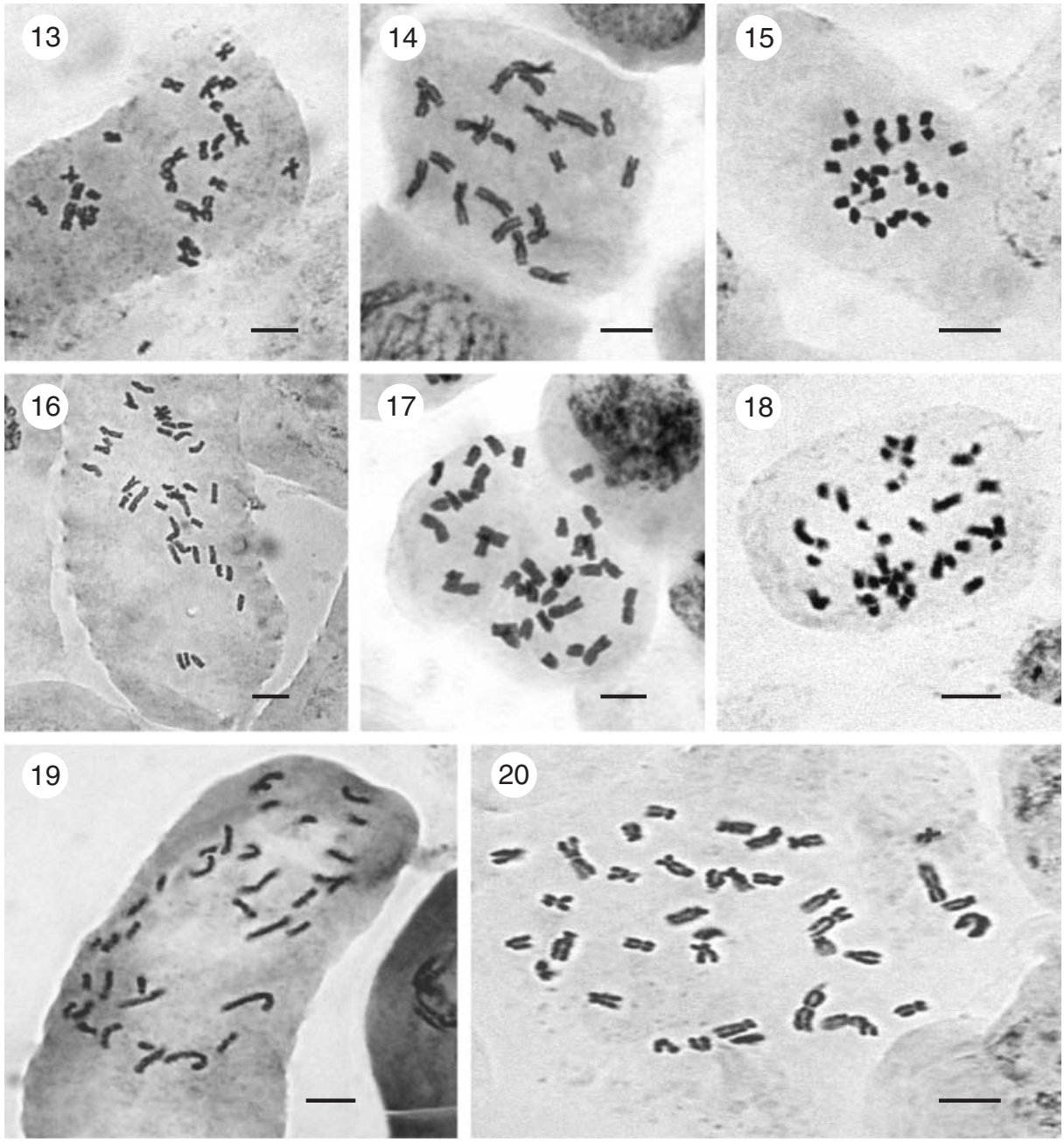


Figures 1–12. Somatic metaphases of *Cousinia* spp. Scale bars = 10 μm. Fig. 1. *Cousinia chrysantha* (2*n* = 24). Fig. 2. *C. tianshanica* (2*n* = 26). Fig. 3. *C. coronata* (2*n* = 26). Fig. 4. *C. congesta* (2*n* = 26). Fig. 5. *C. minkwitziae* (2*n* = 24). Fig. 6. *C. polycephala* (2*n* = 24). Fig. 7. *C. syrdariensis* (2*n* = 24). Fig. 8. *C. canescens* (2*n* = 24). Fig. 9. *C. purpurea* (2*n* = 24). Fig. 10. *C. caespitosa* (2*n* = 22). Fig. 11. *C. astracanica* (2*n* = 24). Fig. 12. *C. arachnoidea* (2*n* = 24).



Figures 13–20. Somatic metaphases of *Cousinia* and *Schmalhausenia* spp. Scale bars = 10 μm. Fig. 13. *Cousinia microcarpa* (2*n* = 26). Fig. 14. *C. platylepis* (2*n* = 22). Fig. 15. *C. tenella* (2*n* = 26). Fig. 16. *C. karatavica* (2*n* = 36). Fig. 17. *C. lappacea* (2*n* = 36). Fig. 18. *C. triflora* (2*n* = 36). Fig. 19. *C. umbrosa* (2*n* = 36). Fig. 20. *Schmalhausenia nidulans* (2*n* = 36).

material from Tadjikistan, and Koul (1964) and Podlech & Dieterle (1969) on material from Afghanistan. There is a different count of $n = 10$ in material from India by Mehra & Remanandan (1969).

Cousinia platylepis Schrenk
Kazakhstan, Žambylskaya oblast: 30 km from the Kurdai pass, *Ivaschenko, Susanna 2158 & Vallès*, 27.viii.2000 (BC). 2*n* = 22 (Fig. 14).

As far as we know, this is the first report for this species.

Our results indicate three different basic numbers for sect. *Microcarpae*: $x = 11$, 12, 13. Previous chromosome counts for this section have revealed $x = 13$ in *C. microcarpa* and $x = 11$ in *C. sewerzowii* Regel (Tscherneva, 1985) and *C. leiocephala* (Regel) Juz. (Aryavand, 1976). As already noted, the existence of