New chromosome counts in the genus Cousinia (Asteraceae, Cardueae) from Iran

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New chromosome counts in the genus *Cousinia (Asteraceae, Cardueae)* from Iran

Abstract

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Meiotic chromosome counts are reported for 20 populations, representing 18 species of the genus *Cousinia* s.str. from Iran. Most of the populations counted are from the type localities of the corresponding species. Counts for fifteen species are new to science, two confirm previous reports, while one count, for *C. hypopolia,* opposes an early report. The chromosome number for the monotypic *C.* sect. *Harazensis* is reported for the first time.

Additional key words: *Compositae, Carduinae,* karyology

Introduction

The genus *Cousinia* Cass., comprising more than 600 species (Rechinger 1986; Susanna & Garcia-Jacas 2006), is one of the largest genera of *Asteraceae* and the largest genus of the *Arctium* group of the tribe *Cardueae* subtribe *Carduinae* (Mabberley 1990; Häffner 2000; Susanna & al. 2003a, b). All members of the *Arctium* group, which also includes the genera *Arctium* L. (11 species), *Hypacanthium* Juz. (3 species) and *Schmalhausenia* C. Winkl. (1 species), are characterised by twisted scales of the receptacle, tigrine surface of the achenes, a missing nectary and free deciduous bristles of the pappus (Susanna & al. 2003b). According to Tscherneva (1988), *Cousinia* is divided into three subgenera; *Cynaroides* (c. 20 species), *Hypacanthodes* (c. 10 species) and *Cousinia* (c. 600 species).

The *Arctium* group is monophyletic and consists of two distinct clades: the arctioid clade (including the genera *Arctium, Hypacanthium, Schmalhausenia* and *Cousinia* subg. *Cynaroides* and subg. *Hypacanthodes*) and the cousinioid clade (including *Cousinia* subg. *Cousinia*). All members of the arctioid clade have a diploid chromosome number of $2n = 36$ (Tscherneva 1985), whereas members of the cousinioid clade are characterised by chromosome numbers of $2n = 18$, 20, 22, 24 and 26 (Susanna & al. 2003b; Ghaffari & al. 2006). The species of the subgenera *Cynaroides* and *Hypacanthodes* have the *Arctiastrum* pollen type, similar to that of *Arctium* (orbicular and spiny pollen), while species of subgenus *Cousinia* are characterised by smooth and oblong pollen, the *Cousinia* pollen type (Susanna & al. 2003b).

More than 400 of the *Cousinia* species are centred in SW Asia, especially in the mountainous regions of the Flora Iranica area (Rechinger 1986; Knapp 1987; López-Vinyallonga & al. 2009). Alongside four important centres of diversification in Central Asia (with a high number of endemics in the eastern part of its distribution area), four other diversity centres could be distinguished in western Asia: the Kopetdagh mountain range, the Elburz range, the northern part of the Zagros range and Azerbaijan (Knapp 1987). Therefore, Iran is one of the main centres of speciation of the genus.

Considering the large size of the genus, *Cousinia* is still poorly known cytologically. So far, chromosome number reports exist only for c. 80 species in Iran, thus roughly a fifth of the *Cousinia* species in the country (Aryavand 1975; Afzal-Rafii 1980; Ghaffari 1984, 1986,

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1987; Ghaffari & Chariat-Panahi 1985; Ghaffari & Djavadi 1998; Djavadi & Ghaffari 1999; Ghaffari & al. 2000, 2006; Susanna & al. 2003b; Sheidai & al. 2005; Djavadi 2005, 2007). The basic chromosome numbers found most frequently in *Cousinia* s.str. are $x = 12$ and $x = 13$; $x = 11$ is found less often, while $x = 9$ and $x = 10$, reported by early studies (see Fedorov 1969), had been thought to be erroneous (Susanna & al. 2003b) but their presence in *Cousinia* s.str. (sect. *Microcousinia,* $x = 10$, sect. *Myriotomae,* $x = 9$) was corroborated by Ghaffari & al. (2006).

Material and methods

Floral buds of living plants were collected from populations in the wild and immediately fixed in Pienaar's solution (ethanol; chloroform; propionic acid; 6 : 3 : 2 v/v, Pienaar 1955) for 24 hours, transferred to 70 % alcohol and stored under refrigeration. Slides were prepared by the squash technique and cells were stained with 2 % acetocarmine (Wilson 1945). Digital photographs were taken using a Canon camera (G5) mounted on a Zeiss microscope. The herbarium vouchers are preserved in the herbaria IRAN and TUH (Thiers 2008+).

Results and discussion

In this study, the morphology-based infrageneric classification by Rechinger (1972, 1979) is used, because no modern classification is available yet.

Cousinia Cass. subg. ***Cousinia***

Cousinia sect. ***Albidae*** Rech. f.

Cousinia archibaldii Rech. f.
Bakhtiari: Farsan to Chelgerd, Ab Barik, 2560 m, 5.7. 2009, *Djavadi, Ghanbari & Torabi 53202* (IRAN), $n = 13$.

Meiosis showed 13 bivalents at metaphase I (Fig. 1). Our results agree with the previous chromosome counts for this species by Afzal-Rafii (1980).

Cousinia bachtiarica Boiss. & Hausskn.
Fars: Eghlid, 2100 m, 1.7.2009, *Djavadi, Ghanbari & Torabi 53319* (IRAN), $n = 13$.

Meiosis showed 13 bivalents at metaphase I (Fig. 2). This is the first chromosome count for this species.

Cousinia oligocephala Boiss.
Kohkilouyeh va Boyer Ahmad: Sisakht, Bijan pass, Dena protected region, 2450–3170 m, 3.7.2009, *Djavadi, Ghanbari & Torabi 53193* (IRAN), $n = 13$.

Thirteen bivalents were observed at metaphase I (Fig. 3). This is the first chromosome count for this species.

The section comprises eight species, all endemic to Iran (Rechinger 1972). The three above-mentioned species are the only ones for which chromosome numbers are known, all of them being $x = 13$.

Cousinia sect. ***Alpinae*** Bunge

Cousinia multiloba DC.
Fars: Eghlid, Bel mountain, 2400–2800 m, 1.7.2009, *Djavadi, Ghanbari & Torabi 53235* (IRAN), $n = 13$.

Meiosis showed 13 bivalents at metaphase I (Fig. 4) and a 13–13 chromosome segregation at anaphase I (Fig. 5). This is a widespread species, distributed in Iran, Turkmenistan, Afghanistan and Pakistan. Our results agree with the previous chromosome counts for this species in material from Afghanistan (Podlech & Bader 1974) and in two populations from northern Iran (Ghaffari & al. 2006).

This section comprises more than 20 species. The only other species counted to date is *Cousinia chrysantha,* for which $2n = 24$ was reported by Tscherneva (1985) and Susanna & al. (2003b), which means that two basic chromosome numbers, $x = 12$ and $x = 13$, are known for this section.

Cousinia sect. ***Cynaroideae*** Bunge

Cousinia barbeyi C. Winkl.
Kohkilouyeh va Boyer Ahmad: Sisakht, Bijan pass, Dena protected region, 2450–3170 m, 3.7.2009, *Djavadi, Ghanbari & Torabi 53865* (IRAN), $n = 12$.

Twelve bivalents were observed at metaphase I (Fig. 6). This is the first chromosome count for this species, which is a narrow endemic of Iran, distributed in the Kohkilouyeh va Boyer Ahmad Province.

Cousinia concinna Boiss. & Hausskn.
Kurdestan: Sanandaj to Marivan, the side road of Tij-Tij, Eslam-Dasht village to Sheykh-Attar, 7.6.2009, *Attar & Zamani 40545* (TUH), $n = 12$.

Twelve bivalents were observed at metaphase I (Fig. 7). Chromosome segregation at anaphase I was 12–12 (Fig. 8). This is the first chromosome count for this species, which is endemic to the Kurdestan Province.

Cousinia farsistanica Bornm.
Fars: Eghlid, Bel mountain, 2400–2800 m, 1.7.2009, *Djavadi, Ghanbari & Torabi 53864* (IRAN), $n = 12$.
Fars: Dedj Kord to Sadeh, 2340 m, 2.7.2009, *Djavadi, Ghanbari & Torabi 53859* (IRAN), $n = 12$.

Twelve bivalents were observed at metaphase I (Fig. 9). Chromosome segregation at anaphase I was 12–12 (Fig. 10). This is the first chromosome count for this species, which is endemic to Iran, distributed in the provinces of Fars, Yazd, Kerman and Bakhtiari.

Cousinia lordeganensis Mehregan
Bakhtiari: Lordegan to Broujen, after Al-Ghadir tunnel, 1880 m, 4.7.2009, *Djavadi, Ghanbari & Torabi 53191* (IRAN), $n = 12$.

Meiosis showed 12 bivalents at metaphase I (Fig. 11). Chromosome segregation at anaphase I of meiosis divi-

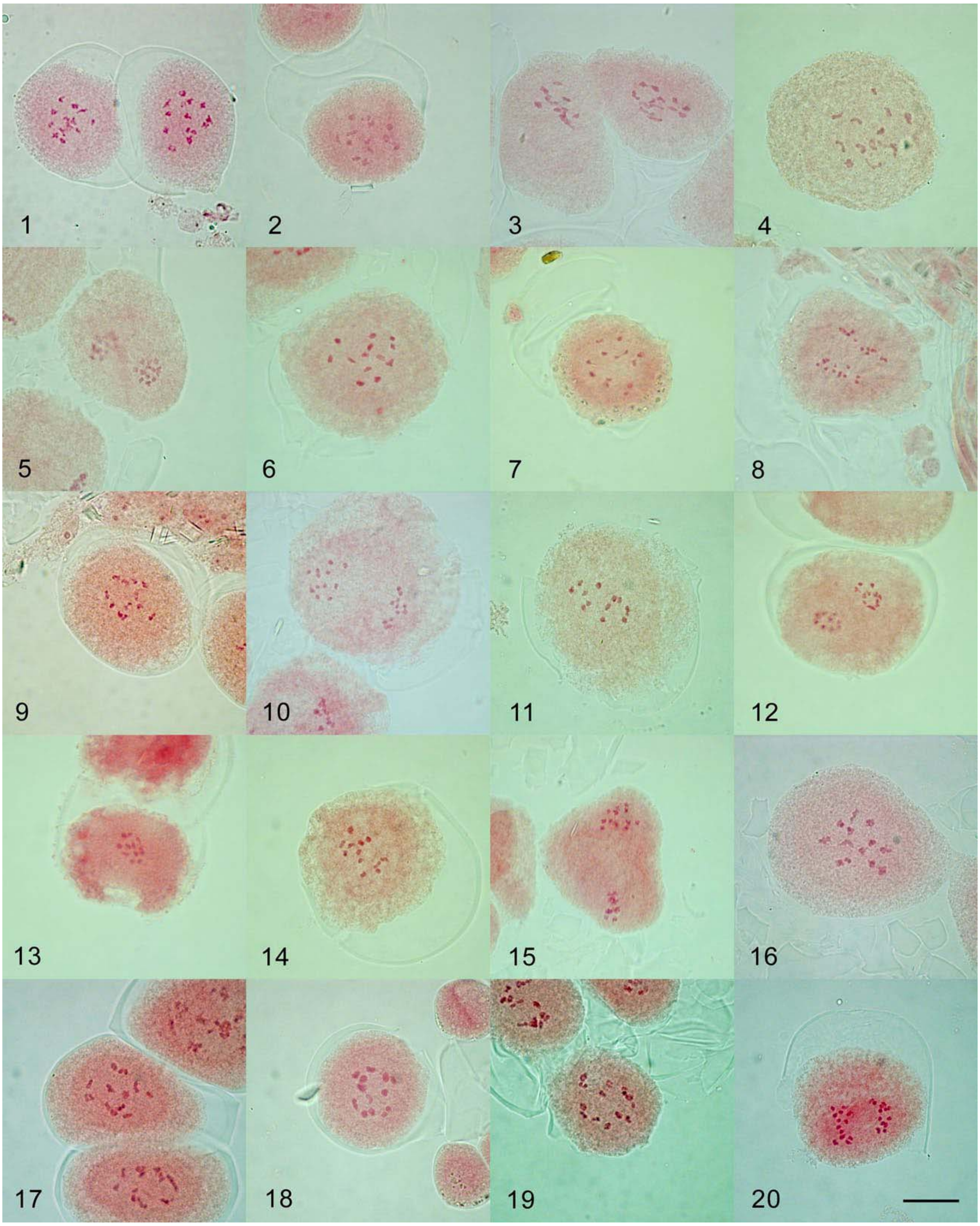

Fig. 1–20. Meiosis in *Cousinia* spp. – 1: *C. archibaldii,* metaphase I, $n=13$; 2: *C. bachtiarica,* metaphase I, $n=13$; 3: *C. oligocephala,* metaphase I, $n=13$; 4–5: *C. multiloba,* metaphase I, $n=13$ and anaphase I (13–13); 6: *C. barbeyi,* metaphase I, $n=12$; 7–8: *C. concinna,* metaphase I, $n=12$, anaphase I (12–12); 9–10: *C. farsistanica,* metaphase I, $n=12$ and anaphase I (12–12); 11–12: *C. lordeganensis,* metaphase I, $n=12$ and anaphase I (12–12); 13: *C. pergamacea,* metaphase I, $n=12$; 14–15: *C. zardkuhensis,* metaphase I and metaphase II, $n=12$; 16: *C. harazensis,* metaphase I, $n=13$; 17: *C. kilouyensis,* metaphase I, $n=12$; 18: *C. gracilis,* metaphase I, $n=13$; 19–20: *C. platyptera,* $n=13$, metaphase I (19), metaphase II (20). – Scale bar = 20 µm.

sion was 12–12 (Fig. 12). This is the first chromosome count for this species, which is a local endemic of the Bakhtiari Province.

Cousinia pergamacea Boiss. & Hausskn.
Kurdestan: 25 km Baneh to Saqqez, 11 km after Mir-Deh toward Pir Amran village, 9.6.2009, *Attar & Zamani 40466* (TUH), $n = 12$.

Meiosis showed 12 bivalents at metaphase I (Fig. 13). This is the first chromosome count for this species, which is endemic to the Kurdestan Province.

Cousinia zardkuhensis Attar & Ghahreman
Bakhtiari: 14 km Shahr-e Kord to Farsan, before Soreshjan, 2250 m, 5.7.2009, *Djavadi, Ghanbari & Torabi 53198* (IRAN), $n = 12$.

Meiosis in this species showed 12 bivalents at metaphase I (Fig. 14) and metaphase II (Fig. 15). This is the first chromosome count for this species.

Cousinia sect. *Cynaroideae* Bunge seems to be monophyletic (Mehregan & Kadereit 2008). On the basis of our results and those of other authors (Aryavand 1975; Afzal-Rafii 1980; Ghaffari 1986, 1987; Tscherneva 1985; Ghaffari & al. 2000, 2006; Susanna & al. 2003b), the section appears to be uniformly characterised by the basic chromosome number $x = 12$.

Cousinia sect. ***Harazensis*** Rech. f.

Cousinia harazensis Rech. f.
Mazandaran: Haraz road, Gazanak, 10.7.2009, *Djavadi 53201* (IRAN), $n = 13$.

Meiosis indicated 13 bivalents at metaphase I (Fig. 16). This is the first chromosome count for this species and section. *Cousinia harazensis,* the only member of this section, is a narrow endemic species restricted to a very small area in the Mazandaran Province (Gazanak).

Cousinia sect. ***Leiocaules*** Bunge

Cousinia kilouyensis Djavadi & Attar
Kohkilouyeh va Boyer Ahmad: Sepidan to Yasouj, 5 km before Vezg village, 2420 m, 2.7.2009, *Djavadi, Ghanbari & Torabi 53185* (IRAN), $n = 12$.

Meiosis showed 12 bivalents at metaphase I (Fig. 17). This is the first chromosome count for this species.

Cousinia sect. *Leiocaules* comprises 21 species (Tscherneva 1962; Rechinger 1972, 1979; Huber-Morath 1975; Djavadi & Attar 2009), out of which eight species are distributed in Iran (six of them being entirely endemic to the area). Almost all other counts, conducted in six species altogether, also revealed $n = 12$ or $2n = 24$, respectively (Ghaffari 1984; Tscherneva 1985; Djavadi 2005; Susanna & al. 2003b; Ghaffari & al. 2006). The odd exception is a single previous count of $2n = 26$ on *C. glandulosa* Kult (Chukdanova, in Fedorov 1969).

Cousinia sect. ***Pugioniferae*** Bunge

Cousinia gracilis Boiss.
Kohkilouyeh va Boyer Ahmad: Chal Kalagh, 2410 m, 2.7. 2009, *Djavadi, Ghanbari & Torabi 48650* (IRAN), $n = 13$.

Thirteen bivalents were observed at metaphase I (Fig. 18). This is the first chromosome count for this species, which is endemic to the provinces Kohkilouyeh va Boyer Ahmad, Lurestan and Fars.

Cousinia platyptera Bornm. & C. Winkl.
Fars: Saghad to Shirin khosrow, Gerdou (Girdu) village, 2100 m, 1.7.2009, *Djavadi, Ghanbari & Torabi 53190* (IRAN), $n = 13$.
Fars: Eghlid, the road toward Bel mountain, 2100 m, 1.7.2009, *Djavadi, Ghanbari & Torabi 53192* (IRAN), $n = 13$.

Meiosis showed 13 bivalents at metaphase I (Fig. 19) and metaphase II (Fig. 20). Chromosome segregation at anaphase I was 13–13 (Fig. 21) and 13–13 + 13–13 monad segregating was observed at anaphase II (Fig. 22). This is the first chromosome count for this species endemic to Iran.

This is a small section, represented by 15 species (Rechinger 1972; Assadi 2009; Attar & Mirtadzadini 2009), out of which 13 species are distributed in Iran (11 of them being endemic to the area). Only four chromosome counts are reported in the literature; three species also show $n = 13$ or $2n = 26$, respectively: *Cousinia belangeri* DC. (Ghaffari & Chariat-Panahi 1985), *C. calcitrapa* Boiss. and *C. macroptera* C. A. Mey (Afzal-Rafii 1980). For *C. pugionifera* Jaub. & Spach $2n = 24$ was reported by Aryavand (1975).

Cousinia sect. ***Stenocephalae*** Bunge

Cousinia bijarensis Rech. f.
Kurdestan: Bijar, Kuh Siah, 6.6.2009, *Attar & Zamani 40482* (TUH), $n = 13$.

Thirteen bivalents were observed at metaphase I (Fig. 23). This is the first chromosome count for this species, which is endemic to the Kurdestan Province.

Cousinia hypopolia Bornm. & Sint.
Khorasan: Bojnourd, Shoghan, Garmak, 6.6.2009, *A. Javadi & Torabi 53812* (IRAN), $n = 13$.

Thirteen bivalents were observed at metaphase I (Fig. 24). This count disagrees with the only previous report of $2n = 18$ by Chuksanova (in Fedorov 1969). This species is endemic to Iran and Turkmenistan.

Cousinia renominata Rech. f.
Golestan: Maraveh Tappeh to Kalaleh, Chenarly, 7.9. 2009, *A. Javadi & Torabi 53811* (IRAN), $n = 13$.

Thirteen bivalents were observed at metaphase I (Fig. 25); chromosome segregation at anaphase I was

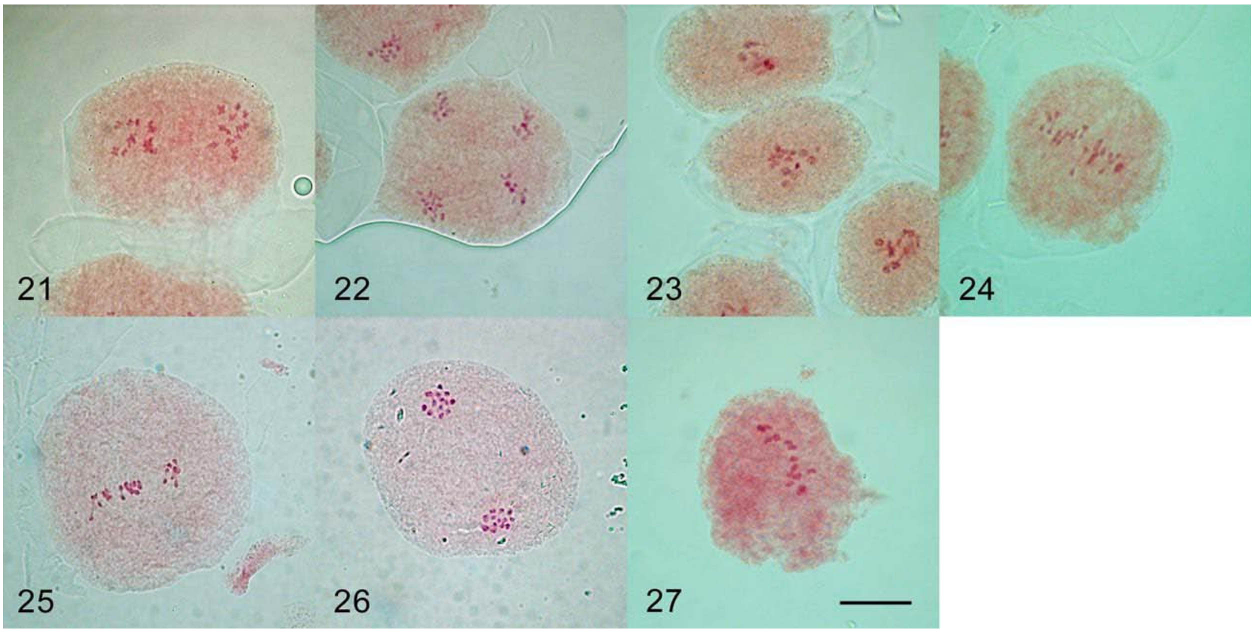

Fig. 21–27. Meiosis in *Cousinia* spp. – 21–22: *C. platyptera,* anaphase I (13–13) (21), anaphase II (22) showing 13–13 +13–13 monad segregation; 23: *C. bijarensis,* metaphase I, $n = 13$; 24: *C. hypopolia,* metaphase I, $n = 13$; 25–26: *C. renominata,* metaphase I, $n = 13$ (25) and anaphase I (13–13) (26); 27: *C. stahliana,* metaphase I, $n = 13$. – Scale bar = 20 μm.

13–13 (Fig. 26). This is the first chromosome count for this species, which is endemic to a very limited area around Maraveh Tappeh in the Golestan Province.

Cousinia stahliana Bornm. & Gauba
Khorasan: Shirvan, Ziarat, 5.6.2009, *A. Javadi & Torabi 53806* (IRAN), $n = 13$.

Thirteen bivalents were observed at metaphase I (Fig. 27). A previous report of $n = 13$ for this species by Ghaffari & Djavadi (1998) was based on a misidentification; the material counted by these authors has been revised by the authors of the present study and determined to be *Cousinia assyriaca* Jaub. & Spach. Therefore, this is the first chromosome count for *C. stahliana,* which is endemic to Iran and Turkmenistan.

Cousinia sect. *Stenocephalae* Bunge is cytologically almost well known. According to our results and those of other authors (Afzal-Rafii 1980; Tscherneva 1985; Ghaffari 1986; Ghaffari & Chariat-Panahi 1985; Ghaffari & Djavadi 1998; Djavadi 2005; Ghaffari & al. 2006; Djavadi 2007), the section has a uniform basic chromosome number of $x = 13$. The early count of $2n = 18$ by Chuksanova appears to be erroneous.

Chromosome numbers and infrageneric systematics of *Cousinia*

Whereas chromosome numbers agree well with the delimitation between the cousinioid and the arctioid clade of the *Cousinia-Arctium* complex, as is indicated by pollen type, morphology and DNA sequences (Susanna & al. 2003a, b; López-Vinyallonga & al. 2009), the case appears somewhat different within the cousinioid clade, or *Cousinia* s.str.

Five basic chromosome numbers, viz. $x = 9$, 10, 11, 12 and 13, are confirmed for *Cousinia* s.str. (Susanna & al. 2003b; Ghaffari & al. 2006). In several cases the basic chromosome numbers do correspond to the current sectional classification. So far, for instance *C.* sect. *Albidae* ($x = 13$, present study), sect. *Carduncellus* ($x = 13$, Susanna & al. 2003b), sect. *Cynaroideae* ($x = 12$, Susanna & al. 2003b, Ghaffari & al. 2006 and present study), sect. *Cousinia* ($x = 12$, Ghaffari & al. 2006), sect. *Helianthae* ($x = 13$, Ghaffari & al. 2006) and sect. *Stenocephala* ($x = 13$, Ghaffari & Djavadi 1998 and present study, also confirming that an earlier count of $2n = 18$ for *C. hypopolia* must be erroneous) seem to be characterised by a single basic number. Such sections are also morphologically well characterised: e.g. all species belonging to *C.* sect. *Cynaroideae* have spiny-winged decurrent leaves and appendaged bracts; all members of sect. *Stenocephalae* have numerous heads, a capitulum with up to 20 flowers, corymbose branches and decurrent leaves. It seems that such morphological and karyological similarities are also reflected in the pollen type (Saber & al. 2009).

In some other cases, sections have more than one basic number, often both $x = 12$ and $x = 13$. This, in the present study, is the case with *Cousinia* sect. *Alpinae,* sect. *Leiocaules* (although it is possible that the earlier finding of $x = 13$ is erroneous) and sect. *Pugioniferae*. *C.* sect. *Microcarpae* has even the three basic numbers $x = 11$, 12 and 13 (Susanna & al. 2003b).

On the basis of such findings, Susanna & al. (2003b) hypothesised that descending dysploidy, moving down from $x = 13$ has independently and frequently occurred in *Cousinia* s.str., which seems to be a highly plausible scenario. It should, however, be kept in mind that the morphology-based infrageneric classification of *Cousinia* s.str. by Rechinger (1972, 1979) probably needs revision in several cases once molecular analyses with higher phylogenetic resolution become available. Currently, relationships among species groups are only inadequately resolved by molecular analyses (López-Vinyallonga & al. 2009; Mehregan & Kadereit 2009).

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