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Original Scientific Report

Chromosome number and meiotic behavior in several plant taxa from Iran

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ABSTRACT:
Original meiotic or both meiotic and mitotic chromosome numbers are reported for ten endemic and one non endemic species in nine vascular plant families from Iran. The chromosome numbers of *Acantholimon schahrudicum*, *A. truncatum*, *Anthochlamys multinervis*, *Campanula perpusilla*, *Cousinia calcitrapa* var. *interrupta*, *Dorema ammoniacum*, *Euphorbia gedrosiaca*, and *Hyocyamus orthocarpus* were determined for the first time. The chromosome counts for *Astrodaucus persicus* and *Hedysarum criniferum* agree with previous ones. The gametic chromosome numbers for *Hedysarum criniferum* and *Allium stipitatum* are reported here for the first time. The occurrence of accessory chromosomes are also reported for *Acantholimon schahrudicum* and *Dorema ammoniacum*, being the first records of B chromosomes in the genera *Acantholimon* and *Dorema*.

Keywords:
karyology, B chromosome, mitosis, meiosis, endemic plants

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Endemic plants constitute valuable floristic elements in every region. Many of them are threatened plant species with a limited distribution range and a small number of individuals. Their identification, protection, ecological and genetic studies are of prime interest for the scientific community. The flora of Iran comprises ca. 8000 flowering plant species, among which more than 2000 species are endemic or subendemic (Ghahreman & Attar 1999; Jalili & Jamzad 1999). Unfortunately, because of the current impact of the expansion of urban development and land use activities in Iran, several species are in danger of extinction.

Chromosome studies on endemic species from Iran include fewer than 100 plant species and are usually based on a very limited number of observations (Ghaffari 1988; Ghaffari *et al*. 2005, 2006; Hesamzadeh - Hejazi & Ziaei Nasab 2007; Shariat *et al*. 2013; Hatami *et al*. 2019; Oroji Salmasi *et al*. 2019; Sadeghian *et al*. 2019). The present study describes the meiotic or both meiotic and mitotic chromosome number of ten endemic and one non endemic species in nine families, aiming to contribute to the cytological knowledge of the endemic flora of Iran.

The plants were collected from different parts of Iran. The chromosome numbers were determined by examining the somatic cells from the root tips or pollen mother cells. More than 10 slides and 50 cells were examined for each species. For the study of mitotic chromosomes the root tips were placed in 0.002 M 8-hydroxyquinoline for 3 h at 20°C and then fixed in 6:3:2 ethyl alcohol (96%): chloroform: propionic acid at 4°C for 48 h. After rinsing in distilled water, the root tips were hydrolyzed in 1N hydrochloric acid for 10 min at 60°C. Staining was carried out by means of the Feulgen reaction enhanced by squashing in 2% acetocarmine (Ghaffari 2006). The nomenclature adopted by Levan *et al.* (1964) was followed for recognizing chromosome types.

For meiotic studies floral buds of appropriate size were fixed in Pienaar's fixing fluid (ethanol 96%, chloroform, propionic acid, 6:3:2 v/v) and stained with 2% acetocarmine. A herbarium specimen of each species with perma-

nent cytological slides is preserved in the plant cytogenetic laboratory of the National Botanical Garden of Iran (Research Institute of Forests and Rangelands, Tehran, Iran). The collector and code number of both the herbarium voucher specimens and the permanent slides for each species are presented in the results.

Alliaceae

Allium stipitatum Regel– $n = 8$, $2n = 16$ (Fig. 1A, B)
Esfahan province: Khoonsar, Mt. Golestan, N 33°09'09.5", E 50°23'11.0", Ghaffari 486

With c. 126 species of *Allium*, Iran is one of the most important diversity centers of the genus *Allium*. Among them 19 species are endemic (Mehrabian *et al.* 2021). *Allium stipitatum* is distributed in Iran, Afghanistan, Pakistan, and Central Asia (Fritsch & Abbasi 2013). Mathew (1996) believed *A. hirtifolium* Boiss. to be a distinct species endemic to Iran. However, recent sources regard *A. hirtifolium* as a synonym of *A. stipitatum* and both names refer to one species (Fritsch *et al.* 2006; Fritsch & Abbasi 2013).

Previous reports for *A. stipitatum* were $2n = 16$ by Pedersen & Wendelboo (1966), Panahandeh & Mahna (2011) and Oroji Salmasi *et al.* (2019) from Iran, and Pogosian (1983) from Armenia, with certain doubts regarding species determination (Ghaffari 2006). Karyotype formula and somatic chromosome counts in all the root tips of our sample (from different parts of previous reports) were $2n = 3m + 5sm = 16$ (Fig. 1A). Meiosis in this taxon showed 8 bivalents at diploten (Fig. 1B). The gametic number is reported here for the first time.

Apiaceae

Astrodaucus persicus (Boiss.) Drude $n = 10$ (Fig. 1C, D)
Qazvin province: 45 km towards Karaj, N 36°04'15.5", E 50°23'54.8", Ghaffari 3085

The flora of Iran includes two species of the genus *Astrodaucus*, *A. orientalis* (L.) Drude and *A. persicus*. *Astrodaucus persicus* is endemic to Iran and mainly distributed in the Mazandaran, Tehran, Semnan and Golestan provinces (Mozaffarian 2007). There is only one report on the meiosis ($n = 10$) of this species by Shner *et al.* (2004), which is in agreement with the present count. Our sample was diploid and showed 10 bivalents at diakinesis and 10 dyads at metaphase II (Fig. 1C, D). The analysis of 28 cells at metaphase I showed the mean number of chiasma was 1.56 per cell in this species.

Dorema ammoniacum D.Don. $n = 11+2B$, $2n = 22+ 4B$ (Fig. 1G, H, I)
Tehran province: Damavand, between Absard and Saran, N 35°33'05.1", E 48°13'49.6", Ghaffari 1173

There are six species of the genus *Dorema* in Iran, among which two are endemic, namely, *D. ammoniacum* and *D. aucheri* Boiss. (Ghahreman & Attar 1999). *Dorema ammoniacum* produces gum as a result of insect bites. The gum of this species is used in pharmacy, perfumery and medicine (Rajaei & Mohamadi 2012). Meiosis in this species was regular and showed 11 bivalents at metaphase I and normal chromatid segregation at anaphase II (Fig. 1E, G). In some cells 2 B chromosomes were observed in addition to the normal set of chromosomes. The B chromosomes appeared as univalents and did not pair with each other or with the A chromosomes. They showed a tendency to lag at first and second anaphase (Fig. 1F, H). The analysis of chiasma frequency in 29 cells with B and 31 cells without B chromosomes at metaphase I showed 1.38 and 1.54 chiasma frequency per bivalent respectively. This result indicated that the presence of B chromosomes decreases chiasma frequency in A chromosomes.

The chromosome number at mitotic division is $2n = 22 + 0\text{-}4B$ (Fig. 1I). To our knowledge, this is the first report of the chromosome number for this species and the presence of B chromosomes in the genus *Dorema*.

Asteraceae

Cousinia calcitrapa Boiss.var. *interrupta* Heimerl $n = 13$ (Fig. 1J, K)
Qom province: Salafchegan towards Qom, N 34°34'02.4", E 50°40'54.7", Ghaffari 177

Cousinia is one of the largest genera of the Asteraceae family in Iran with 294 species, 81% of them being endemic (Noroozi *et al.* 2019).

Cousinia calcitrapa var. *interrupta* is endemic to Iran and belongs to the section Pugioniferae. There are 13 bivalents at metaphase I showing regular chromosome segregation at anaphase I (Fig. 1J, K). The chiasma frequency obtained from 28 cells was 1.32 per bivalent. The previous mitotic count for *C. calcitrapa* was $2n = 26$ from a different part of Iran (Mt. Tash-sefid) as recorded by Afzal-Rafii (1980). The genus *Cousinia* exhibits a complex basic chromosome number series ranging from $x = 9$ to $x = 13$ (Ghaffari *et al.* 2006). According to our data, this is the first chromosome count for this variety.

Campanulaceae

Campanula perpusilla DC. $n = 11$ (Fig. 1L)
Khuzestan province: Iyzeh, Eshkaft-e Solyman, N 31°49'04.0", E 49°50'57.7", Ghaffari 885

In Iran the genus *Campanula* consists of 46 taxa, 14 of them being endemic (Aghabeigi 2010; Advay & Maroof 2015). *Campanula perpusilla* is a common endemic species to Iran and Iraq. Eleven bivalents at metaphase I were observed (Fig. 1L). This is the first chromosome number report for this taxon.

Chenopodiaceae

Anthochlamys multinervis Rech.f. $n = 9$ (Fig. 2A, B)
Tehran province: 60 km towards Qom, N 35°13'03.5", E 51°05'50.7", Ghaffari 3385

The genus *Anthochlamys* comprises 5-6 species distributed in the Irano-Turanian floristic region. Almost

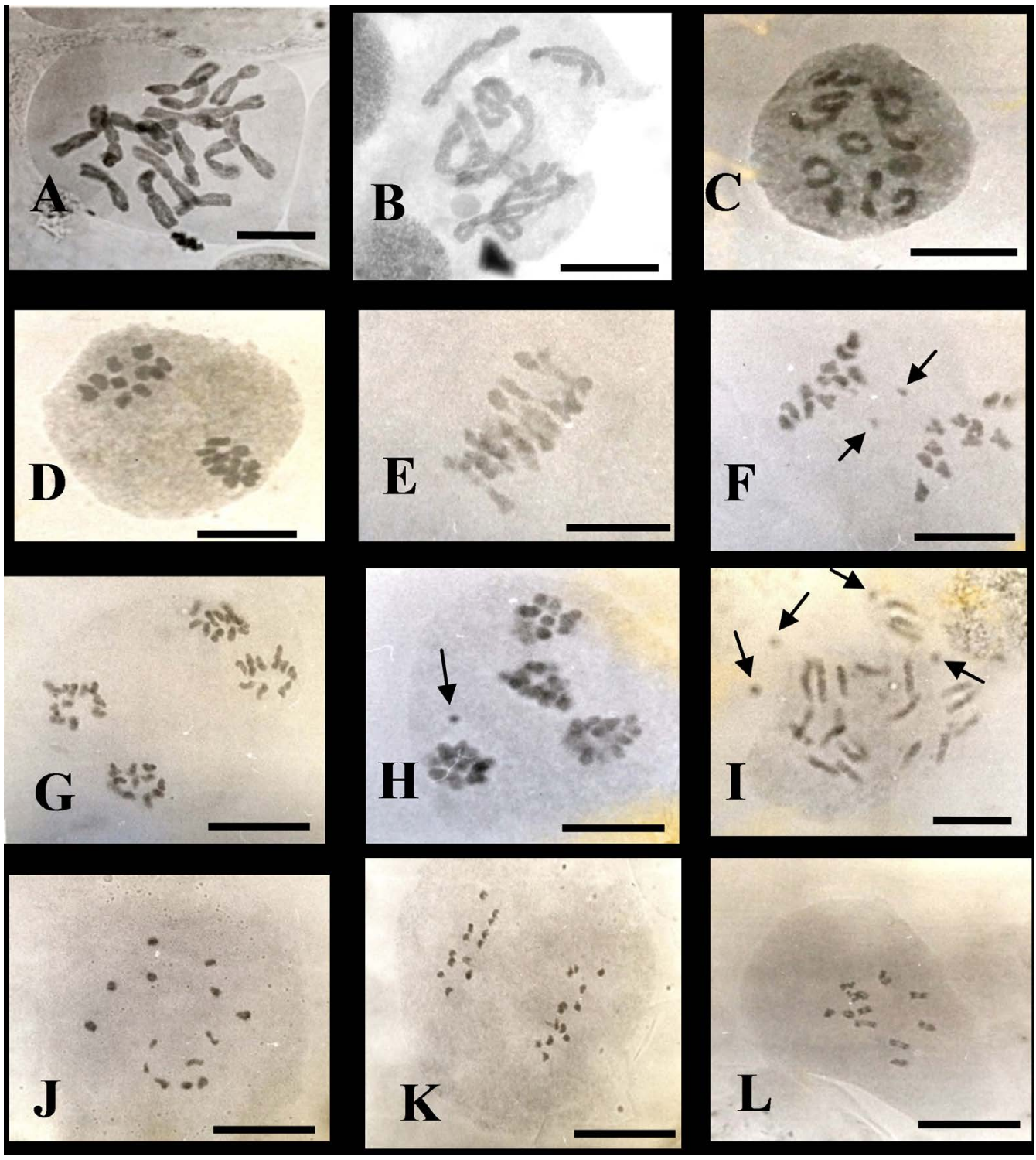

Fig. 1. Chromosome slides of: *Allium stipitatum* - A) metaphase of mitotic chromosomes (2*n* = 16), and B) metaphase I (*n* = 8); *Astrodaucus persicus* - C) diakinesis, and D) metaphase II (*n* = 10); *Dorema ammoniacum* - E) metaphase I (*n* = 11), F) anaphase I, showing B chromosome laggards (arrows), G) anaphase II, showing normal chromatid segregation, H) anaphase II, showing B-chromosome laggards (arrow), and I) metaphase of mitotic chromosomes (2*n* = 22) showing 4 B chromosomes (arrows); *Cousinia calcitrapa* var. *interrupta* - J) metaphase I, and K) anaphase I (*n* = 13); L) *Campanula perpusilla* metaphase I (*n* = 11). Scale bar 5µm.

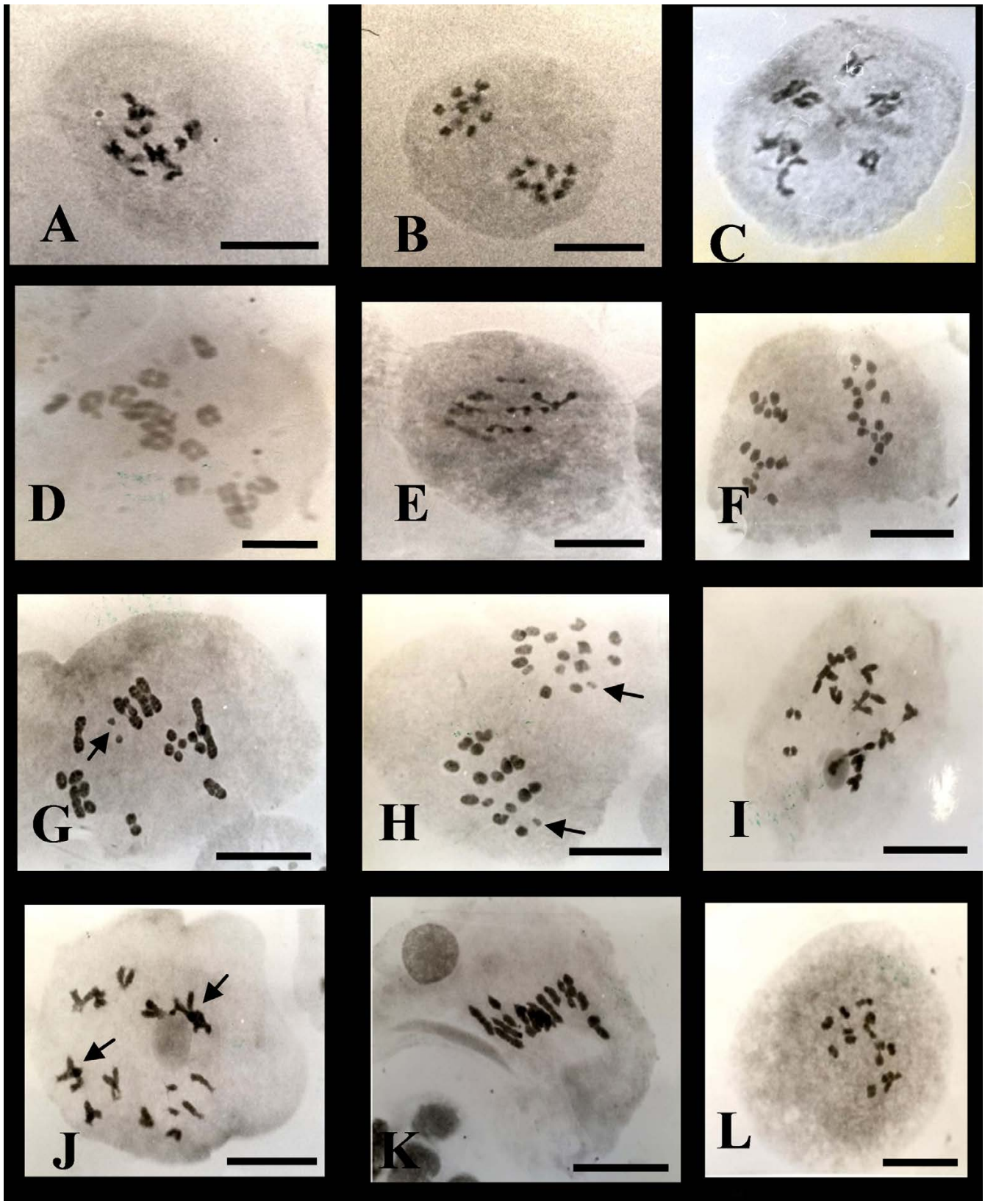

Fig. 2. *Anthochlamys multinervis* - A) diakinesis, and B) anaphase I (n = 9); C) *Euphorbia gedrosiaca* - diakinesis (n = 9); *Hedysarum criniferum* - D) metaphase of mitotic chromosomes ($2n$ = 16), and E) metaphase I (n = 8); *Acantholimon schahrudicum* - F) anaphase I (n = 15), G) metaphase I, showing one pair of B chromosomes (arrow), and H) metaphase II showing normal segregation of B chromosomes (arrows); *Acantholimon truncatum* - I) diakinesis, J) diakinesis showing tetravalent and hexavalent (arrows) and K) metaphase I (n = 15); L) *Hyocyamus orthocarpus* - metaphase I (n = 14). Scale bar 5µm.

all the taxa can be found in Iran and West Afghanistan (Hedge *et al.* 1997).

Anthochlamys multinervis is endemic to Iran and distributed in limited areas of the Semnan and Tehran provinces. Meiosis in this species was regular and showed nine bivalents at diakinesis and (9-9) segregation at anaphase I (Fig. 2A, B). This is the second chromosome number for the genus. The first chromosome count for the species *A. polygaloides* (Fisch. & C.A.Mey.) Fenzl. (*2n* = 18) was reported by Ghaffari *et al.* (2015). According to our data, this is the first chromosome number report for this species.

Euphorbiaceae

Euphorbia gedrosiaca Reach. f., Aellen & Esfand. *n* = 9 (Fig. 2C)

Tehran province: 60 km towards Qom, N 35°13'03.5", E 51°05'50.7", Ghaffari 2485

The countries with the highest diversity of *Euphorbia* taxa are Turkey (102 taxa), Iran (92 taxa), Syria (50 taxa), Pakistan and Yemen (45 taxa each), while the highest number of endemics occur in Iran (21 taxa), Turkey (12 taxa), Yemen (7 taxa), and Afghanistan (5 taxa) (Govaerts *et al.* 2000; Pahlevani *et al.* 2020). The genus *Euphorbia* has a wide range of chromosome numbers (Fedorov 1974; Goldblatt & Johnson 2006, 2010). Our specimen was diploid and showed 9 bivalents at diakinesis (Fig. 2C). According to current information, this is the first chromosome count for this species.

Fabaceae

Hedysarum criniferum Boiss. *2n* = 16 (Fig. 2D, E)

Hamadan province: between Nowbaran and Tajrak, N 34°51'19.3", E 48°13'49.6", Ghaffari 481

Rechinger (1984) classified 25 Iranian species of *Hedysarum* into four sections. Fifteen species among them are endemic to Iran. According to recent findings (Ranjbar 2010; Dehshiri & Goodarzi 2016; Nafisi *et al.* 2019), the number of *Hedysaum* species in Iran has increased to 38, of which 23 are endemic.

Hedysarum criniferum is endemic to Iran and belongs to the section *Crinifera*. The chromosome number at metaphase in this species was 2*n* = 16 based on *x* = 8 (Fig. 2D). The formula of the karyotype is 2*n* = 7m+1sm = 16. The lengths of the chromosomes pairs were 4.27, 3.76, 3.52, 3.52, 3.24, 3.22, 2.89 and 2.65 μm, respectively. Secondary constrictions were found on the short arm of the largest chromosome pair at early metaphase (the micrograph was not clear for all chromosomes). This count confirms the only previous one, from a population collected in Norwest Iran by Hesamzadeh-Hejazi & Ziaei Nasab (2007). Meiosis in this species showed eight bivalents at metaphase I, of which most were rod-shaped with a single chiasma (Fig. 2E). The gametic number (*n* = 8) is reported here for the first time. According to the literature, two basic numbers of *x* = 7 and *x* = 8 are assumed for the genus *Hedysarum* (Rani *et al.* 2014).

Plumbaginaceae

Acantholimon schahrudicum Bunge n=15+0-2B (Fig. 2F, G, H)

Tehran province: 60 km towards Qom, N 35°13'03.5", E 51°05'50.7", Ghaffari 1484

The genus *Acantholimon* comprising 22 species was first described by Boissier (1846). von Bunge (1872) increased the number of species to 83, 45 of which were reported from Iran. In his monograph, Mobayen (1964) recognized 119 *Acantholimon* species all around the world and 84 in Iran. In his treatment for the Flora of Iran Assadi (2005) subdivided the genus into 8 sections with 79 species of which 65 are endemic to the flora of Iran.

Acantholimon schahrudicum is endemic to Iran. Consistent with meiotic chromosome counts, an examination of more than 100 pollen mother cells revealed 15 bivalents, the majority of which were rod-shaped. Normal chromosome segregation (15-15) at anaphase I was observed (Fig. 2F). Also, 2 B chromosomes at metaphase I were observed in numerous cells (Fig. 2G). Bivalent B chromosomes showed normal segregation at metaphase II (Fig. 2H). According to the previous reports three diploid chromosome numbers of 2*n* = 28, 30, and 32 occur among the species of the genus *Acantholimon*, most of them being 2*n* = 30 (Nazarova 1984; Zakharjeva 1993; Daniella 1997; Moradpoor *et al.* 2010). According to our information, this is the first report of the chromosome number for this species and the presence of B chromosomes in the genus *Acantholimon*.

Acantholimon truncatum Bunge *n* = 15 (Fig. 2I, J, K)

Tehran province: 60 km towards Qom, N 35°13'03.5", E 51°05'50.7", Ghaffari 1585

This taxon is endemic to Iran. Meiosis in this taxon showed 15 bivalents at diakinesis and metaphase I (Fig. 2 I, K). Meiotic configurations in some cells at diakinesis showed an absence of pairing, characterized by the presence of univalent, or different associations of homologous chromosomes: tetravalent and hexavalent (Fig. 2J). To our knowledge, this is the first chromosome number report for this species.

Solanaceae

Hyoscyamus orthocarpus Schonbeck- Temesy *n* = 14 (Fig. 2L)

Khuzestan province: Ramhormoz, 3 km towards Iyzeh, N 31°18'17.0", E 49°37'08.2", Ghaffari 467

Hyoscyamus is a small herbaceous genus with 26 species all over the world (Yousaf *et al.* 2008). This genus comprises 12-19 species in Iran with a wide range of distribution.

According to Schonbeck-Temesy (1972), *H. orthocarpus* is endemic to Iran. However, in the flora of Iran, Khatamsaz (1998) believed this species to be synonymous with *H. insanus* Stocks. Meiosis in this species was regular and showed 14 bivalents at metaphase I (Fig. 2L). This is the first chromosome number report for this species.

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REZIME

Botanica SERBICA

Broj hromozoma i mejotičko ponašanje nekoliko biljnih vrsta iz Irana

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Prikazani su originalni mejotički ili i mejotički i mitotički brojevi hromozoma kod 10 endemičnih i neendemičnih vrsta biljaka iz devet familija sa područja Irana. Za taksone *Acantholimon schahrudicum*, *A. truncatum*, *Anthochlamys multinervis*, *Campanula perpusilla*, *Cousinia calcitrapa* var. *interrupta*, *Dorema ammoniacum*, *Euphorbia gedrosiaca*, i *Hyocyamus orthocarpus* broj hromozoma je dat prvi put. Broj hromozoma kod *Astrodaucus persicus* i *Hedysarum criniferum* se slaže sa predhodno utvrđenim. Brojevi gametskih hromozoma kod *Hedysarum criniferum* i *Allium stipitatum* su dati prvi put. Pojava akscesornih hromozoma je uočena kod *Acantholimon schahrudicum* i *Dorema ammoniacum*, što je prvi zapis o B hromozomu kod vrsta iz rodova *Acantholimon* i *Dorema*.

Ključne reči: kariologija, B hromozom, mitoza, mejoza, endemične biljke