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Chromosome counts of some species of wetland plants from Northwest Iran

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Abstract. Wetlands scatter as microclimates in mountain areas of Iran. In this investigation, the chromosome number of species and populations from Azerbaijan provinces were studied. After seed germination and root fixation, meristem cells were stained and photographs were taken for cells in the metaphase stage by light microscope. Data were analyzed by Micromeasure and excel softwares. As many as 28 populations of 24 species were studied. The chromosome numbers of two species (viz. *Ranunculus kotschyi*) were reported for the first time. 11 populations in 9 species were reported for the first time from Iranian populations (viz. *Alisma plantago-aquatica*, *Prunella vulgaris*, *Scrophularia umbrosa*). The range of haploid chromosome numbers is between n=6 and n=21. Ideograms were depicted for each species.

Keywords: chromosome number, wetland, ideogram, Azerbaijan.

INTRODUCTION

Wetlands are considerate as microclimates in alpine areas with special species in Iran. In the steppic mountain areas of Iran are a wide range of wetland vegetation. The wetland habitats are sharply embedded within vegetation of the IranoTuranian steppes that are more characteristic of this region and are of interest both in themselves and for wider comparison with EuroSiberian wetlands (Naqinezhad, 2012).

Most of the wetlands in the northwest part of Iran develop when snowmelt and rain fill the pockmarks left on the landscape by glaciers. This kind of wetlands was categorized in Prairie potholes class (EPA 2001).

In angiosperms, the haploid chromosome number varies between $n = 2$ and $n = 132$, but the majority of them show a range between $n = 7$ and $n = 12$(Sharma, 2009). Variation or constancy in the chromosome number within taxa of different categories has been proven to be important characters for taxonomic groupings (Sharma, 2009).

We studied on chromosome number of 28 accessions of 24 species belonging to 21 genera of 12 families wetland plants of Azerbaijan Mountains of Iran. The aim of this study was to investigate on chromosome number of Wetland plants of Azerbaijan provinces.

MATERIALS AND METHODS

Plant materials

Specimens and seeds were collected from natural habitats of Azerbaijan provinces (West, East Azerbaijan and Ardebil) in 2013 and 2014. Vouchers are deposited in TARI (Table 1). The plant samples were identified by Flora of Iran (Assadi *et al*., 2018) and Flora Iranica (Rechinger, 2005).

Chromosome counts

The seeds were used for chromosome counts. Seeds were germinated at 20-25°C on moist filter paper in Petri dishes. Root tips of seeds had grown 1–1.5 cm in length were stopped from the germinating by pretreating with *alpha-bromonaphthalene* for two hours. Then fixed with acetic alcohol (1:3) for 4 h and stored in 70% alcohol at 2–4°C. Then the root tips were rinsed in water and hydrolyzed with 1 N HCl for 10–18 min at 60°C and rinsed in running water for a minimum of 3–5 min. Staining of root tips was carried out for 1–2 h and root tips were put in hematoxylin for 5–10 min to improve staining. Finally, squash preparations were made. Chromosome nomenclature follows Levan *et al*. (1964) and Stebbins (1971). The photos were taken by light microscope (BH2 Olympus × 1000).

We measured the chromosomes using MicroMeasure (version 3.3 (Reeves and Tear, 2000).

RESULTS

Our goal in this project was to create a chromosome index for the wetland plants of Azerbaijan. It is so difficult to discussion about several species from several families that collected for common goal.

Table 1. List of species examined, Family, Locality, Altitude, Voucher number, and Habitat.

Voucher no.	Alt.	Locality	Family	Species
102715	2392	East Azerbaijan, Tabriz, Arshad chamani	Alismataceae	*Alisma plantago- aquatica* L.
102769	2281	West Azerbaijan, Salmas, Jam Valley	Poaceae	*Alopecurus arundinaceus* Poir.
102752	1900	Aligo Village	Brasicaceae	*Barbarea plantaginea* DC.
8228	----	East Azerbaijan, Bostanabad	Poaceae	*Catabrosa aquatica* (L.) P.Beauv.
102750	2266	Ardebil province, Salmas, Jam Valley	Asteraceae	*Erigeron acris* L. subsp. *pycnotrichus* (Vierh.) Rech. f.
101699	2266	West Azerbaijan, Dalamper Village	Geraniaceae	*Geranium sylvaticum* L.
102751	2266	West Azerbaijan, Dalamper Village	Hypericaceae	*Hypericum perforatum* L.
102807	2266	West Azerbaijan, Dalamper Village	Asteraceae	*Inula aucheriana* DC.
102762	1688	East Azerbaijan, Hashtroud, Zalbil	Asteraceae	*Mulgedium tataricum* (L.) DC.
8239	1819	East Azerbaijan, Hashtroud, Baboneh village	Poaceae	*Phragmites australis* Trin. ex Steud.
101691	2281	West Azerbaijan, Salmas, Jam Valley	Plantaginaceae	*Plantago atrata* Hoppe.
101601	2266	West Azerbaijan, Dalamper Village	Lamiaceae	*Prunella vulgaris* L. (Dalamper)
8242	----	East Azerbaijan, Bostanabad	Lamiaceae	*Prunella vulgaris* L. (Bostanabad)
101668	2296	Ardebil, Agh Ghabali	Rannunculaceae	*Ranunculus aquatilis* var. *diffusus* With.
101670		East Azerbaijan, Varzaghan	Rannunculaceae	*Ranunculus dolusus* Fisch . & C. A. Mey.
101674	1648	Ardebil province, Ardebil to Bolaghvar	Rannunculaceae	*Ranunculus kotschyi* Boiss.
102738	1900	East Azerbaijan, Bostanabad	Scrophulariaceae	*Scrophularia umbrosa* Dumort.
102805	1688	East Azerbaijan, Hashtroud, Zalbil	Asteraceae	*Senecio pseudoorientalis* Schischk. (Hashtroud)
102761	1648	Ardebil province, Ardebil to Bolaghvar	Asteraceae	*Senecio pseudoorientalis* Schischk.)Bolaghvar)
101688	2266	West Azerbaijan, Dalamper Village	Fabaceae	*Trifolium pratense* L.
102724	2281	West Azerbaijan, Salmas, Jam Valley	Juncaginaceae	*Triglochin maritima* L. (Jam valley(
102728	1650	Ardebil province, Ardebil to Bolaghvar	Juncaginaceae	*Triglochin maritima* L. (Bolaghvar)
102753	1900	East Azerbaijan, Bostanabad	Asteraceae	*Tripleurospermum disciforme* Sch.Bip. (Bostanabad)
102784	1900	East Azerbaijan, Tabriz, Arshad chamani	Asteraceae	*Tripleurospermum disciforme* Sch.Bip. (Jam valley)
102713	2392	East Azerbaijan, Tabriz, Arshad chamani	Scrophulariaceae	*Veronica orientalis* Miller (Arshad Chamani)
102716	2281	East Azerbaijan, Bostanabad	Scrophulariaceae	*Veronica orientalis* Miller (Bostanabad)
102729	2281	East Azerbaijan, Bostanabad	Scrophulariaceae	*Veronica filiformis* Sm.
101677	1650	Ardebil province, Ardebil to Bolaghvar	Fabaceae	*Vicia variabilis* Freyn & Sint. ex Freyn

Table 2. Chromosome counts of studied species previously described in the literature.

References (viz.)	2*n*	References	N	Species
(Dobes *et al.,* 1997)(Wulff, 1939)(Love and Love., 1942)	10, 12, 14	(Kaur et al., 2011)	14	*Alisma plantago- aquatica*
(Kuzmanov, 1993)(Amosova *et al.,* 2019)(Sheidai *et al.,* 2009)	28, 42	(Koull and Gohil 1991)	14	*Alopecurus arundinaceus*
(Astanova, 1999)(Ørgaard and Linde-laursen, 2014)	16	(Aryavand, 1977) (Ghaffari, 2007)	8	*Barbarea plantaginea*
(Sawicka, 1991)(Lövkvist and Hultgård., 1999)(Sheidai *et al.,* 2009)	20			*Catabrosa aquatica.*
(Kaur *et al.,* 2011)(Bala and Gupta, 2013)(Paule *et al.*, 2017)	18			*Erigeron acris* (other subspecies)
(Petrova and Stanimirova, 2001) (Lövkvist and Hultgård 1999)(Dmitrieva, 1986)	28, 24	(Clifford Odets, 1954)	12	*Geranium sylvaticum*
(Ciccarelli *et. al.* 2001)(Lövkvist and Hultgård, 1999)(Krasnikov and Schaulo 1990)(Kalinka *et al.*, 2014((Baltisberger and Widmer, 2009((Brutovska et al., 2000)	32	(Ghaffari, 2006)	16	*Hypericum perforatum*
		(Chehregani and Hajisadeghian, 2009)	9	*Inula aucheriana*
(Probatova, 2004)	36			*Mulgedium tataricum*
(Lövkvist and Hultgård 1999)(Panahi., 1979)(Gervais *et al.*, 1993)	42,48, 72,96			*Phragmites australis*
(Petrova and Stanimirova, 2001)(Lessani and Chariat-panahi., 1979)	12, 24			*Plantago atrata*
(Lövkvist and Hultgård 1999((Krasnikov and Schaulo., 1990)	28			Prunella vulgaris
(Dahlgren and Cronberg, 1996)	32,48			*Ranunculus aquatilis* var. *diffusus*
(Baltisberger, 1991) (Agapova, 1981)(Assadi, 1989) (Ghasemi *et al.*, 2015)	28, 32			*Ranunculus dolusus*
Not reported				*Ranunculus kotschyi*
(Javurkova, 1979)(Grau, 1979)(Vitek *et al.* 1992)	26,52	(Grau, 1979)	13, 26	*Scrophularia umbrosa*
		(Ghaffari, 1999)	20	*Senecio pseudoorientalis*
(Probatova, 2000) (Krasnikov and Schaulo, 1990)(Zhang *et al.,* 1993)(Sheidai *et al.,* 1998)	14, 16			*Trifolium pratense*
(Lövkvist and Hultgård. 1999)(Krasnikov, 1991)) Rotreklova, 2004((Uchiyama, 1989)(Iwatsubo *et al.*, 1998)	48,120			*Triglochin maritima*
(Ghaffari, 1999) (Hayirlioglu-Ayaz, 2011)	18	(Ghaffari, 1999) (Razaq, et al., 1994)	9	Tripleurospermum disciforme
(Ghaffari, 1986)	32	(Ghaffari, 1987)	32	Veronica orientalis
(Pogan *et al.*, 1990)(Dzhus and Dmitrieva, 2001)(Albach *et al.*, 2009)	14	(Dobes and Vitek, 2000)	7	*Veronica filiformis*
(Hesamzadeh Hejazi and Rasuli, 2006)(Hesamzadeh Hajazi and Ziaei Nasab, 2009)	14			*Vicia variabilis*

Of 24 examined species (Table 2), 5 species have two populations (*Prunella vulgaris, Tripleurospermum disciforme*, *Pedicularis sibthorpii*, *Senecio pseudoorientalis* and *Veronica orientalis*). Another species has just one population. Asteraceae (5 species), Scrophulariaceae (3 species), Poaceae and Ranunculaceae (3 species) families have the most species respectively.

The chromosomes counts of the taxa previously reported are in Table 2. Most of them confirm our results.

Chromosome numbers of *Ranunculus kotschyi* (Fig. 2P) and *Erigeron acris* subsp. *pycnotrichus* (Fig. 1F) were reported for the first time. *Alisma plantago-aquatica* (Fig. 1A), *Prunella vulgaris* (Fig. 2M & N), *Scrophularia umbrosa* (Fig. 2Q), *Mulgedium tataricum* (Fig. 1J), *Ranunculus aquatilis* var. *diffusus* (Fig.1D), *Erigeron acris* subsp. *pycnotrichus* (Fig.1F), *Geranium sylvaticum* (Fig.1G), *Triglochin maritima* (Fig.3U&V), *Veronica filiformis* (Fig. 3Y) were reported for the first time of Iranian populations. Sporophytic count of *Barbarea plantaginea* (Fig. 1C) was reported for the first time from Iran.

The details of each taxon reported as in Table 3.

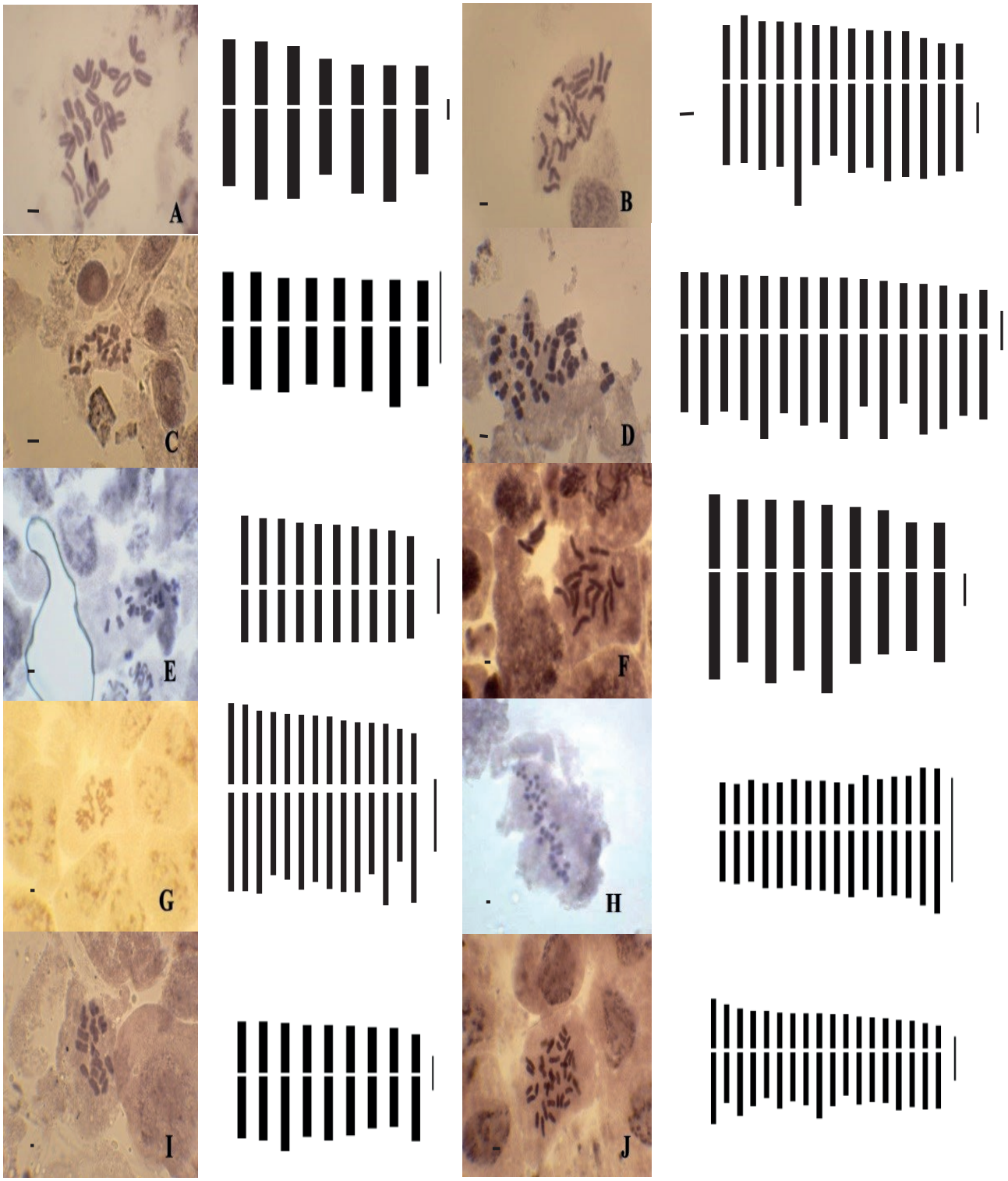

Figure 1. Somatic metaphase chromosome, Ideogram of each population is on the right side of the image: A: *Alisma plantago- aquatica* (2*n* = 14); B: *Alopecurus arundinaceus* (2*n* = 28); C: *Barbarea plantaginea* (2*n* = 16); D: *Ranunculus aquatilis* var. *diffusus* (2*n* = 32); E: *Catabrosa aquatica* (2*n* = 20); F: *Erigeron acris* subsp. *pycnotrichus* (2*n* = 18); G: *Geranium sylvaticum* (2*n* = 28); H: *Hypericum perforatum* (2*n* = 32); I: *Inula aucheriana* (2*n* = 18); J: *Mulgedium tataricum* (2*n* = 36). Scale bar: 1 μm.

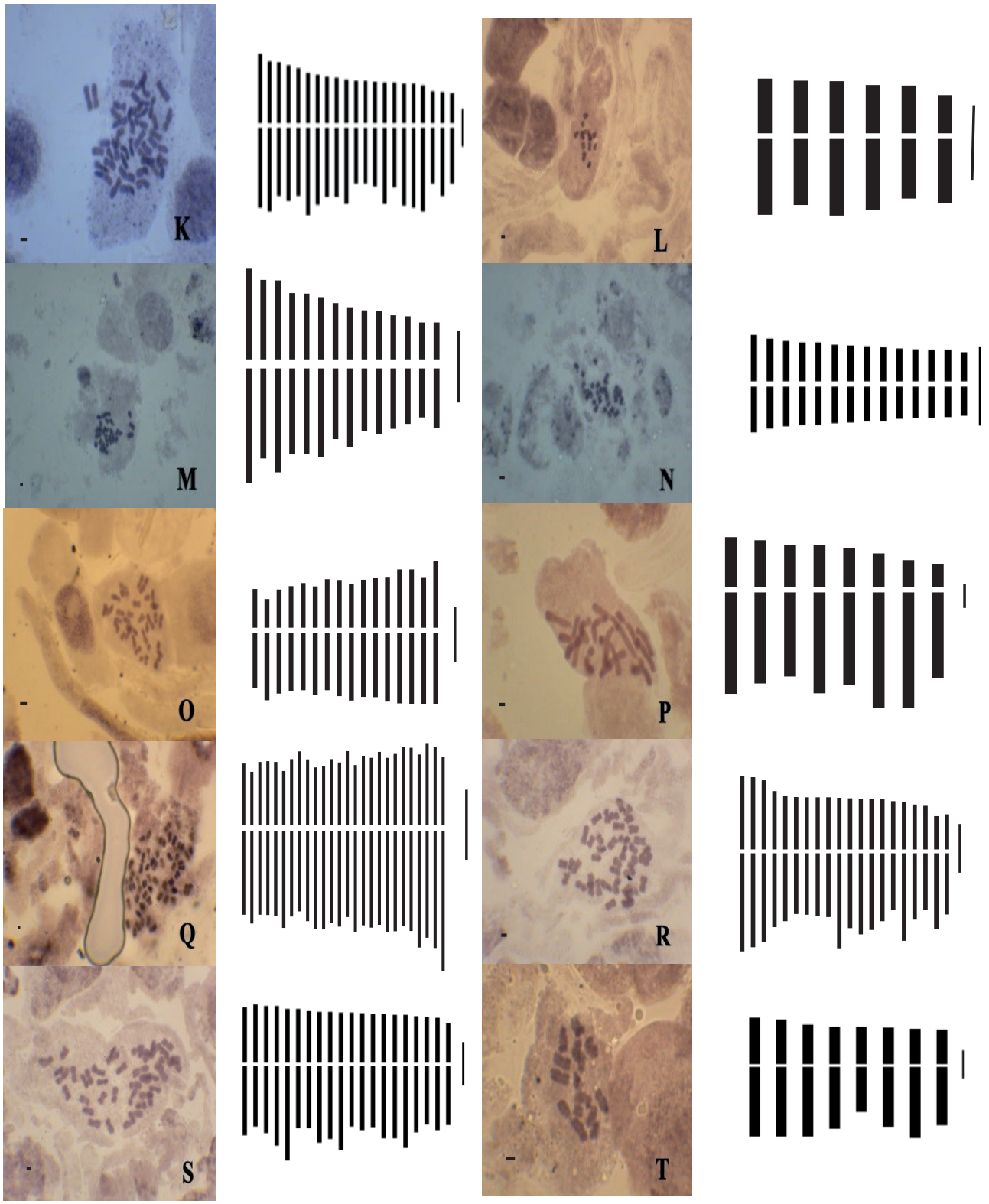

Figure 2. Somatic metaphase chromosome, Ideogram of each population is on the right side of the image: K: *Phragmites australis* (2*n* = 42); L: *Plantago atrata* (2*n* = 12); M: *Prunella vulgaris* (Dalamper) (2*n* = 28); N: *Prunella vulgaris* (Bostanabad) (2*n* = 28); O: *Ranunculus dolusus* (2*n* = 32); P: *Ranunculus kotschyi* (2*n* = 16); Q: *Scrophularia umbrosa* (2*n* = 52); R: *Senecio pseudoorientalis* (Hashtroud) (2*n* = 40); S: *Senecio pseudoorientalis*)Bolaghvar) (2*n* = 40); T: *Trifolium pratense* (2*n* = 16). Scale bar: 1 µm.

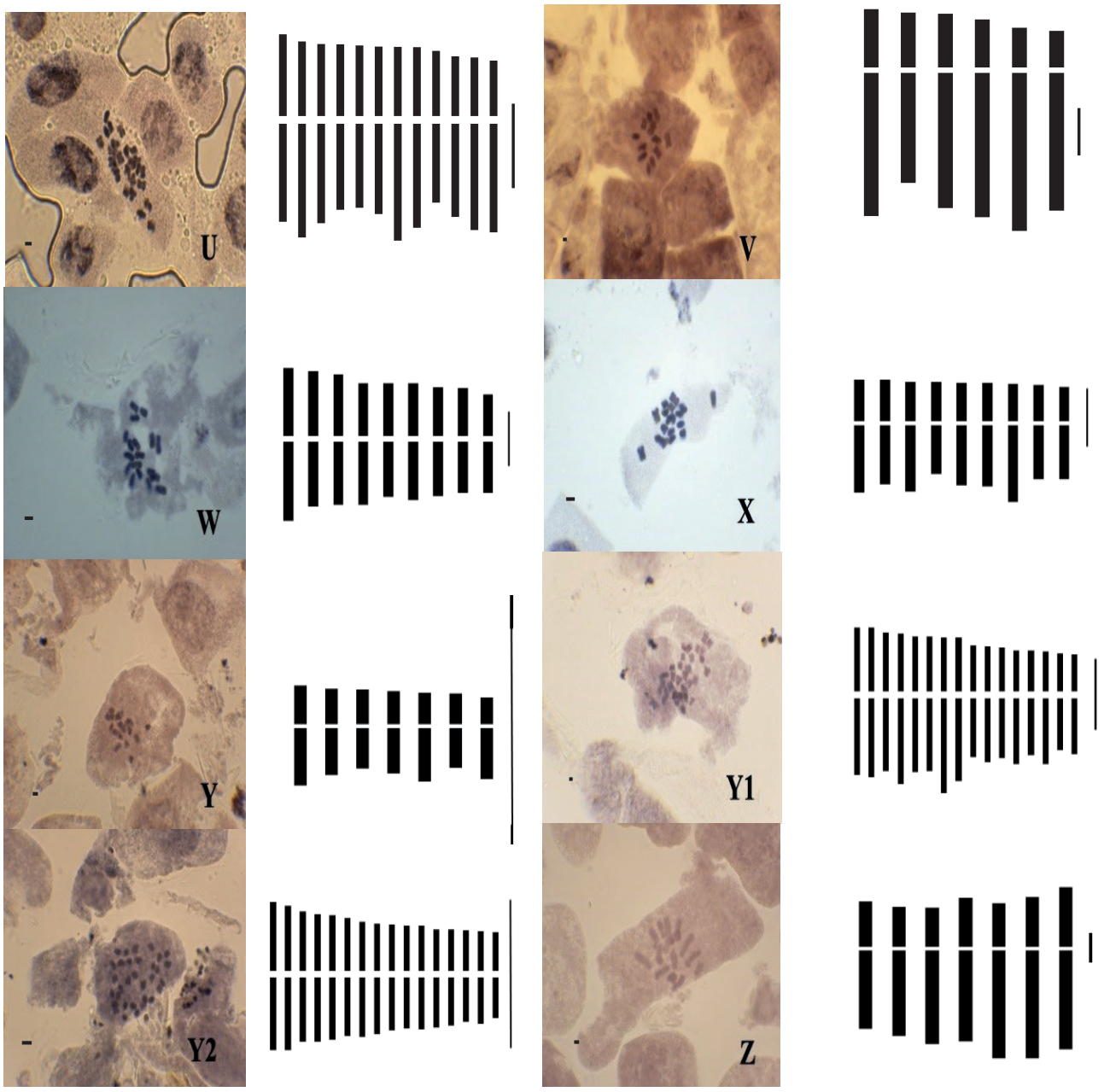

Figure 3. Somatic metaphase chromosome, Ideogram of each population is on the right side of the image: U: *Triglochin maritima* (Jam valley) (2*n* = 24); V: *Triglochin maritima* (Bolaghvar) (2*n* = 12); W: *Tripleurospermum disciforme* (Bostanabad). (2*n* = 18); X: *Tripleurospermum disciforme* (Jam valley((2*n* = 18); Y: *Veronica filiformis* (2*n* = 14); Y1: *Veronica orientalis* (Arshad Chamani) (2*n* = 32); Y2: *Veronica orientalis* (Bostanabad) (2*n* = 32); Z: *Vicia variabilis* (2*n* = 14). Scale bar: 1 µm.

Somatic metaphase and Ideograms of taxa were shown (Figs 1-3).

DISCUSSION

Only some interesting results are discussed here.

The chromosome number of the most of the species support by former studies (Table 2). The variation ranges of haploid chromosome numbers in studied species are between *n*=6 and *n*=21.

Populations in *Triglochin maritima* (Fig. 3U & V) comprise different ploidy levels (2*n* = 24, 12). Some species like *Prunella vulgaris* (Fig. 2M & N), *Veronica ori-*

Table 3. Somatic chromosome number (2n), Ploidy level, karyotype formula, ranges of chromosome length and degree of Asymmetry according to STEBBINS (1971) for the studied taxa, Figure numbers are related images in Fig 1

Figure number (Figs.1-3)	Stebbins	Chromosome range length (μm)	Karyotype formula	Ploidy level	x	2*n*	Taxa
A	B1	5.3-7.83	2sm+5m	2	7	14	*Alisma plantago- aquatica*
B	A2	4.1-5.95	8sm+6m	4	7	28	*Alopecurus arundinaceus*
C	A1	1.06-1.29	2sm+6m	2	8	16	*Barbarea plantaginea*
E	A1	2.25-3.09	10m	4	5	20	*Catabrosa aquatica*
F	A1	3.44-5.10	3sm+6m	2	9	18	*Erigeron acris* subsp. *pycnotrichus*
G	A1	1.51-2.19	2sm+12m	2	14	28	*Geranium sylvaticum*
H	A1	0.92-1.37	16m	4	8	32	*Hypericum perforatum*
I	A1	2.91-3.8	9m	*2*	9	18	*Inula aucheriana*
J	A2	2.14-3.30	5sm+13m	4	9	36	*Mulgedium tataricum*
K	A2	2.8-4.89	10sm+11m	4	An.	42	*Phragmites australis*
L	A1		6m	2	6	12	*Plantago atrata*
M	B1	0.95-2.28	14m	2	14	28	*Prunella vulgaris* (Dalamper)
N	A1	0.7-1.11	14m	2	14	28	*Prunella vulgaris (*Bostanabad)
D	A2	2.01-3.31	9sm+7m	4	8	32	*Ranunculus aquatilis* var. *diffusus*
O	A2	1.89-2.87a	4sm+12m	4	8	32	*Ranunculus dolusus*
P	A3	3.67-5.38	5sm+3st	2	8	16	*Ranunculus kotschyi*
Q	A1	1.51-3.15	4sm+22m	4	13	52	*Scrophularia umbrosa*
R	A1	2.41-4.31	4sm+16m	4	10	40	*Senecio pseudoorientalis* (Hashtroud)
S	A2	2.6-3.66	2sm+18m	4	10	40	*Senecio pseudoorientalis*)Bolaghvar)
T	A1	3.41-4.80	2sm+6m	2	8	16	*Trifolium pratense*
U	A1	1.88-2.46	3sm+9m	4	6	24	*Triglochin maritima* (Jam valley)
V	A3	4.23-5.16	3sm+3st	2	6	12	*Triglochin maritima* (Bolaghvar)
W	A1	2.14-2.7	9m	2	8	18	*Tripleurospermum disciforme* (Bostanabad).
X	B1	1.81-2.86	9m	2	9	18	*Tripleurospermum disciforme* (Jam valley(
Y1	A1	0.73-1.31	16m	4	8	32	*Veronica orientalis* (Arshad Chamani)
Y2	A1	1.38-2.19	16m	4	8	32	*Veronica orientalis* (Bostanabad)
Y	A1	0.28-0.48	1sm+6m	2	7	14	Veronica filiformis
Z	A3	1.66-2.37	3sm+4m	2	7	14	*Vicia variabilis*

An.= Aneuploidy.

entalis (Fig. 3Y1 & Y2), *Senecio pseudoorientalis* (Fig. 2R & S), *Tripleurospermum disciforme* (Fig. 3W & X) have invariant ploidy level in this study.

Alisma plantago-aquatica n = 7 (Fig. 1A), is not particularly variable; nevertheless three different chromosome numbers have been found for it viz. *n* = 5 (Wulff, 1939), 6 (Liehr, 1916; Love and Love, 1942) and 7 (Love and Love, 1942).

Combining the available chromosome studies, the *Erigeron* has relatively consistent chromosome diversification with a basic number of 9, and most species contain diploid individuals, which suggested *Erigeron* at the initial phase of polyploid diversification (Baldwin and Speese, 1955).

Phragmatis australis is represented by many polyploids, cuploids from 3x = 36 to 8x = 96 (without 5 x) and aneuploidies (2*n* = 42, 44, 46, 49, 50, 51, 52, 54). Tetraploidy and octoploidy are in majority (Gorenflot, 1979). Chromosome numbers of *Phragmatis australis* showed a high degree of aneuploidy and varied between 2*n*=42 and 2*n*=59 (Gervais *et al*., 1993). The degree of polyploidy is not in direct relation with the individual's habitus. Meiosis study shows that this complex has already passed the maturity, the diploid forms having disappeared (Gorenflot, 1979). Base on this study our case is aneuploidy.

Hypericum perforatum has the smallest chromosomes than other species of this genus. In other studies it had 2n=32 with median centromeric chromosomes as our result (Brutovska *et al*., 2000).

Most of the subgenera of *Veronic*a exhibit only one single basic number, i.e., x = 6, 7, 8, 9, 12, 17, or 20/21.

In this genus, the putative ancestral base number of 9 has been reduced several times to 8 and 7, respectively (aneuploidy/dysploidy), often associated with transition to annual life history. In contrast, no unambiguous increase of chromosome base number has been inferred (Albach *et al*., 2008). A base chromosome number reduction to x = 8 (aneuploidy), seems to have occurred in *Veronica orientalis* (tetraploid, 2*n*=4x=32*).* Previous results confirm our result (Ghaffari, 1986).

Basic chromosome number(x)

The frequency of 2x (53.57%) and 4x (46.43%) in this study are almost identical. 6x and more another nx not found. 2x and 4x are most common in flowering plants (Bala and Gupta, 2013). The present result is in agreement with the reports of earlier investigators.

Polyploidy and habit

The overall chromosome numbers during present study lie on two different levels of ploidy i.e. 2x, 4x. Among these, the diploids are the most common in terms of frequency (53.57 %), followed by tetraploids (46.43 %) (Table 3).

As reported at least 47% of species have undergone a recent polyploidy event (Wood *et al*., 2009)polyploidy has been recognized as an important phenomenon in vascular plants, and several lines of evidence indicate that most, if not all, plant species ultimately have a polyploid ancestry. However, previous estimates of the frequency of polyploid speciation suggest that the formation and establishment of neopolyploid species is rare. By combining information from the botanical community's vast cytogenetic and phylogenetic databases, we establish that 15% of angiosperm and 31% of fern speciation events are accompanied by ploidy increase. These frequency estimates are higher by a factor of four than earlier estimates and lead to a standing incidence of polyploid species within genera of 35% (n = 1,506. As Ramsey and Ramsey,(2014) indicated that; Polyploids are able to colonize larger geographic ranges and/or occur in more habitats than related diploids, similar picture occurred in our study: those species with higher ploidy level have wide spread geographical distribution than their related diploid populations or species. For example in Iran, some species such as; *Alopecurus arundinaceus, Phragmites australis, Catabrosa aquatica, Hypericum perforatum, Veronica orientalis* with higher polyploidy level showed same attitudes (Table 3) (Alinejad *et al*., 2017; Ghahremaninejad *et al*.; 2012, Khanhasani *et al*., 2021; Safikhani *et al*., 2018; Aref Tabad *et al*., 2016; Jalili *et al*., 2014)

Karyotype

Metacentric and submetacentric are commonly observed. Karyotype of ten species (35.7%) consists of chromosomes with the centromere in median regions (m). 14 species (50%) have metacentric and submetacentric chromosomes in their formula. Only 2 species have karyotypes with *submetacentric* and *subtelocentric chromosomes*. Karyotypes of most species (78.58%) were classified in the 1A and 2A Stebbins classes (Table 3).

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