Karyotype analysis and karyological relationships of Turkish *Bunium* species (Apiaceae)

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Abstract: Chromosomal data and karyological relationships provide valuable information about karyotype evolution and speciation. For the genus *Bunium*, the chromosomal data are limited. In the present study, the chromosomal data of 10 taxa are provided, 6 of which are given for the first time, 2 present new chromosome numbers, and 2 agree with previous reports. Four different chromosome numbers (2*n*=18, 20, 22 and 40) were detected, and 2*n*=40 is a new number in the genus *Bunium*. *B. brachyactis* is the first polyploid species of the genus with a ploidy level of 4*x.* The most asymmetric karyotypes are those of *B. pinnatifolium* and *B. sayae.* Regarding karyological relationships, *B. pinnatifolium* forms a monophyletic group by quite different karyological features such as large chromosomes, more submedian chromosomes and the most asymmetric karyotypes. In addition, the other 5 taxa form a strong monophyletic group. *B. verruculosum* and *B. ferulaceum* are cytotaxonomically very close species, as are *B. sayae* and *B. elegans* var. *elegans*. The chromosome numbers of 2 Turkish species, *B. nudum* and *B. sivasicum*, remain unknown. The presented results provide important contributions to the cytotaxonomy of *Bunium*.

Keywords: *Bunium*; chromosome; karyotype asymmetry; Mediterranean Basin; polyploidy

INTRODUCTION

Metric and meristic characters in some cases are inadequate in the classification of species, subspecies and isolated groups that are similar and morphologically indistinguishable in systematic studies. Systematic studies are supported by cytogenetic and molecular studies in order to overcome these deficiencies. Therefore, chromosomal data remain important parameters that support morphological data. The major chromosomal data are the basic number (*x*), the diploid number (2*n*) and the total chromosome length. These data can be alterable numerically with aneuploidy or polyploidy, but also structurally with deletion, inversion and translocation. All these mechanisms generate karyotypic variations by affecting the centromere position, which changes the intra- and interchromosomal asymmetry [1-4]. Speciation and diversification could be the result of karyotypic variations. The families and genera of higher plants are important systems for determining speciation and diversification.

Apiaceae is a cosmopolitan family represented by approximately 434 genera and 3780 species. Although a cosmopolitan family, it has a more intensive distribution in the subtropical climate zone and northern temperate regions [5]. *Bunium* L. is placed in the subfamily Apioideae in the family Apiaceae. The genus consists of about 30 species worldwide and spreads in Europe, West, South and South-East Asia, North and North-West Africa, which are temperate zones of the northern hemisphere. In addition, *B. bulbocastanum* L. is known from North America. The main center of *Bunium* diversity is the Mediterranean Basin, and in particular Turkey, which is a major center with 15 species including 7 endemics. In *Bunium*, uncommon characters for Apiaceae are useful at infrageneric levels. These characters are diploidy by variable basic numbers

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(from 6 to 11), and pseudomonocotyly in seedlings and embryos [6-8]. From this perspective, *Bunium* is an important model to examine chromosomal and karyotypic variations.

A cytotaxonomic study reported the somatic chromosome numbers and chromosomal properties of 23 *Bunium* species [9], with some species belonging to the genus *Elwendia* Boiss. The chromosome numbers of Turkish *Bunium* are 2*n*=17, 18 in *B. elegans* (Fenzl) Freyn; 2*n*=18 in *B. simplex* (C. Koch) Kljuykov; 2*n*=20 in *B. paucifolium* DC, *B. cylindricum* Drude, *B. ferulaceum* Sibt. & Sm. and *B. microcarpum* (Boiss.) Freyn & Bornm. ex Freyn; and 2*n*=22 in *Elwendia caroides* Boiss. (=*B. caroides* Hausskn. ex Bornm.) and *B. pinnatifolium* Kljuykov [9-14].

The karyological data of most Turkish *Bunium* have not yet been described in detail. In the present study, we provide a contribution to the cytotaxonomy and interspecific relationships of the genus *Bunium*.

MATERIALS AND METHODS

Plant material

The distribution map of *Bunium* taxa is given in Supplementary Fig. S1. Collection details are as follows: *Bunium allioides* B. Bani, Pimenov & Adıgüzel. Adana: Tufanbeyli, Güzelim village, Kumlupınar location, 1432 m a.s.l., 14.08.2016, [M. Çelik 478]; *Bunium brachyactis* (Post) H.Wolff. Kahramanmaraş: Ahır Mountain, Çallıbalma location, 1908 m a.s.l., 10.07.2015, [M. Çelik 423 et al.]; *Bunium elegans* var. *elegans*. Kayseri: Sarız, 3 km west of Yeşilkent, 1529 m a.s.l., 10.07.2015, [M. Çelik 434 et al.]; *Bunium elegans* var. *brevipes* Freyn & Sint. Van: Başkale, Çaldıran village, Muz Tepesi location, 2045 m a.s.l., 29.06.2015, [M. Çelik 364 et al.]; *Bunium ferulaceum*. Denizli: Honaz, 2 km from Karateke to Honaz, 502 m a.s.l., 13.06.2015, [M. Çelik 278 et al.]; *Bunium microcarpum* subsp. *bourgaei* (Boiss.) Hedge & Lamond. Rize: İkizdere, Cimil, between Ortaköy and Başköy, 1960 m a.s.l., 12.08.2015, [M. Çelik 480]; *Bunium pestalozzae* Boiss. Kayseri: Yahyalı, from Çubukharmanı village to mountain road, 1746 m a.s.l., 09.07.2015, [M. Çelik 401 et al.]; *Bunium pinnatifolium* Mersin: Silifke, 2 km from Paslı castle, 474 m a.s.l., 12.07.2015, [M. Çelik 407 et al.]; *Bunium sayae* Yıld. Hakkari: between Depin and Çimenli, Zap valley, 1165 m a.s.l., 28.06.2015, [M. Çelik 312 et al.]; *Bunium verruculosum* C.C. Towns. Malatya: between Erkenek and Doğanşehir, 1492 m a.s.l., 19.06.2017, [M. Çelik 501 & Ö.Çetin]. The specimens were deposited in Selçuk University Konya Herbarium (KNYA).

Cytogenetic procedure

Chromosome spreads were made from root tips pretreated with α-mono-bromonaphthalene at 4°C for 16 h, fixed in fixative solution containing absolute alcohol:glacial acetic acid (3:1, v:v) for 24 h, and stored in ethanol (70%) at 4°C until use. The roots were hydrolyzed in 1 N HCl at 60°C for 10-12 min and stained in 2% aceto-orcein. Preparations were made by the squash method [15-16].

Karyotype analysis

At least 10 metaphase plates were analyzed by Software Image Analyses (Bs200ProP). The following abbreviations and formulae were evaluated to characterize the chromosomes: LA – long arm, SA – short arm, LA+SA – total length, LA/SA – arm ratio, [(SA)/(LA+SA)×100] –the centromeric index, THL – total haploid length, RL=[(LA+SA)×100/THL] – relative length, and MCL – mean chromosome length. The karyotype formulae were determined by chromosome morphology based on centromere position according to the Levan classification [17]. The ideograms were drawn based on chromosome lengths.

Karyological relationships and karyotype asymmetry

The following parameters were evaluated to characterize the karyological relationships: *x* – basic chromosome number; 2*n* – diploid chromosome number; PL – ploidy level; KF – karyotype formula; THL – total haploid length; M_{CA}=[mean(L–S)/(L+S)]×100 – mean centromeric asymmetry [18]; CV_{CL}=(S_{CL}/X_{CL})×100 – coefficient of variation of chromosome length [19], and S/A_I=[(1×M)+(2×SM)+(3×ST)+(4×T)]/2*n* – symmetry/asymmetry index [20]. The meanings of the abbreviations are as follows: L (total length of long arms), S (total length of short arms), S_{CL} (standard deviation), X_{CL} (mean chromosome length in a chromosome set),

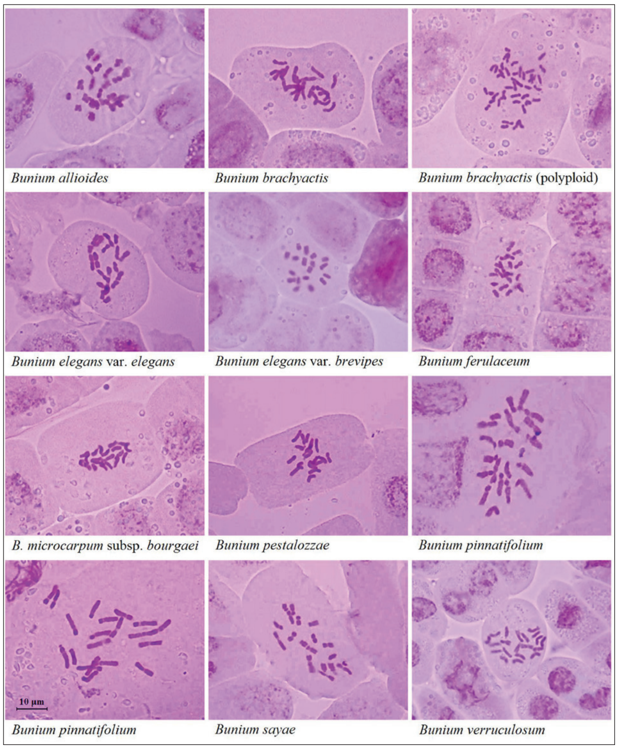

Fig. 1. Somatic metaphase chromosomes of the genus *Bunium*.

M (median chromosome number), SM (submedian chromosome number), ST (subtelocentric chromosome number), and T (telocentric chromosome number). A scatter diagram was drawn between M_{CA} and CV_{CL}. A dendrogram showing karyological relationships was drawn using bootstrap values (BV) with UPGMA software. The dendrogram contains 6 *Bunium* taxa with detailed chromosomal data and *Carum carvi* L. as a sister outgroup.

RESULTS

Chromosomal data

The chromosomal data of 10 taxa are provided (Fig. 1), 6 of which are given for the first time, 2 present new chromosome numbers, and 2 agree with previous reports (*B. ferulaceum* and *B. microcarpum* subsp. *bourgaei*). Table 1 presents the chromosome numbers of present and previous studies. Four different chromosome numbers (2*n*=18, 20, 22 and 40) were detected

Table 1. Chromosome counts of the investigated species in this and previous studies.

Taxa (alphabetically)	Previous results (2*n*) *x*: basic number;	References	Present Results (2*n*)+ Karyotype Formula	Explanation
B. allioides			*x*=11 2*n*=22	First report
B. brachyactis			*x*=10 2*n*=20, 40	First report Polyploidy
B. elegans var. *elegans*	*x*=9 2*n*=17, 18	[9, 11, 13]	*x*=10 2*n*=20=18m + 2sm	New count
B. elegans var. *brevipes*			*x*=9 2*n*=18=18m	First report
B. ferulaceum	*x*=10 2*n*=20	[10, 14]	*x*=10 2*n*=20=20m	Equal count
B. microcarpum subsp. *bourgaei*	*x*=10 2*n*=20	[9, 12]	*x*=10 2*n*=20	Equal count
B. pestalozzae			*x*=10 2*n*=20	First report
B. pinnatifolium	*x*=11 2*n*=22	[12]	*x*=10 2*n*=20=6m + 14sm	New count
B. sayae			*x*=10 2*n*=20=16m + 4sm	First report
B. verruculosum			*x*=10 2*n*=20=20m	First report
B. paucifolium	*x*=10 2*n*=20	[9]		no grown fruit
B. simplex	*x*=9 2*n*=18	[9]		not germinated
B. nudum				not collected
B. sivasicum				not germinated

Table 2. The detailed chromosome measurements of *Bunium* taxa.

Taxa	Pair	L + S	L	S	L / S	RL %	CI %	Type
B. elegans var. *elegans*	1	6.53	3.62	2.91	1.24	16.69	44.56	median
	2	5.61	3.10	2.50	1.24	14.34	44.64	median
	3	4.79	2.53	2.26	1.12	12.25	47.18	median
	4	4.25	2.38	1.87	1.28	10.87	44.00	median
	5	3.61	2.21	1.41	1.57	9.24	38.95	median
	6	3.27	1.89	1.38	1.36	8.35	42.20	median
	7	3.10	1.98	1.12	1.78	7.93	36.13	submedian
	8	2.95	1.60	1.35	1.19	7.55	45.76	median
	9	2.67	1.53	1.14	1.35	6.83	42.70	median
	10	2.33	1.22	1.11	1.10	5.95	47.64	median
B. elegans var. *brevipes*	1	3.49	1.91	1.58	1.21	13.86	45.27	median
	2	3.22	1.84	1.38	1.33	12.79	42.86	median
	3	3.10	1.73	1.37	1.26	12.31	44.19	median
	4	2.95	1.55	1.40	1.11	11.72	47.46	median
	5	2.76	1.60	1.16	1.38	10.96	42.03	median
	6	2.67	1.46	1.21	1.20	10.60	45.32	median
	7	2.58	1.48	1.10	1.34	10.24	42.64	median
	8	2.32	1.38	0.94	1.47	9.22	40.52	median
	9	2.09	1.28	0.81	1.58	8.30	38.76	median
B. ferulaceum	1	4.55	2.62	1.93	1.35	15.05	42.42	median
	2	3.73	2.21	1.52	1.46	12.35	40.75	median
	3	3.49	1.82	1.67	1.09	11.55	47.85	median
	4	3.35	1.91	1.45	1.32	11.09	43.15	median
	5	3.05	1.87	1.19	1.57	10.09	38.89	median
	6	2.85	1.66	1.19	1.39	9.42	41.75	median
	7	2.66	1.45	1.21	1.20	8.78	45.49	median
	8	2.43	1.46	0.97	1.51	8.04	39.92	median
	9	2.19	1.21	0.98	1.23	7.23	44.75	median
	10	1.93	1.07	0.87	1.23	6.40	44.85	median
B. pinnatifolium	1	10.30	7.27	3.03	2.40	14.36	29.42	submedian
	2^{sat}	8.69	5.66	3.03	1.87	12.11	34.87	submedian
	3	8.11	4.90	3.21	1.53	11.31	39.58	median
	4	7.60	4.51	3.09	1.46	10.60	40.66	median
	5	7.40	5.04	2.36	2.14	10.32	31.89	submedian
	6	7.08	4.87	2.21	2.20	9.87	31.21	submedian
	7	6.38	4.03	2.35	1.71	8.89	36.83	submedian
	8	5.97	3.93	2.04	1.93	8.32	34.17	submedian
	9	5.56	3.57	1.99	1.79	7.75	35.79	submedian
	10	5.04	2.62	2.02	1.27	6.47	43.53	median
B. sayae	1	6.72	4.05	2.67	1.52	17.73	39.73	median
	2	5.10	3.30	1.80	1.84	13.47	35.29	submedian
	3	4.35	2.45	1.90	1.29	11.48	43.68	median
	4	4.07	2.62	1.46	1.80	10.75	35.78	submedian
	5	3.80	2.21	1.58	1.40	10.03	41.69	median
	6	3.42	1.88	1.54	1.23	9.02	45.03	median
	7	3.24	1.96	1.28	1.53	8.54	39.51	median
	8	2.94	1.84	1.10	1.67	7.75	37.41	median
	9	2.46	1.39	1.07	1.30	6.49	43.50	median
	10	1.80	1.03	0.77	1.35	4.75	42.78	median
B. verruculosum	1	4.92	2.95	1.97	1.50	12.85	40.04	median
	2	4.38	2.55	1.82	1.40	11.44	41.65	median
	3	4.24	2.27	1.97	1.15	11.07	46.46	median
	4	4.06	2.20	1.86	1.18	10.61	45.81	median
	5	3.80	2.06	1.73	1.19	9.93	45.65	median
	6	3.75	2.19	1.57	1.40	9.82	41.76	median
	7	3.66	1.92	1.73	1.11	9.56	47.40	median
	8	3.52	1.93	1.59	1.21	9.21	45.17	median
	9	3.06	1.79	1.27	1.41	7.99	41.50	median
	10	2.88	1.75	1.30	1.55	7.53	42.62	median

The lengths (μm) of total (L + S), long arm (L), and short arm (S), arm ratio (L/S), relative length (RL); centromeric index (CI).

and 2*n*=40 is a new number in the genus *Bunium*. Six taxa have only median and submedian chromosomes (Table 2). The smallest chromosome is 1.80 μm in *B. sayae*. The largest chromosome is 10.30 μm in *B. pinnatifolium*. The smallest total haploid length is 25.18 μm in *B. elegans* var. *brevipes*, and the highest value is 71.73 μm in *B. pinnatifolium* (Table 3).

Basic numbers, ploidy levels and polyploidy

In the genus *Bunium*, common basic numbers are *x*=9, 10 and 11. In the present study, the basic numbers are *x*=9 in *B. elegans* var. *brevipes*, ploidy level 2*x*; *x*=10 in 8 taxa, ploidy levels 2*x* and 4*x*; *x*=11 in *B. allioides*, ploidy level 2*x*. The monoploid ideograms are generated by *x*=9 and 10 (Fig. 2). Polyploidy is demonstrated by the prevalence of cells with 2*n*=4*x*=40 (tetraploid) in *B. brachyactis* (Fig. 1).

Karyotype asymmetry and karyological relationships

The M_{CA} value, which refers to intrachromosomal asymmetry, ranges from 12.39 (*B. verruculosum*) to 28.41 (*B. pinnatifolium*). The CV_{CL} value, which refers to interchromosomal asymmetry, ranges from 15.12 (*B. verruculosum*) to 36.89 (*B. sayae*). *B. pinnatifolium* and *B. sayae* have the most asymmetric karyotypes, while *B. verruculosum* has the most symmetric karyotype (Table 3).

Fig. 3. presents a dendrogram including chromosomal data of 6 *Bunium* taxa. The dendrogram consists

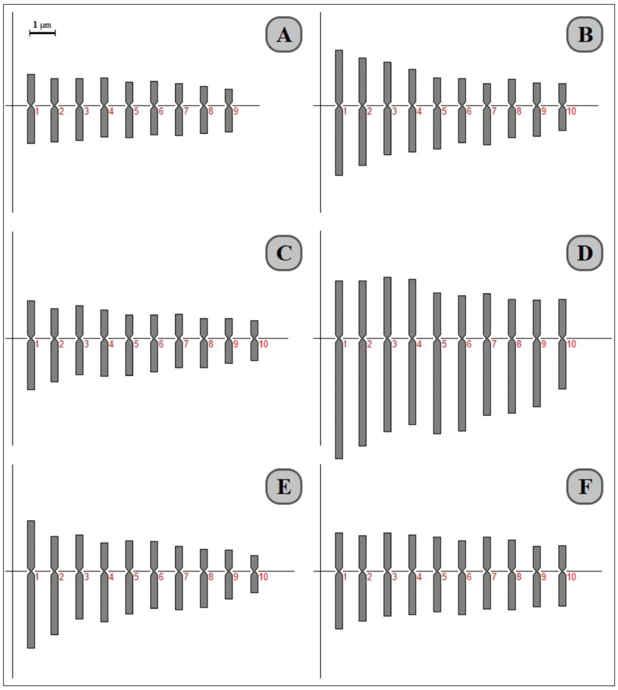

Fig. 2. Monoploid ideograms of **A** – *B. elegans* var. *brevipes*, x=9; **B** – *B. elegans* var. *elegans*; **C** – *B. ferulaceum*; **D** – *B. pinnatifolium*; **E** – *B. sayae*; **F** – *B. verruculosum*, x=10.

Table 3. The total haploid length (THL), mean chromosome length (MCL) and asymmetry index values of *Bunium* taxa.

Taxa	THL	MCL	CV_{CL}	M_{CA}
B. elegans var. *elegans*	39.11	3.91	34.85	13.25
B. elegans var. *brevipes*	25.18	2.80	15.82	13.55
B. ferulaceum	30.26	3.03	25.94	14.04
B. pinnatifolium	71.73	7.17	22.90	28.41
B. sayae	37.90	3.79	36.89	19.12
B. verruculosum	38.42	3.84	15.12	12.39

of 2 main clades. In clade I, *B. pinnatifolium* forms a monophyletic group with quite different karyological features such as large chromosomes, more submedian chromosomes and the most asymmetric karyotype. Clade II consists of 2 subclades with a strong monophyletic group (BV=82). Subclade 1 contains different basic number (x=9) and relatively more symmetrical karyotypes. Subclade 2 contains the most asymmetric karyotypes of interchromosomal asymmetry.

DISCUSSION

The chromosome numbers of 6 taxa are reported for the first time in *B. elegans* var. *brevipes* (2n=18), *B. brachyactis*, *B. pestalozzae*, *B. sayae*, *B. verruculosum* (2n=20), and *B. allioides* (2n=22). The chromosome numbers of 2 taxa are the same as in previous records. The chromosome number of *B. ferulaceum* is 2n=20 in Greece and Syria. The chromosome number of *B. microcarpum* is 2n=20 in Crimea and Turkey [9-10,12,14]. However, subspecies may be different from *B. microcarpum* subsp. *bourgaei*.

The chromosome numbers of *B. elegans* var. *elegans* and *B. pinnatifolium* represent new cytotypes (2n=20). In the literature, the chromosome numbers are 2n=17, 18 in *B. elegans* and 2n=22 in *B. pinnatifolium* [9,11-13]. The metaphase microphotographs of some *Bunium* species are shown in [9] while *B. elegans* is not included, and an image of metaphase chromosomes of *B. elegans* is presented in [13], however, cell borders are not shown. In the present study, the chromosome number is 2n=20 in cells with clear cell boundaries.

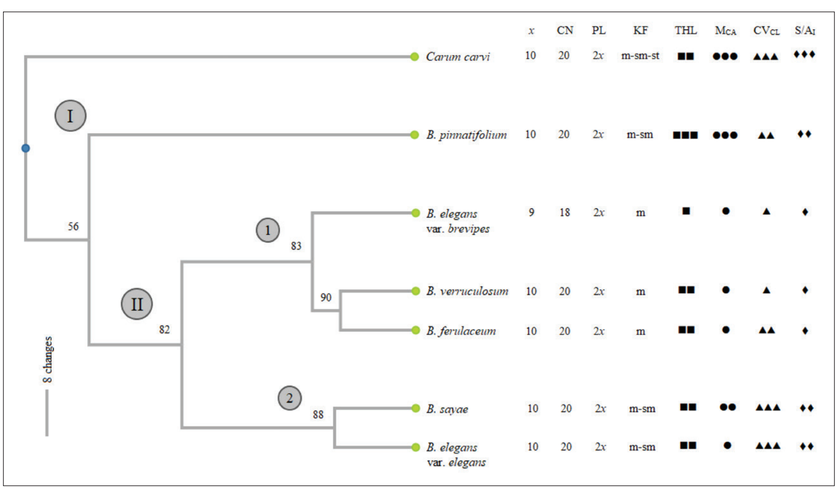

Fig. 3. Dendrogram showing karyological relationships of 6 *Bunium* taxa. Numbers above branches indicate bootstrap values. Main clades and subclades are shown in circles. x: basic chromosome number, CN: chromosome number, PL: ploidy level, KF: karyotype formula, THL: total haploid length, M_{CA}: mean centromeric asymmetry, CV_{CL}: coefficient of variation of chromosome length, S/A_I: symmetry/asymmetry index. 0<THL≤30.00 (▪), 30.00<THL≤60.00 (▪▪), 60.00<THL (▪▪▪); 0<M_{CA}≤15.00 (•), 15.00<M_{CA}≤25.00 (••), 25.00<M_{CA} (•••); 0<CV_{CL} ≤ 20.00 (▲), 20.00<CV_{CL}≤30.00 (▲▲), 30.00<CV_{CL} (▲▲▲); 1.0=S/A_I (♦, full symmetric), 1.0<S/A_I≤2.0 (♦♦, symmetric).

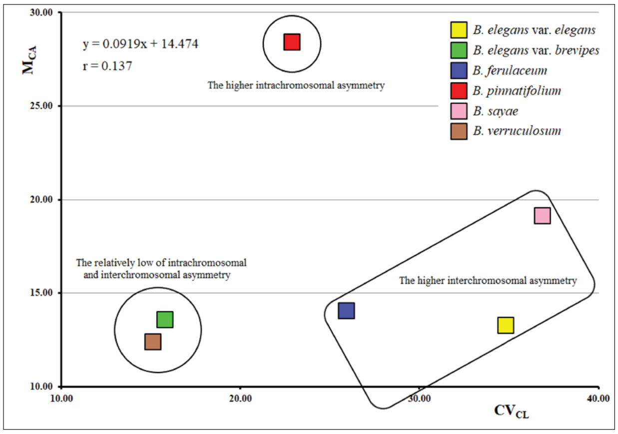

Fig. 4. Scatter diagram between intrachromosomal asymmetry (M_{CA}) and interchromosomal asymmetry (CV_{CL}).

In addition to the new cytotypes, satellites are found in the second chromosome pairs of *B. pinnatifolium*. It was reported that *B. persicum* (Boiss.) B. Fedtsch had a single satellite [21].

Chromosomal data could not be obtained in *B. paucifolium*, which does not have ripe fruits and *B. nudum* (Post) H. Wolff, which could not be collected. In addition, *B. sivasicum* M. Çelik & Y. Bağcı *sp. nov.* and *B. simplex* could not be germinated. The chromosome numbers of *B. simplex* and *B. paucifolium* are 2*n*=18 and 2*n*=20, respectively [9]. There is no information about the chromosome numbers of *B. nudum* and *B. sivasicum*.

A basic number of *x*=10 dominates in Turkish *Bunium* taxa, but the basic numbers of *x*=9 and 11 characterize several taxa. In addition, the basic numbers of *x*=6, 7 and 8 have been reported in other regions. For example, the basic number is *x*=6 in *B. afghanicum* Beauverd, *B. intermedium* Korovin, *B. kuhitangi* Nevski, and *B. latilobum* Korovin; *x*=7 in *B. hissaricum* Korovin, *B. persicum* (Boiss.) B. Fedtsch., and *B. seravschanicum* Korovin; and *x*=8 in *B. capusii* (Franch.) Korovin [9,22-23].

B. brachyactis is the first polyploid species of the genus with a ploidy level of 4*x*. The polyploidy or genome duplication, originating from autopolyploidy and allopolyploidy, is one of the most effective mechanisms in speciation and karyotype evolution [24]. Polyploidy levels increase at high altitudes, although this is not the only reason. For example, *B. brachyactis* spreads between 1700 and 3150 m a.s.l. In addition, the adjacent cells of *B. brachyactis* present different patterns in terms of chromosome set, which is referred to as mixoploidy (2*n*=2*x* =20 and 2*n*=4*x*=40).

There is no detailed study showing karyological relationships and karyotype evolution among *Bunium* species. The karyological studies consist of basic chromosome numbers, diploid chromosome numbers and several karyotype analyses. *B. pinnatifolium* forms a monophyletic group (clade I) by quite different karyological features such as large chromosomes, more submedian chromosomes and a mostly asymmetric karyotype. In addition, the other 5 taxa form a strong monophyletic group (clade II). *B. elegans* var. *brevipes* shapes the subclade 1 by some variations, which have a different basic number, different diploid number and small chromosomes. *B. verruculosum* and *B. ferulaceum* are cytotaxonomically very close species, as are *B. sayae* and *B. elegans* var. *elegans*. Two varieties of *B. elegans* are cytotaxonomically distant taxa located in different subclades. Comparative karyotype analysis can be used to describe the chromosomal evolution patterns in a group. In particular, karyological variations can produce postzygotic-crossover barriers for diversification and speciation. The data will provide important contributions to understanding the karyotype evolution of *Bunium*, however, it must be supported by molecular data.

In intrachromosomal asymmetry, all karyotypes are symmetrical except the karyotype of *B. pinnatifolium*. The most symmetrical karyotypes are the karyotypes of *B. verruculosum*, *B. elegans* var. *elegans*, and *B. elegans* var. *brevipes*, with $0<M_{CA}\leq 15.00$. In interchromosomal asymmetry, all karyotypes are symmetrical except the karyotypes of *B. sayae* and *B. elegans* var. *elegans*. The most symmetrical karyotypes are karyotypes of *B. verruculosum* and *B. brevipes*, with $0<CV_{CL}\leq 20.00$. *Bunium* taxa have different karyotypes in terms of asymmetry degrees: *B. elegans* var. *brevipes* and *B. verruculosum* possess relatively low intrachromosomal and interchromosomal asymmetry; *B. elegans* var. *elegans*, *B. ferulaceum* and *B. sayae* have a higher interchromosomal asymmetry, and *B. pinnatifolium* has higher intrachromosomal asymmetry.

In the genus *Bunium*, the asymmetry data are limited to several reports. Karyotype asymmetry of 23 species is highly variable [9]. *B. elegans* has a symmetrical karyotype in both [9] and the present study. *B. microcarpum* also has a symmetrical karyotype, however, karyotype asymmetry could not be determined in the present study. Karyotype asymmetry of 3 species, *B. persicum*, *B. cylindricum* and *B. chaerophylloides* Drude, was shown [22].

CONCLUSIONS

New chromosomal data are reported for the genus *Bunium* as follows: (i) new chromosome numbers and polyploidy for the genus, (ii) in 6 taxa, the first report of chromosome numbers, (iii) in 2 taxa, different numbers from previous reports, (iv) in 6 taxa, detailed chromosomal data, (v) karyotype asymmetry and generally symmetric karyotypes, (vi) interspecific relationships and karyological variations. The chromosome numbers of 2 Turkish species, *B. nudum* and *B. sivasicum,* are still unknown. The results provide an important contribution to the cytotaxonomy of *Bunium*.

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Supplementary Material

The Supplementary Material is available at: http://serbiosoc.org.rs/NewUploads/Uploads/Celik%20et%20al_5010_Supplementary%20Material.pdf