

## Cytogenetic studies on some *Scorzonera* L. s.l. (Asteraceae) taxa from Turkey

Fahim ALTINORDU<sup>1\*</sup>, Esra MARTİN<sup>1</sup>, Serdar MAKBUL<sup>2</sup>, Kamil COŞKUNÇELEBİ<sup>3</sup>, Mutlu GÜLTEPE<sup>3</sup>

<sup>1</sup>Department of Biotechnology, Faculty of Science, Necmettin Erbakan University, Konya, Turkey

<sup>2</sup>Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdoğan University, Rize, Turkey

<sup>3</sup>Department of Biology, Faculty of Science, Karadeniz Technical University, Trabzon, Turkey

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**Abstract:** In the present study, chromosome morphology of 32 *Scorzonera* L. taxa, including 19 species endemic to Turkey, was analyzed. The plant materials were collected from different natural populations of Turkey between 2005 and 2011. The classification of chromosomes, the length of the long and short arm, haploid chromosome length, arm ratio, and relative chromosomal length were measured by software image analyses. The basic chromosome numbers were determined as  $x = 6$  and  $x = 7$ . Two types of ploidy levels were observed as diploidy and tetraploidy. Karyotype asymmetry indices, TF%, As K%, Syi, Rec, A, A1, and A2 were also calculated. *Scorzonera ahmet-duranii* S.Makbul & Coskuncelebi, *S. laciniata* subsp. *calcitrapifolia* (Vahl) Marie, and *Scorzonera suberosa* C.Koch subsp. *cariensis* (Boiss.) Chamberlain had the most symmetrical karyotypes for the subgenera *Scorzonera* L., *Podospermum* (L.) DC., and *Pseudopodospermum* (Lipsch. & Krasch.) Lipsch., respectively.

**Key words:** Asteraceae, cytogenetic, karyotype, karyotype asymmetry, *Scorzonera*

### 1. Introduction

The family Asteraceae is the richest vascular plant family in the world with 1600–1700 genera and 24,000–30,000 species. Many naturalized members of Asteraceae have been recorded in recent years (Funk et al., 2005; Moreira-Muñoz and Muñoz-Schick, 2007). With 134 genera and about 1200 species, the family represents the largest number of species in the flora of Turkey and the East Aegean islands. Out of these taxa, 447 are endemic, with an endemism rate of 37% for Turkey (Davis et al., 1988; Özhatay and Kültür, 2006).

The ancient Mediterranean genus *Scorzonera* L. s.l., with about 160 species and belonging to the subtribe Scorzonerinae Dumort. (Lactuceae Cass., Cichorioideae), is widespread in the arid regions of Eurasia and Africa (Bremer and Anderberg, 1994; Nazarova, 1997). *Scorzonera* is represented by 28 species in the flora of Europe (Chater, 1976), 11 species in the flora of Greece (Tutin et al., 1976), 9 species in the flora of Austria (Adler et al., 1994), 25 species in the flora of Germany (Jäger and Werner, 2002), and 2 species in the flora of Sweden (Binz and Heitz, 1990). According to Chamberlain (1975), the genus *Scorzonera* is represented by 42 species in Turkey. However, the number reaches 52 species (59 taxa), 31 of which are endemic, when taken together with the newly described taxa. This

means that Turkey is an important center of diversity for this genus (Coşkunçelebi et al., 2015).

A karyotype characterization usually includes the chromosome number and the absolute or relative length of chromosomes (Levan et al., 1964; Peruzzi et al., 2009). The position of primary and secondary constrictions (Levan et al., 1964; Peruzzi et al., 2009) and the distribution of material with different staining properties (Stebbins, 1971; Stace, 2000; Levin, 2002; Lysak and Lexer, 2006; Peruzzi et al., 2009) are also included in karyotype characterization. In describing the genome of a species, chromosome number is the most fundamental feature and it is also the easiest to measure. Consequently, since 1882 (Garbari et al., 2012; Peruzzi et al., 2012), chromosome number data for many plant species have been recorded worldwide; currently, one-third of all plant chromosomes have been characterized in this respect (Stace, 2000; Peruzzi et al., 2012). Chromosome number is also an important characteristic for plant evolutionary studies and may provide information on polyploidy and other highly significant genome changes (Guerra, 2008; Louzada, 2010). Plant chromosome number databases are useful tools for systematic comparisons of geographical or taxonomical groups of plants (Peruzzi et al., 2012). Moreover, chromosome counts can enhance our understanding of

\* Correspondence: altinordu.fahim@gmail.com

phylogenetic relationships at different taxonomic levels (Yang et al., 2009).

*Scorzonera* s.l. is one of the most complicated plant genera with a basic chromosome number of  $x = 6$  and  $x = 7$  (Mavrodiev et al., 2004; Owen et al., 2006). The taxonomic difficulties are caused by the high morphological variation and presence of different ploidy levels (Mavrodiev et al., 2004). Although the majority of *Scorzonera* taxa are diploids, several polyploids and/or polyploid cytotypes are also found in the genus (Nazarova, 1997; Owen et al., 2006). Although there are several studies on chromosome counts in the genus *Scorzonera* (Martin et al., 2012), there are fewer regarding karyotype analysis. Martin et al. (2012) reported that diploid chromosome counts are  $2n = 12, 14,$  and  $28$  for some *Scorzonera* taxa. Similarly, De la Guardia and Blanca (1987) reported that the basic chromosome number in the genus is  $x = 6$  and  $x = 7$ , and that polyploidy is very important for the evolution of the genus *Scorzonera* s.l. The most comprehensive cytological study on *Scorzonera* and related genera was performed by Nazarova (1997), who reported that karyotype analysis supports sectional delimitation in the genus *Scorzonera*. Nazarova also indicated that *Scorzonera*, *Podospermum* (L.) DC., *Epilasia* Benth., *Tourneuxia* Cass., *Pterachaenia* Lipsch., and *Takhtajantha* Nazarova are related taxa based on karyotype analysis. She also emphasized that *Podospermum* should be a different genus from the genus *Scorzonera*, according to the cytological data.

The aim of the present study was to establish the karyotypes and karyotype asymmetry indices of 32 *Scorzonera* taxa from Turkey, which have not been studied to date, and evaluate the taxonomic value within *Scorzonera* s.l.

## 2. Materials and methods

### 2.1. Plant materials

Ripe cypselae were collected from natural populations of *Scorzonera* s.l. in Turkey. Herbarium materials were deposited in the herbarium of Recep Tayyip Erdoğan University, Department of Biology, and Karadeniz Technical University, Department of Biology.

### 2.2. Karyotype analysis

For somatic chromosome study, ripe cypselae were germinated on wet filter paper in petri dishes, which were pretreated in distilled water at  $20 \pm 1$  °C for several days. When cypselae germinated (1–1.5 mm), root tips were initially pretreated for 16 h in  $\alpha$ -monobromonaphthalene at 4 °C and then fixed in 3:1 absolute alcohol–glacial acetic acid. The root tips were then hydrolyzed with 1 N HCl for 5 min at 60 °C and stained with 2% aceto orcein for 2 h at room temperature. Stained root tips were squashed in a drop of 45% acetic acid, and permanent slides were made with the standard liquid nitrogen method. The slides

were dried for 24 h at room temperature and mounted in DEPEX.

For karyotype analysis,  $10 \times 100$  enlarged photographs were taken using an Olympus BX51 microscope with a Pixera PVC 100C camera attachment. The classification of chromosomes, the length of long and short arms, arm ratio, and relative chromosomal length were measured by software image analyses (Bs200Pro) on a personal computer. Chromosomes were classified using the nomenclature of Levan et al. (1964). Ideograms of these taxa were arranged in decreasing length (Martin et al., 2009).

### 2.3. Karyotype asymmetry

To describe karyotype asymmetry and to determine the karyotypic relationships between species, Huziwaru (1962) developed the total form percent (TF%). Arano (1963) developed another karyotype asymmetry index, As K%. Greilhuber and Speta (1976) developed 2 indices to evaluate karyotype asymmetry, which were termed as the Syi index and the Rec index by Venora et al. (2002). Zarco (1986) provided a different method to measure karyotype asymmetry, the intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2). Watanabe et al. (1999) defined the degree of asymmetry for karyotypes (A).

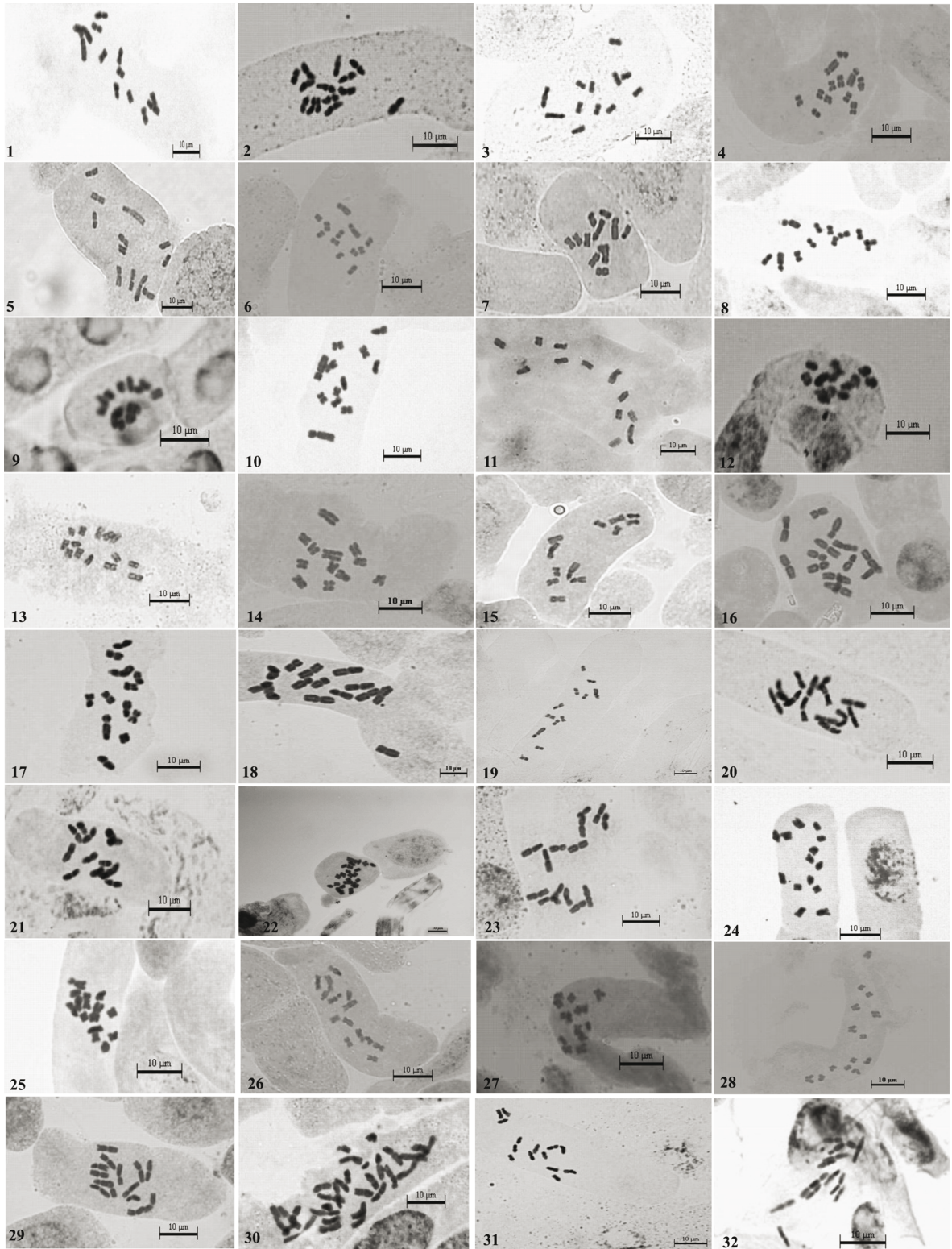
Furthermore, Stebbins (1971) distinguished 12 categories of karyotype asymmetry, only 10 of which were known to occur in higher plants. He established these by recognizing 3 degrees of difference (A–C) between the largest and smallest chromosome of the complement and 4 degrees (1–4) with respect to the proportion of chromosomes that are median pairs with an arm ratio of  $<2:1$ .

Based on the karyological data, a cluster analysis was carried out to examine karyotype similarity among the species. Statistical analysis was carried out using Microsoft Office Excel 2010.

## 3. Results

In the present study, we evaluated the results for subgenera *Scorzonera*, *Podospermum*, and *Pseudopodospermum*. Karyological features, asymmetry indices, metaphase plates (Figure 1), karyograms (Figure 2), and ideograms (Figure 3) were determined for each taxon.

In this cytogenetical study we determined the chromosome morphology of *Scorzonera* taxa (Table 1). The chromosome morphology shows important differences among taxa in the subgenera of *Scorzonera*. In subgenus *Scorzonera*, the smallest chromosome length (1.88  $\mu\text{m}$ ) was observed in *S. ahmet-duranii*. The largest (9.63  $\mu\text{m}$ ) was observed in *S. karabelensis*. Regarding haploid chromosome length, *S. davisii* (15.63  $\mu\text{m}$ ) was shortest and *S. longiana* (46.06  $\mu\text{m}$ ) was longest. The smallest arm



**Figure 1.** Somatic metaphase chromosomes: 1- *Scorzonera acuminata*, 2- *S. boissieri*, 3- *S. dzhawakhetica*, 4- *S. sublanata*, 5- *S. karabelensis*, 6- *S. seidlitzii*, 7- *S. sandrasica*, 8- *S. rigida*, 9- *S. davisii*, 10- *S. lasiocarpa*, 11- *S. pygmaea*, 12- *S. sericea*, 13- *S. ahmet-duranii*, 14- *S. tuzgoluensis*, 15- *S. ulrichii*, 16- *S. violacea*, 17- *S. pisidica*, 18- *S. longiana*, 19- *S. aucheriana*, 20- *S. amasiana*, 21- *S. cinerea*, 22- *S. latifolia* var. *latifolia*, 23- *S. armeniaca*, 24- *S. cana* var. *cana*, 25- *S. cana* var. *alpina*, 26- *S. cana* var. *radicosa*, 27- *S. laciniata* subsp. *calcitrapifolia*, 28- *S. hieraciifolia*, 29- *S. mollis* subsp. *szowitzii*, 30- *S. semicana*, 31- *S. inaequiscapa*, 32- *S. suberosa* subsp. *cariensis*.





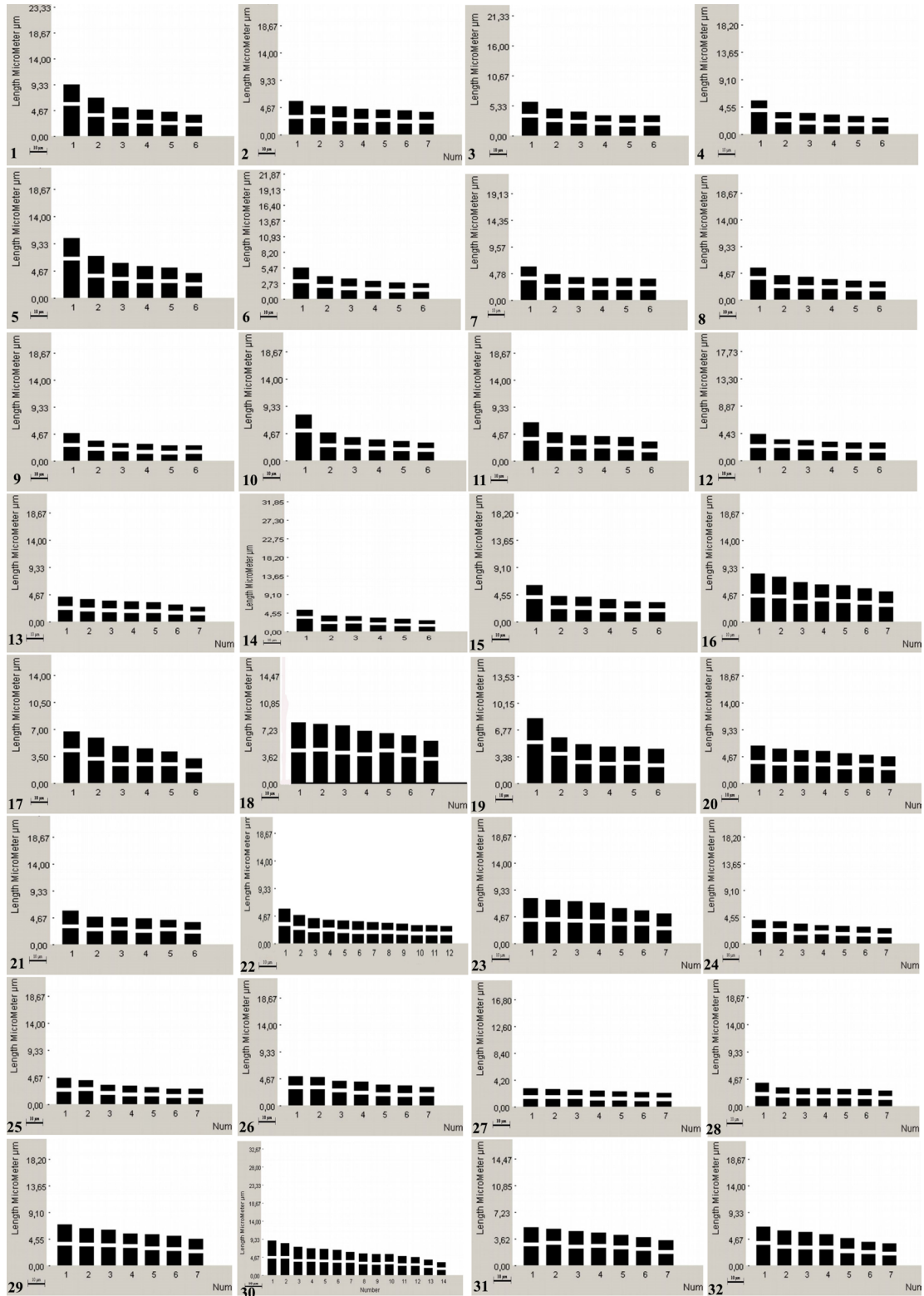
**Figure 2.** Karyograms: 1- *Scorzonera acuminata*, 2- *S. boissieri*, 3- *S. dzhawakhetica*, 4- *S. sublanata*, 5- *S. karabelensis*, 6- *S. seidlitzii*, 7- *S. sandrasica*, 8- *S. rigida*, 9- *S. davisii*, 10- *S. lasiocarpa*, 11- *S. pygmaea*, 12- *S. sericea*, 13- *S. ahmet-duranii*, 14- *S. tuzgoluenensis*, 15- *S. ulrichii*, 16- *S. violacea*, 17- *S. pisidica*, 18- *S. longiana*, 19- *S. aucheriana*, 20- *S. amasiana*, 21- *S. cinerea*, 22- *S. latifolia* var. *latifolia*, 23- *S. armeniaca*, 24- *S. cana* var. *cana*, 25- *S. cana* var. *alpina*, 26- *S. cana* var. *radicosa*, 27- *S. laciniata* subsp. *calcitrapifolia*, 28- *S. hieraciifolia*, 29- *S. mollis* subsp. *szowitzii*, 30- *S. semicana*, 31- *S. inaequiscapa*, 32- *S. suberosa* subsp. *cariensis*.

ratio was observed in *S. violacea* (1.03) and the largest was observed in *S. sublanata* (2.70). The smallest relative length value was measured in *S. latifolia* var. *latifolia* (5.93) and the largest relative value was measured in *S. lasiocarpa* (31.26). The metaphase chromosome pairs were usually of the median and submedian type. In this study, the karyotype formulae were  $5m + 1sm$  for *S. cinerea*, *S. acuminata*, *S. dzhawakhetica*, *S. karabelensis*, *S. rigida*, *S. pisidica*, *S. davisii*, *S. lasiocarpa*, *S. pygmaea*, *S. sericea*, and *S. ulrichii*;  $6m + 1sm$  for *S. boissieri*, *S. longiana*, and *S. amasiana*;  $4m + 2sm$  for *S. sublanata*, *S. sandrasica*, *S. tuzgoluenensis*, and *S. aucheriana*;  $6m$  for *S. seidlitzii*;  $7m$  for *S. ahmet-duranii* and *S. violacea*; and  $12m$  for *S. latifolia* var. *latifolia*.

In subgenus *Podospermum*, the shortest chromosome length (1.58  $\mu\text{m}$ ) was observed in *S. laciniata* subsp. *calcitrapifolia*. In contrast, the longest (7.23  $\mu\text{m}$ ) was observed in *S. armeniaca*. Concerning haploid chromosome length, *S. laciniata* subsp. *calcitrapifolia*

(13.44  $\mu\text{m}$ ) was shortest and *S. armeniaca* (41.97  $\mu\text{m}$ ) was longest. The smallest arm ratio was observed in *S. laciniata* subsp. *calcitrapifolia* (1.02) and the largest was observed in *S. cana* var. *radicosa* (1.90). The smallest and largest relative length values were measured in *S. cana* var. *alpina* (10.14 and 20.71). The karyotype formulae were  $7m$  for *S. armeniaca*, *S. laciniata* subsp. *calcitrapifolia*, and *S. hieraciifolia*, and  $6m + 1sm$  for *S. cana* var. *radicosa*, *S. cana* var. *alpina*, and *S. cana* var. *cana*.

In subgenus *Pseudopodospermum*, the smallest and largest chromosome length (2.62  $\mu\text{m}$  and 8.29  $\mu\text{m}$ ) was observed in the taxon *S. semicana*. The largest haploid chromosome length, 75.02  $\mu\text{m}$ , was found in *S. semicana*. The smallest arm ratio was observed in *S. semicana* (1.07), and the largest was observed in *S. inaequiscapa* (1.71). The smallest relative length value was measured in *S. semicana* (3.49) and the largest relative value was measured in *S. suberosa* (18.98). The karyotype formulae were  $7m$



**Figure 3.** Ideograms: 1- *Scorzonera acuminata*, 2- *S. boissieri*, 3- *S. dzhawakhetica*, 4- *S. sublanata*, 5- *S. karabelensis*, 6- *S. seidlitzii*, 7- *S. sandrasica*, 8- *S. rigida*, 9- *S. davisii*, 10- *S. lasiocarpa*, 11- *S. pygmaea*, 12- *S. sericea*, 13- *S. ahmet-duranii*, 14- *S. tuzgoluensis*, 15- *S. ulrichii*, 16- *S. violacea*, 17- *S. pisidica*, 18- *S. longiana*, 19- *S. aucheriana*, 20- *S. amasiana*, 21- *S. cinerea*, 22- *S. latifolia* var. *latifolia*, 23- *S. armeniaca*, 24- *S. cana* var. *cana*, 25- *S. cana* var. *alpina*, 26- *S. cana* var. *radicosa*, 27- *S. laciniata* subsp. *calcitrapifolia*, 28- *S. hieraciifolia*, 29- *S. mollis* subsp. *szowitzii*, 30- *S. semicana*, 31- *S. inaequiscapa*, 32- *S. suberosa* subsp. *cariensis*.

**Table 1.** Karyological features of the studied *Scorzonera* taxa.

Taxa	2n	Chromosome length (µm)		Haploid chromosome length (µm)	Arm ratio		Relative length		Karyotype formulae
		Min	Max		Min	Max	Min	Max	
<i>Scorzonera acuminata</i> Boiss.	12	3.17	8.65	30.54	1.11	1.73	10.36	28.31	5 m + 1 sm
<i>S. boissieri</i> Lipsch.	14	3.18	5.08	27.49	1.14	2.02	11.57	18.48	6 m + 1 sm
<i>S. dzhawakhetica</i> Sosn. ex Grossh.	12	2.85	5.34	21.80	1.19	1.72	13.07	24.49	5 m + 1 sm
<i>S. sublanata</i> Lipschitz	12	2.07	5.05	17.98	1.10	2.70	11.51	28.08	4 m + 2 sm
<i>S. karabelensis</i> Parolly & Kilian	12	3.52	9.63	34.30	1.09	2.04	10.26	28.09	5 m + 1 sm
<i>S. seidlitzii</i> Boiss.	12	2.16	4.73	17.60	1.08	1.47	12.27	26.87	6 m
<i>S. sandrasica</i> Hartvig & Strid	12	3.15	5.22	22.48	1.19	2.04	14.01	23.24	4 m + 2 sm
<i>S. rigida</i> Aucher	12	2.50	4.92	20.13	1.29	2.28	12.44	24.42	5 m + 1 sm
<i>S. davisii</i> Lipschitz	12	1.99	4.08	15.63	1.08	1.98	12.00	26.28	5 m + 1 sm
<i>S. lasiocarpa</i> Chamberlain	12	2.44	7.21	22.70	1.17	2.02	10.57	31.26	5 m + 1 sm
<i>S. pygmaea</i> Sm.	12	2.77	5.99	23.83	1.15	1.43	11.63	25.12	5 m + 1 sm
<i>S. sericea</i> DC.	12	2.23	3.64	16.07	1.23	2.63	13.88	22.68	5 m + 1 sm
<i>S. ahmet-duranii</i> S.Makbul & Coşkunçelebi	14	1.88	3.67	19.64	1.07	1.26	9.60	18.67	7 m
<i>S. tuzgoluensis</i> A.Duran, B.Doğan & S.Makbul	12	2.09	4.65	18.22	1.09	2.04	11.50	25.52	4 m + 2 sm
<i>S. ulrichii</i> Parolly & Kilian	12	2.70	5.46	21.03	1.08	2.21	12.82	25.97	5 m + 1 sm
<i>S. violacea</i> Chamberlain	14	4.55	7.63	41.88	1.03	1.50	10.87	18.21	7 m
<i>S. pisidica</i> Hub.-Mor.	12	2.71	6.16	26.25	1.04	1.83	10.32	23.49	5 m + 1 sm
<i>S. longiana</i> Sümbül	14	5.21	7.61	46.06	1.13	2.03	11.32	16.52	6 m + 1 sm
<i>S. aucheriana</i> DC.	12	3.85	7.79	29.62	1.10	1.85	13.01	26.29	4 m + 2 sm
<i>S. amasiana</i> Hausskn. & Born.	14	3.92	5.82	33.84	1.12	1.92	11.57	17.20	6 m + 1 sm
<i>S. cinerea</i> Boiss.	12	3.13	5.13	23.82	1.10	1.74	13.16	21.56	5 m + 1 sm
<i>S. latifolia</i> (Fisch. & Mey.) DC. var. <i>latifolia</i> (Fisch. & Mey.)	24	2.24	5.09	37.74	1.08	1.52	5.93	13.49	12 m
<i>S. armeniaca</i> (Boiss. & A.Huet) Boiss.	14	4.49	7.23	41.97	1.04	1.55	10.70	17.22	7 m
<i>S. cana</i> (C.A. Meyer) Hoffm. var. <i>cana</i> (C.A.Mey) Hoffm.	14	1.94	3.38	18.11	1.03	1.73	10.72	18.67	6 m + 1 sm
<i>S. cana</i> (C.A.Mey) Hoffm. var. <i>alpina</i> (Boiss.) Chamberlain	14	1.91	3.89	18.78	1.13	1.89	10.14	20.71	6 m + 1 sm
<i>S. cana</i> (C.A.Mey) Hoffm. var. <i>radicosa</i> (Boiss.) Chamb.	14	2.52	4.31	18.14	1.11	1.90	10.63	20.71	6 m + 1 sm
<i>S. laciniata</i> L. subsp. <i>calcitrapifolia</i> (Vahl) Marie	14	1.58	2.34	13.44	1.02	1.41	11.76	17.45	7 m
<i>S. hieracifolia</i> Hayek	14	1.97	3.23	17.18	1.09	1.41	11.47	18.83	7 m
<i>S. mollis</i> Bieb. subsp. <i>szowitzii</i> (DC.) Chamb.	14	3.90	6.30	35.27	1.10	1.59	11.04	17.86	7 m
<i>S. semicana</i> DC.	28	2.62	8.29	75.02	1.07	1.56	3.49	11.05	14 m
<i>S. inaequiscapa</i> Boiss.	14	2.82	4.68	26.78	1.14	1.71	10.55	17.47	6 m + 1 sm
<i>S. suberosa</i> C.Koch subsp. <i>cariensis</i> (Boiss.) Chamberlain	14	3.19	6.12	32.22	1.15	1.42	9.90	18.98	7 m

for *S. suberosa* and *S. mollis* subsp. *szowitzii*, 14 m for *S. semicana*, and 6 m + 1 sm for *S. inaequiscapa*.

#### 4. Discussion

In the present study, 22 taxa belonging to subgenus *Scorzonera*, 15 of which are endemic to Turkey, were investigated. The basic chromosome number was determined as  $x = 6$  and  $x = 7$ ;  $x = 6$  is widespread for subg. *Scorzonera*. In subgenus *Podospermum*, 6 taxa were

investigated, one of which is endemic to Turkey. The basic chromosome number was determined as  $x = 7$  for all taxa in subg. *Podospermum*. In subgenus *Pseudopodospermum*, 4 taxa were studied, 3 of which are endemic to Turkey. The basic chromosome number was determined as  $x = 7$  for all taxa in subg. *Pseudopodospermum*.

Chromosome number is an important indicator for cytotaxonomy in *Scorzonera*. Coşkunçelebi et al. (2015) reported that *S. hieracifolia* ( $x = 7$ ) should be transferred to

subgenus *Podospermum* ( $x = 7$ ), based on morphological, anatomical, and other studies. Chromosome number is in accordance with the basic chromosome number of *Podospermum* and thus supports this opinion. Furthermore, according to this revisional study, *S. inaequiscapa* ( $x = 7$ ) and *S. semicana* ( $x = 7$ ) should also be transferred under the subgenus *Pseudopodospermum* ( $x = 7$ ). The basic chromosome numbers of these 2 taxa also support the transfer of the last 2 taxa under the subgenus *Pseudopodospermum* (Coşkunçelebi et al., 2015).

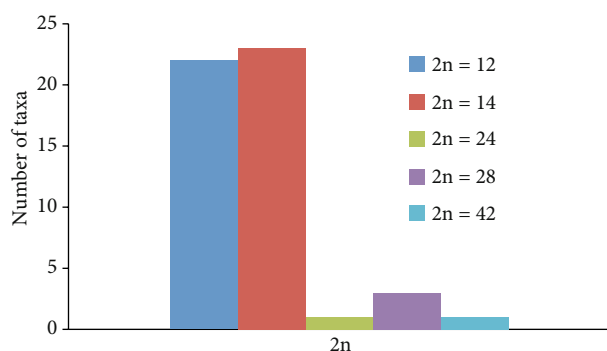
In our study, chromosome numbers of 32 *Scorzonera* taxa were reported. These numbers are  $2n = 12, 14, 24,$  and  $28$ , which are detailed in the results. In addition, Martin et al. (2012) reported the chromosome numbers of 13 *Scorzonera* taxa as  $2n = 12, 14,$  and  $28$ . These are  $2n = 14$  for *Scorzonera laciniata* L. subsp. *laciniata*, *S. cana* var. *jacquiniana* (W.Koch) Chamb., *S. suberosa* C.Koch subsp. *suberosa*, *S. mollis* M.Bieb. subsp. *mollis*, *S. papposa* DC., *S. lacera* Boiss. & Bal., *S. elata* Boiss., and *S. parviflora* Jacq.;  $2n = 28$  for *S. phaeopappa* (Boiss.) Boiss.; and  $2n = 12$  for *S. eriophora* DC., *S. pseudolanata* Grossh., *S. tomentosa* L., and *S. kotschy* Boiss. Dinç et al. (2008) reported the chromosome number and determined the karyotype of *S. argyria* Boiss. This study reported the chromosome number as  $2n = 12$ . Hamzaoğlu et al. (2010) reported the chromosome number of *Scorzonera ketzkhoveli* Grossh as  $2n = 12$ . Chromosome numbers of many taxa belonging to *Scorzonera* have been studied and published previously. According to these reports, the chromosome numbers of *Scorzonera* vary between  $2n = 12$  and  $2n = 42$ . The frequency of chromosome numbers for Turkey is given in Figure 4.

According to Martin et al. (2012), the karyotype formulas reported were  $3m + 4sm$  for *S. laciniata* subsp. *laciniata*;  $4m + 3sm$  for *S. cana* var. *jacquiniana*;  $5m + 2sm$  for *S. suberosa* subsp. *suberosa*, *S. elata*, and *S. parviflora*;  $7m$  for *S. mollis* subsp. *mollis*, *S. papposa*, and *S. lacera*;  $13m + 1sm$  for *S. phaeopappa*;  $5m + 1sm$  for *S. eriophora*, *S. pseudolanata*, and *S. kotschy*; and

$3m + 3sm$  for *S. tomentosa*. *S. laciniata* subsp. *laciniata* had the shortest chromosome length at  $1.20 \mu\text{m}$ , while *S. eriophora* had the longest chromosome length at  $7.63 \mu\text{m}$ . *S. laciniata* subsp. *laciniata* had the shortest haploid chromosome length ( $11.44 \mu\text{m}$ ), while *S. phaeopappa* had the longest ( $38.28 \mu\text{m}$ ). The lowest arm ratio value was obtained from *S. kotschy* (1.00) and the highest from *S. laciniata* subsp. *laciniata* (2.73). In terms of relative length, *S. phaeopappa* had the lowest value (4.48), while *S. kotschy* had the highest (28.56). Dinç et al. (2008) determined the karyotype of *S. argyria*; total chromosome length varied between  $3.52$  and  $8.36 \mu\text{m}$ . In addition, arm ratios were determined as  $1.28 \mu\text{m}$ , which was lowest, and  $1.87 \mu\text{m}$ , which was highest. Total haploid chromosome length was calculated as  $31.07 \mu\text{m}$ .

The concept of karyotype asymmetry is typified by a karyotype marked by the predominance of chromosomes with terminal/subterminal centromeres (intrachromosomal asymmetry) and by highly heterogeneous chromosome sizes (interchromosomal asymmetry) (Peruzzi and Eroğlu, 2013). The karyotype asymmetry index is a good expression of the general morphology of plant chromosomes. It would therefore be advantageous to have a uniform system whereby the karyotypes of related genotypes and species could be compared (Paszko, 2006). Karyotype asymmetry indices were estimated by different methods in this study (Table 2).

When we compare the karyotype asymmetry in subgenus *Scorzonera* according to Stebbins's (1971) classification, *S. violacea* and *S. ahmet-duranii* are classified to symmetry class 1A, indicating the most symmetrical karyotype features. On the other hand, *S. sericea* is classified to symmetry class 3A, indicating the most asymmetrical karyotype features. *S. seidlitzii* is in class 1B, *S. cinerea* and *S. longiana* are in class 2A, and *S. sublanata* is in class 2B. According to Stebbins's (1971) classification, *S. seidlitzii* has a more symmetrical karyotype than *S. cinerea* and *S. longiana*, and *S. sublanata* is more asymmetrical than the others. We can say that 3A is more asymmetrical than 1A and that 2B is more symmetrical than 2C, but we cannot determine which members of class 1A have higher symmetry; Stebbins's classification does not clarify this situation. Thus, we used other indices to determine the most symmetrical or asymmetrical karyotype. The TF% and Syi-Rec values decrease with increasing asymmetry, while the As K %, the A1-A2, and the A values increase with increasing asymmetry (Zuo and Yuan, 2011; Eroğlu et al., 2013). When we compare *S. violacea* and *S. ahmet-duranii* based on these indices, *S. ahmet-duranii* has the most symmetrical karyotype and *S. sericea* has the most asymmetrical karyotype in subgenus *Scorzonera*. The TF%, As K%, Syi, A1, and A indices are more definitive than Stebbins's classification.



**Figure 4.** Frequency of chromosome numbers of *Scorzonera* taxa in Turkey.

**Table 2.** Values of asymmetry indices in the studied *Scorzonera* taxa.

Taxa	Stebbins's classification	TF%	As K%	Syi	Rec	A	A1	A2
<i>Scorzonera acuminata</i>	2B	48	52	77	59	0.11	0.18	0.40
<i>S. boissieri</i>	2A	43	57	75	77	0.14	0.24	0.16
<i>S. dzhawakhetica</i>	2A	41	59	69	68	0.18	0.31	0.27
<i>S. sublanata</i>	2B	38	62	63	59	0.19	0.30	0.36
<i>S. karabelensis</i>	2B	42	58	73	59	0.13	0.21	0.38
<i>S. seidlitzii</i>	1B	44	56	79	62	0.11	0.20	0.33
<i>S. sandrasica</i>	2A	41	59	69	72	0.17	0.28	0.21
<i>S. rigida</i>	2A	40	60	68	68	0.17	0.28	0.26
<i>S. davisii</i>	2B	41	59	70	64	0.17	0.29	0.30
<i>S. lasiocarpa</i>	2B	39	61	64	52	0.20	0.33	0.47
<i>S. pygmaea</i>	1B	44	56	77	66	0.12	0.21	0.28
<i>S. sericea</i>	3A	39	61	63	74	0.23	0.36	0.20
<i>S. ahmet-duranii</i>	1A	46	54	85	76	0.08	0.15	0.20
<i>S. tuzgoluensis</i>	2B	41	59	68	65	0.16	0.27	0.30
<i>S. ulrichii</i>	2B	40	60	68	64	0.17	0.28	0.29
<i>S. violacea</i>	1A	44	56	79	78	0.12	0.21	0.18
<i>S. pisidica</i>	2B	43	57	77	71	0.12	0.20	0.28
<i>S. longiana</i>	2A	43	57	63	86	0.15	0.25	0.14
<i>S. aucheriana</i>	2B	42	58	71	63	0.15	0.24	0.30
<i>S. amasiana</i>	2A	41	59	71	83	0.17	0.29	0.14
<i>S. cinerea</i>	2A	43	57	76	77	0.14	0.24	0.17
<i>S. latifolia</i> var. <i>latifolia</i>	1B	45	55	80	62	0.11	0.20	0.26
<i>S. armeniaca</i>	1A	45	55	83	83	0.10	0.17	0.17
<i>S. cana</i> var. <i>cana</i>	2A	43	57	76	77	0.13	0.22	0.19
<i>S. cana</i> var. <i>alpina</i>	2B	43	57	74	69	0.14	0.24	0.27
<i>S. cana</i> var. <i>radicosa</i>	2A	40	60	67	79	0.19	0.30	0.20
<i>S. laciniata</i> subsp. <i>calcitrapifolia</i>	1A	46	54	86	82	0.12	0.14	0.14
<i>S. hieraciifolia</i>	1A	45	55	81	76	0.10	0.18	0.16
<i>S. mollis</i> subsp. <i>szowitzii</i>	1A	44	56	78	80	0.12	0.22	0.16
<i>S. semicana</i>	1B	45	55	81	65	0.11	0.20	0.29
<i>S. inaequiscapa</i>	2A	42	58	72	82	0.16	0.28	0.17
<i>S. suberosa</i> subsp. <i>cariensis</i>	1A	44	56	79	75	0.11	0.20	0.23

In subgenus *Podospermum*, when we compare the karyotype asymmetry according to Stebbins's classification, *S. armeniaca*, *S. laciniata* subsp. *calcitrapifolia*, and *S. hieraciifolia* are classified according to the symmetry classes of Stebbins as 1A, indicating the most symmetrical karyotypic features. *S. cana* var. *alpina* belongs to class 2B and has the most asymmetrical karyotype. Once again, it is unclear which taxa (*S. armeniaca*, *S. laciniata* subsp. *calcitrapifolia*, or *S. hieraciifolia*) has the most symmetrical karyotype. Consequently, TF%, Syi-Rec, A1-A2, and A values were used to determine the most symmetrical karyotype. According to these indices, *S. laciniata* subsp.

*calcitrapifolia* has the most symmetrical karyotype and *S. cana* var. *alpina* has the most asymmetrical karyotype in this subgenus. Upon evaluating the karyotype asymmetry among subspecies of *S. cana*, it was revealed that *S. cana* var. *cana* has the most symmetrical karyotype and *S. cana* var. *alpina* has the most asymmetrical karyotype. *S. cana* var. *cana* is more symmetrical than *S. cana* var. *radicosa*.

In subgenus *Pseudopodospermum*, *S. suberosa* subsp. *cariensis* has the most symmetrical karyotype and *S. inaequiscapa* has the most asymmetrical karyotype. For *Scorzonera* taxa belonging to 3 subgenera, the scatter diagram of population dispersion was determined based on 2 components (A1-A2) (Figure 5).



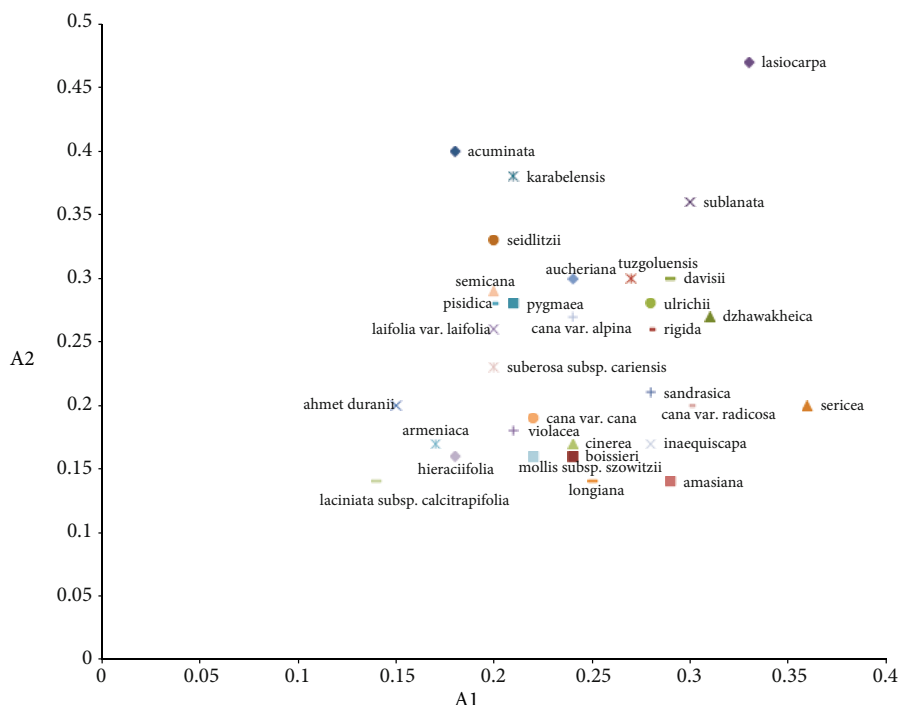


Figure 5. Scatter diagram for *Scorzonera* taxa based on A1–A2 parameters.

In conclusion, the present study defined and evaluated the karyotypic features of 32 *Scorzonera* taxa. The relationships among *Scorzonera* taxa were also determined, based on karyomorphological characters and karyotype symmetry indices.

## References

- Adler W, Oswald K, Fischer R (1994). Exkursionsflora von Österreich. 1st ed. Stuttgart, Germany: Ulmer (in German).
- Arano H (1963). Cytological studies in subfamily Carduoideae (Compositae) of Japan. IX. The karyotype analysis and phylogenetic considerations on *Pertya* and *Ainsliaea*. Bot Mag Tokyo 76: 32–39.
- Binz A, Heitz C (1990). Schul- und Exkursionsflora für die Schweiz. Basel, Switzerland: Schwabe (in German).
- Bremer K, Anderberg AA (1994). Asteraceae: Cladistics and Classification. 1st ed. Portland, OR, USA: Timber Press.
- Chamberlain DF (1975). *Scorzonera* L. In: Davis PH, editor. Flora of Turkey and the East Aegean Islands, Vol. 5. 1st ed. Edinburgh, UK: Edinburgh University Press, pp. 632–657.
- Chater AO (1976). *Scorzonera* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Moore DM, Walters SM, Webb DA, editors. Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae). Vol. 4. Cambridge, UK: Cambridge University Press, pp. 317–322.
- Coşkunçelebi K, Makbul S, Gültepe M, Okur S, Güzel ME (2015). A conspectus of *Scorzonera* s.l. in Turkey. Turk J Bot 39: 76–87.
- Davis PH, Mill RR, Tan K (1988). *Scorzonera* L. In: Davis PH, Mill RR, Tan K, editors. Flora of Turkey and the East Aegean Islands (Suppl. 1), Vol. 10. 1st ed. Edinburgh, UK: Edinburgh University Press, pp. 169–170.
- De la Guardia CD, Blanca G (1987). Karyology of the *Scorzonera* (Compositae) species from the Iberian Peninsula. Plant Syst Evol 156: 29–42.
- Dinç M, Duran A, Martin E (2008). Rediscovery of the poorly known *Scorzonera argyria* and its relationships in Turkey. Biologia 63: 1078–1084.
- Eroğlu HE, Simşek N, Koç M, Hamzaoğlu E (2013). Karyotype analysis of some *Minuartia* L. (Caryophyllaceae) taxa. Plant Syst Evol 299: 67–73.
- Funk VA, Bayer RJ, Keeley S, Chan R, Watson L, Gemeinholzer B, Schilling E, Panero JL, Baldwin BG, Garcia-Jacas N et al. (2005). Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. Biol Skrif 55: 343–374.
- Garbari F, Bedini G, Peruzzi L (2012). Chromosome numbers of the Italian flora. From the Caryologia foundation to present. Caryologia 65: 62–71.

- Greilhuber J, Speta F (1976). C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica* and *Puschkinia* (Liliaceae). *Plant Syst Evol* 126: 149–188.
- Guerra M (2008). Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenet Genome Res* 120: 339–350.
- Hamzaoglu E, Aksoy A, Martin E, Pinar NM, Çölgeçen H (2010). A new record for the flora of Turkey: *Scorzonera ketzkhovellii* Grossh. (Asteraceae). *Turk J Bot* 34: 57–61.
- Huziwara Y (1962). Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of *Aster*. *Am J Bot* 49: 116–119.
- Jäger EJ, Werner K (2002). *Exkursionsflora von Deutschland*. 1st ed. Heidelberg, Germany: Spektrum Akademischer Verlag (in German).
- Levan A, Fredga K, Sandberg AA (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220.
- Levin DA (2002). *The Role of Chromosomal Change in Plant Evolution*. 1st ed. New York, NY, USA: Oxford University Press.
- Louzada RB, Palma-Silva C, Corrêa AM, Kaltchuk-Santos E, Wanderley MDGL (2010). Chromosome number of *Orthophytum* species (Bromeliaceae). *Kew Bulletin* 65: 53–58.
- Lysak MA, Lexer C (2006). Towards the era of comparative evolutionary genomics in Brassicaceae. *Plant Syst Evol* 259: 175–198.
- Martin E, Çetin Ö, Makbul S, Duran A, Öztürk M, Boduroğlu D, Eşmekaya B (2012). Karyology of the *Scorzonera* L. (Asteraceae) taxa from Turkey. *Turk J Biol* 36: 187–199.
- Martin E, Dinç M, Duran A (2009). Karyomorphological study of eight *Centaurea* L. taxa (Asteraceae) from Turkey. *Turk J Bot* 33: 97–104.
- Mavrodiev EV, Edwards CE, Albach DC, Gitzendanner MA, Soltis PS, Soltis DE (2004). Phylogenetic relationships in subtribe Scorzonerinae (Asteraceae: Cichorioideae: Cichorieae) based on ITS sequence data. *Taxon* 53: 699–712.
- Moreira-Muñoz A, Muñoz-Schick M (2007). Classification, diversity, and distribution of Chilean Asteraceae: implications for biogeography and conservation. *Divers Distrib* 13: 818–828.
- Nazarova EA (1997). Karyosystematic investigation of the genus *Scorzonera* L. s.l. (Lactuceae, Asteraceae). *Caryologia* 50: 239–261.
- Owen WM, D'Amato G, De Dominicis RI, Salimbeni P, Tucci GF (2006). A cytological and molecular study of the genera *Scorzonera* L. and *Podospermum* (L.) DC (Asteraceae). *Caryologia* 59: 153–163.
- Özhatay N, Kültür Ş (2006). Check-list of additional taxa to the supplement Flora of Turkey III. *Turk J Bot* 30: 281–316.
- Paszko B (2006). A critical review and a new proposal of karyotype asymmetry indices. *Plant Syst Evol* 258: 39–48.
- Peruzzi L, Eroğlu HE (2013). Karyotype asymmetry: again, how to measure and what to measure? *Comp Cytogen* 7: 1–9.
- Peruzzi L, Góralski G, Joachimiak AJ, Bedini G (2012). Does actually mean chromosome number increase with latitude in vascular plants? An answer from the comparison of Italian, Slovak and Polish floras. *Comp Cytogen* 6: 371–377.
- Peruzzi L, Leitch IJ, Caparelli KF (2009). Chromosome diversity and evolution in Liliaceae. *Ann Bot-London* 103: 459–475.
- Stace CA (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20<sup>th</sup> and 21<sup>st</sup> centuries. *Taxon* 49: 451–477.
- Stebbins GL (1971). *Chromosomal Evolution in Higher Plants*. 1st ed. London, UK: Edward Arnold Press.
- Tutin TG, Heywood VH, Burges NA, Valentine DH (1976). *Flora Europaea*, Vol. 4. 1st ed. Cambridge, UK: Cambridge University Press.
- Venora G, Blangiforti S, Ruffini Castiglione M, Pignone D, Losavio F, Cremonini R (2002). Chromatin organisation and computer aided karyotyping of *Triticum durum* Desf. cv. Timilia. *Caryologia* 55: 91–98.
- Watanabe K, Yahara T, Denda T, Kosuge K (1999). Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae): statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. *J Plant Res* 112: 145–161.
- Yang Z, Zhang L, Zhao H, Yang R, Ding C, Zhou Y, Wan D (2009). Chromosome numbers of some species of *Salvia* (Lamiaceae) from the Sichuan Province, China. *Nord J Bot* 27: 287–291.
- Zarco CR (1986). A new method for estimating karyotype asymmetry. *Taxon* 35: 526–530.
- Zuo L, Yuan Q (2011). The difference between the heterogeneity of the centromeric index and intrachromosomal asymmetry. *Plant Syst Evol* 297: 141–145.