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Karyological study of the genus *Gundelia* (Compositae) in Turkey

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Abstract. Karyotypes in 12 taxa of *Gundelia* are compared, based on Feulgen-stained somatic metaphase chromosomes. The karyotypes of *G. anatolica*, *G. asperrima*, *G. cilicica*, *G. colemerikensis*, *G. dersim*, *G. glabra*, *G. komagenensis*, *G. mesopotamica*, *G. munzuriensis* and *G. vitekii* are described for the first time. Karyological analyses indicate relationships among the species with respect to their asymmetry indices. All *Gundelia* species studied were diploid with $2n = 2x = 18$ chromosomes. All karyotypes are symmetrical, consisting of metacentric and submetacentric chromosomes. The submetacentric chromosomes of all the investigated specimens contain a secondary constriction. Three chromosome types were identified according to the position of the secondary constrictions. The chromosomes ranged in size from 2.00 μm to 7.02 μm . The total haploid chromosome length (THL) varied from 24.97 μm (*G. asperrima*) to 42.56 μm (*G. rosea*). To determine the karyological relationships among taxa, PCoA (Principal Coordinate Analysis) with six uncorrelated parameters was performed.

Keywords. Chromosome number, karyotype asymmetry, secondary constriction, *Cichorieae*, *Scolyminae*.

INTRODUCTION

Gundelia L. was first recorded in one of the earliest natural history collections made in the Near East by the German physician, botanist and traveller, Leonhard Rauwolf (1535–1596) at 16. century. However, the plant was first evaluated in *Silybum* then *Eryngium* groups. About 125 years later, in the early years of the 18th century, Joseph Pitton de Tournefort saw the plant in the natural habitat. Moreover, he concluded that the plant should be called *Gundelia* (Hind 2013). Finally, the plant was named *Gundelia tournefortii* by Linnaeus, in accordance with the binomial nomenclature (Linnaeus 1753).

The infrafamilial position of *Gundelia* has also changed over time (Hind 2013). The last tribal position of genus *Gundelia* was *Cichorieae* Lam. & DC., subtribe *Scolyminae* Less., along with *Catananche* L., *Hymenonema* Cass., and *Scolymus* L. (Kilian et al. 2009). The genus grows in the semi-desert areas, and it is distributed in the Mediterranean to Central/Eastern Asia (Karis et al. 2001).

Some different species and varieties have been described over the years, although many authors have treated *Gundelia* as monospecific and the wide variation in corolla colour was considered unrelated to gross morphology (Komarov 1961; Kupicha 1975; Feinbrun Dothan 1978; Meikle 1985; Rechinger 1989). However, in the last decades, researches on *Gundelia* increased. Numerous new species have been published as a result of research on live and more abundant materials. The genus is currently represented by 16 species, of which 12 (10 endemic) in Turkey (Vitek et al. 2010, 2014, 2017; Nersesyan 2014; Armağan 2016; Firat 2016, 2017a, 2017b, 2017c; Vitek and Noroozii 2017; Vitek 2018). These are *Gundelia anatolica* Firat, *G. asperrima* (Trautv.) Firat, *G. cilicica* Firat, *G. colemerikensis* Firat, *G. dersim* Vitek, Yüce & Ergin, *G. glabra* Mill., *G. komagenensis* Firat, *G. mesopotamica* Firat, *G. munzurien-*

sis Vitek, Yüce & Ergin, *G. rosea* M.Hossain & Al-Taey (non-endemic), *G. tournefortii* L. (non-endemic), and *G. vitekii* Armağan.

The chromosome numbers of *Cichorieae* range between $2n = 14x = 126$ chromosomes in *Sonchus* (Beuzenberg and Hair 1984; Dawson 2000) and $2n = 2x = 6$ in some species of *Crepis* (Ikeda 1988; Gupta and Gill 1989; Dimitrova and Greilhuber 2001). The basic chromosome number in the majority of the subtribes is $x = 9$, or a descending series starting with $x = 9$ to $x = 3$. Subtribe *Scolyminae* has the basic chromosome numbers $x = 9$ and 10 (Kilian et al. 2009). The only chromosome number *Gundelia* is $2n = 18$ (Waisel 1962; Al-Taey and Hossain 1984; Ghaffari and Chariat-Panahi 1985; Nersesyan and Nazarova. 1989; Ghukasyan and Janjughazyan 2015).



Fig. 1. Synflorescences of the studied taxa. (a) *G. anatolica*; (b) *G. asperrima*; (c) *G. cilicica*; (d) *G. colemerikensis*; (e) *G. dersim*; (f) *G. glabra*; (g) *G. komagenensis*; (h) *G. mesopotamica*; (i) *G. munzuriensis*; (j) *G. rosea*; (k) *G. tournefortii*; (l) *G. vitekii*.

This study aimed to determine the chromosome numbers and karyomorphology of all the 12 *Gundelia* species occurring in Turkey (Figure 1).

MATERIALS AND METHODS

Twelve *Gundelia* species were analysed in this study. A list of examined specimens is provided in Table 1. All endemic taxa were collected from their type localities. Voucher specimens were deposited in the Herbarium of University of Van Yüzüncü Yıl (VANF) and in a private herbarium (Herb. M. Fırat). For karyological observations, four to eight individuals were used for each species in this study. Mitotic metaphase cells of root tips were obtained from germinated seeds which were collected in natural habitats from Turkey.

Mitotic chromosomes were prepared from root tips and pre-treated with 0.002 M 8-Hydroxyquinoline at +4 °C for 24 h. Roots were fixed for a minimum of 2 h in absolute ethanol:glacial acetic acid, (3:1,v/v), hydrolysed at 60 °C in 1 N HCl for 12 min. and stained with the Feulgen method. Finally, root tips were squashed in 1% aceto-orcein. Permanent slides were prepared with entellan mounting medium. Microphotographs of good quality metaphase plates were taken using an Olympus BX53 (Tokyo, Japan) microscope equipped with a high-resolution digital camera. Metaphase observations and chromosome measures were made using the image

analysis systems KAMERAM (ARGENİT Microsystems, İstanbul, Turkey). The somatic chromosome number and karyotype details were studied in five to eighteen well-spread metaphase plates from different individuals; mean values were used for the analysis.

Chromosome pairs were identified and arranged on the basis of their length and any other evident karyomorphological features. The nomenclature used for describing karyotype composition followed Levan et al. (1964). To determine the karyological relationships among taxa, we performed a PCoA (Principal Coordinate Analysis) with six uncorrelated parameters as suggested by Peruzzi and Altınordu (2014). These parameters are chromosome number ($2n$), basic chromosome number (x), total haploid length (THL), mean centromeric asymmetry (M_{CA}), coefficient of variation of chromosome length (CV_{CL}), and coefficient of variation of centromeric index (CV_{CI}) (Paszko 2006; Peruzzi et al. 2009; Zuo and Yuan 2011, Peruzzi and Eroğlu 2013). The software Past 3.03 (Hammer et al. 2001, Hammer 2018) was used to perform this analysis.

Mitotic metaphase chromosomes are given in Figure 3. Idiograms of these taxa are arranged in order of centromere position and then decreasing the length of homologue chromosome pairs (Figure 4).

In this study, chromosome types were determined according to the position of the secondary constrictions of *Gundelia* chromosomes for the purpose of chromosome comparison. General description of these chromo-

Table 1. Localities and voucher specimens of *Gundelia* taxa examined in the present study.

| Taxa | Locality | Collector (M. Fırat) /voucher |
|---|---|-------------------------------|
| <i>G. anatolica</i> Fırat | Kırıkkale: Delice province, Tuzkayası region, dry steppe, 700 m, 10 July 2017 | 33866 |
| <i>G. asperrima</i> (Trautv.) Fırat | Erzurum: Palandöken mountain, mountain slope, steppe, 2444 m, 30 July 2017 | 33845 |
| <i>G. cilicica</i> Fırat | Mersin: Erdemli province, Tozlu village, open forrest, 1460 m, 11 July 2017 | 33868 |
| <i>G. colemerikensis</i> Fırat | Hakkâri: Hakkâri Province (Colemerik) Berçelan plateau, open erode region and steppe, 2284 m, 7 July 2017 | 33861 |
| <i>G. dersim</i> Vitek, Yüce & Ergin | Tunceli (Dersim): Ovacık, c. 11.7 km SW Ovacık, 1.9 km NE Ziyaret (fountains of river Munzur), 1300 m, 28 July 2017 | 33872 |
| <i>G. glabra</i> Mill. | Bayburt: Kop mountain arround, near Kop Village, 1897 m, 19 July 2017 | 33867 |
| <i>G. komagenensis</i> Fırat | Adıyaman: Kahta Province, Nemrut mountain, rocky steppe, 1445 m, 30 July 2017 | 33783 |
| <i>G. mesopotamica</i> Fırat | Mardin: 2-3 km from Mardin to Nusaybin (Nisêbîn), eroded slopes, aride steppe, 807 m, 11 July 2017 | 33865 |
| <i>G. munzurensis</i> Vitek, Yüce & Ergin | Tunceli (Dersim): Ovacık, c. 2 km SW Ovacık, near road Ovacık plain, 1275 m, 28 July 2017 | 33871 |
| <i>G. rosea</i> M.Hossain & Al-Taey | Hakkari: Şemdinli Province, Sad Mountain, Deriyê Kera region, meadows and stony slopes, 1662 m, 7 July 2017 | 33862 |
| <i>G. tournefortii</i> L. | Hatay: Reyhanli district, near Syria (Aleppe) border, 121 m, 9 July 2017 | 33864 |
| <i>G. vitekii</i> Armağan | Tunceli (Dersim): c. 8-9 km N of Tunceli, mountain slope NW of Tüllük Bucağı, rocky steppe, 1760 m, 28 July 2017 | 33870 |

some types is given below, followed by the karyotype description.

Type A: Longest metacentric chromosomes with two constrictions, secondary constrictions in the distal position of the long arm.

Type B: Submetacentric chromosomes with two constrictions, secondary constrictions nearly in the median position of the long arm.

Type C: Submetacentric chromosomes with two constrictions and secondary constrictions located very close to the centromere on the short arm.

RESULTS

All species showed basic chromosome number $x = 9$ and diploids with $2n = 18$ (Figures 3 and 4). Chromosome measurements and karyotype formula of the twelve analysed species are indicated in Table 2. Total haploid length, asymmetry indices, chromosome types and flower number within the partial synflorescences in the middle part are summarised in Table 3.

Secondary constrictions were observed at the long or short arms of all submetacentric chromosomes, and in the distal regions of the long arms of some of the longest

Table 3. Karyo-morphometric parameters, symmetry indices, Chromosome types and cephaloid flowers number for investigated taxa (THL: total haploid length; M_{CA} : mean centromeric asymmetry; CV_{CL} : coefficient of variation of chromosome length; CV_{CI} : coefficient of variation of centromeric index).

| | THL | M_{CA} | CV_{CL} | CV_{CI} | Chromosome Types | Cephaloid. Flowers numbers |
|--------------------------|-------|----------|-----------|-----------|------------------|----------------------------|
| <i>G. anatolica</i> | 34.48 | 14.13 | 16.87 | 9.73 | /B/_ | 6 |
| <i>G. asperrima</i> | 24.97 | 13.08 | 21.58 | 9.83 | A/B/C | 3(-4) |
| <i>G. cilicica</i> | 34.26 | 12.06 | 20.01 | 7.44 | _/_/C | 6(-7) |
| <i>G. colemerikensis</i> | 29.84 | 13.03 | 22.42 | 10.75 | _/_/C | (3-)5(-6) |
| <i>G. dersim</i> | 34.52 | 13.27 | 21.57 | 9.56 | A/_/C | 6-7 |
| <i>G. glabra</i> | 35.60 | 13.27 | 20.84 | 9.94 | _/_/C | 3-5(-6) |
| <i>G. komagenensis</i> | 37.90 | 13.95 | 20.56 | 11.94 | _/_/C | 3(-4) |
| <i>G. mesopotamica</i> | 32.64 | 14.38 | 19.70 | 12.44 | A/B/C | 6-7 |
| <i>G. munzurensis</i> | 27.87 | 13.27 | 20.24 | 10.21 | A/B/C | 3-5 |
| <i>G. rosea</i> | 42.56 | 14.40 | 21.42 | 8.12 | A/_/C | (6-) 7-8 |
| <i>G. tournefortii</i> | 27.16 | 11.41 | 20.20 | 8.83 | A/_/C | (5-)6(-7) |
| <i>G. vitekii</i> | 30.57 | 13.76 | 21.64 | 11.82 | A/B/C | 3(-5) |

Table 2. Karyotype formula according to Levan et al. (1964) and measurements of the investigated taxa. (SC: the shortest chromosome length; LC: the longest chromosome length; p: mean long arm length; q: mean short arm length; SD: standard deviation; m: metacentric; sm: submetacentric).

| | SC-LC | q (μ m) Mean (\pm SD) | p (μ m) Mean (\pm SD) | p+q Mean (\pm SD) | Karyotype formula |
|--------------------------|-------------|-------------------------------|-------------------------------|----------------------|-------------------|
| <i>G. anatolica</i> | 2.90 - 5.15 | 1.64(\pm 0.30) | 2.19(\pm 0.44) | 3.83(\pm 0.65) | 16 m + 2 sm |
| <i>G. asperrima</i> | 2.00 - 4.01 | 1.19(\pm 0.23) | 1.58(\pm 0.42) | 2.77(\pm 0.60) | 14 m + 4 sm |
| <i>G. cilicica</i> | 2.77 - 5.35 | 1.67(\pm 0.35) | 2.14(\pm 0.46) | 3.81(\pm 0.76) | 16 m + 2 sm |
| <i>G. colemerikensis</i> | 2.40 - 4.90 | 1.43(\pm 0.33) | 1.89(\pm 0.50) | 3.32(\pm 0.74) | 14 m + 4 sm |
| <i>G. dersim</i> | 2.78 - 5.78 | 1.66(\pm 0.40) | 2.18(\pm 0.50) | 3.84(\pm 0.83) | 16 m + 2 sm |
| <i>G. glabra</i> | 2.86 - 5.61 | 1.70(\pm 0.35) | 2.25(\pm 0.56) | 3.96(\pm 0.82) | 14 m + 4 sm |
| <i>G. komagenensis</i> | 3.10 - 6.08 | 1.79(\pm 0.37) | 2.42(\pm 0.61) | 4.21(\pm 0.87) | 14 m + 4 sm |
| <i>G. mesopotamica</i> | 2.73 - 5.27 | 1.55(\pm 0.36) | 2.08(\pm 0.47) | 3.63(\pm 0.71) | 14 m + 4 sm |
| <i>G. munzurensis</i> | 2.28 - 4.43 | 1.33(\pm 0.26) | 1.77(\pm 0.43) | 3.10(\pm 0.63) | 14 m + 4 sm |
| <i>G. rosea</i> | 3.30 - 7.02 | 2.03(\pm 0.49) | 2.70(\pm 0.58) | 4.73(\pm 1.01) | 16 m + 2 sm |
| <i>G. tournefortii</i> | 2.31 - 4.45 | 1.33(\pm 0.30) | 1.68(\pm 0.37) | 3.02(\pm 0.61) | 16 m + 2 sm |
| <i>G. vitekii</i> | 2.49 - 5.04 | 1.45(\pm 0.31) | 1.95(\pm 0.51) | 3.40(\pm 0.74) | 14 m + 4 sm |

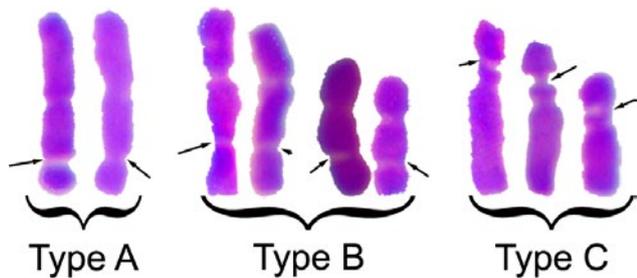


Fig. 2. Chromosome types according to the position of the secondary constrictions (indicated by arrows).

metacentric chromosomes (Figure 2). Moreover, three chromosome types were determined according to the position of the secondary constrictions (Figure 2).

The chromosomes ranged in size from 2.00 μm to 7.02 μm . *G. asperrima* showed the smallest mean chromosome length (2.77 μm), while *G. rosea* the biggest (4.73 μm).

Similarly, the smallest mean short arm length (q) was observed in *G. asperrima* (1.19 μm) and the largest mean long arm length (p) was observed in *G. rosea* (2.70 μm). The idiograms of the analysed species are shown in Figure 3.

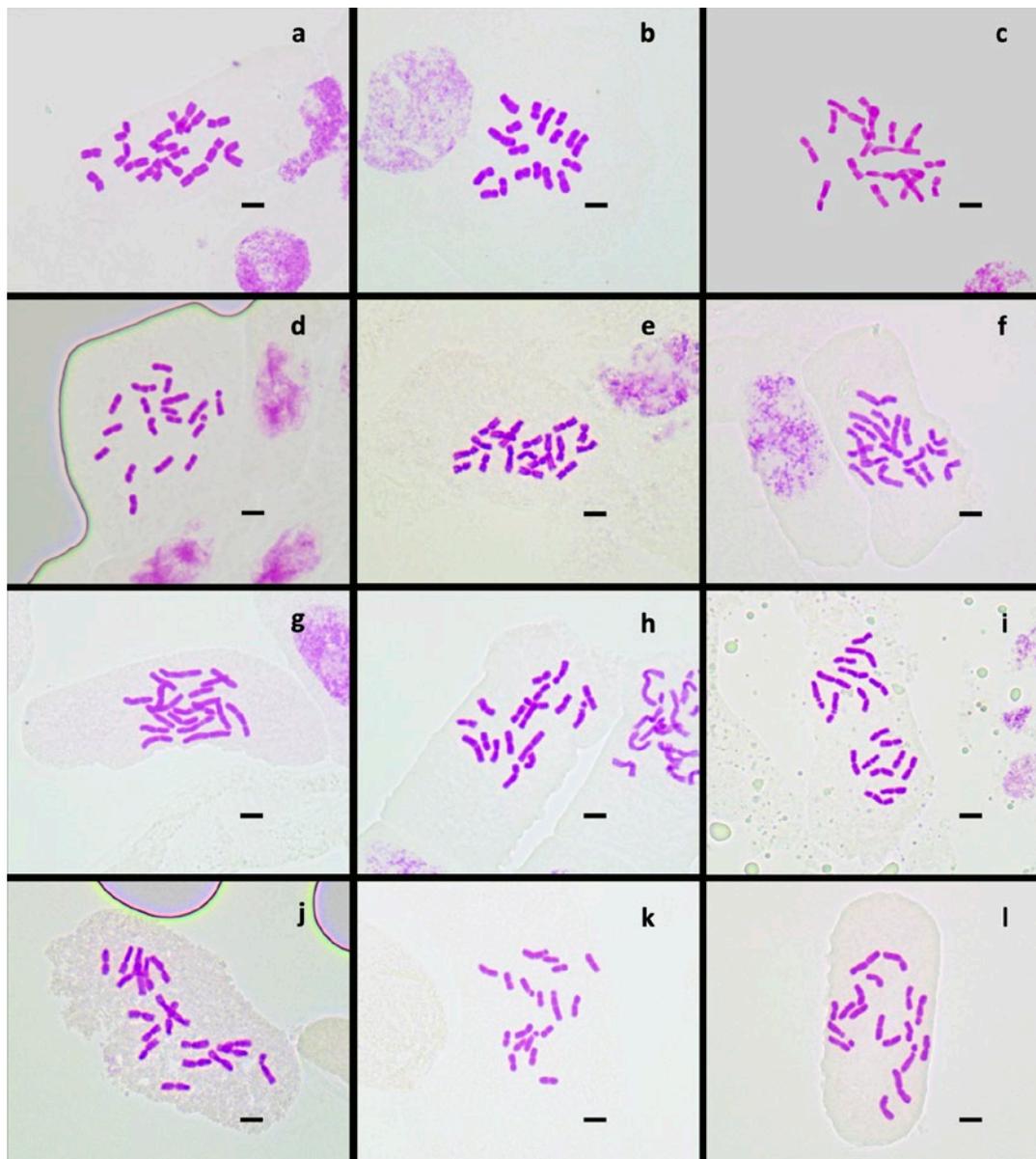


Fig. 3. Somatic chromosomes ($2n = 18$) in the studied taxa. (a) *G. anatolica*; (b) *G. asperrima*; (c) *G. cilicica*; (d) *G. colemerikensis*; (e) *G. dersim*; (f) *G. glabra*; (g) *G. komagenensis*; (h) *G. mesopotamica*; (i) *G. munzuriensis*; (j) *G. rosea*; (k) *G. tournefortii*; (l) *G. vitekii*. Scale bars 3 μm .

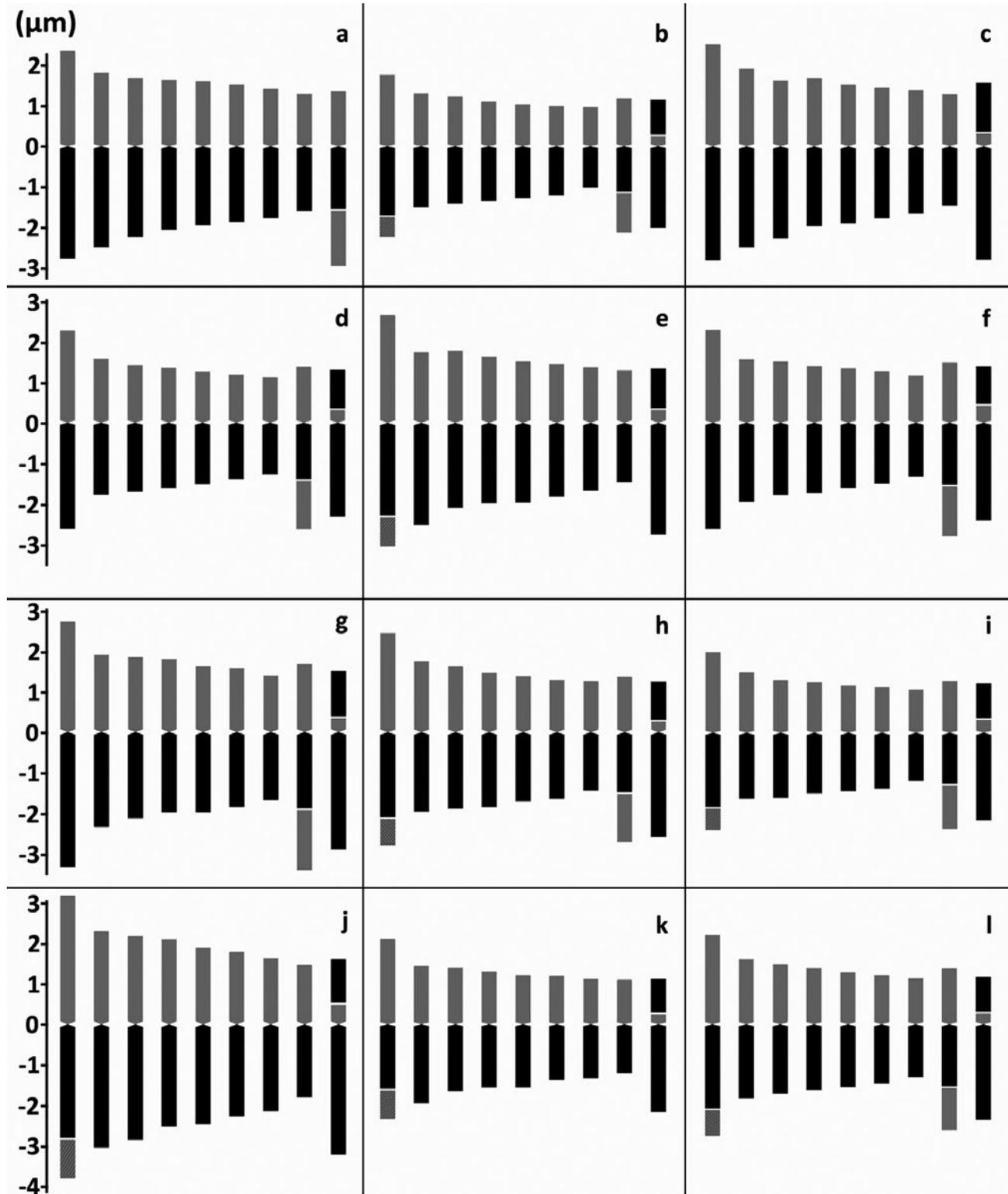


Fig. 4. Haploid idiograms in the studied taxa. (a) *G. anatolica*; (b) *G. asperrima*; (c) *G. cilicica*; (d) *G. colemerikensis*; (e) *G. dersim*; (f) *G. glabra*; (g) *G. komagenensis*; (h) *G. mesopotamica*; (i) *G. munzuriensis*; (j) *G. rosea*; (k) *G. tournefortii*; (l) *G. vitekii*.

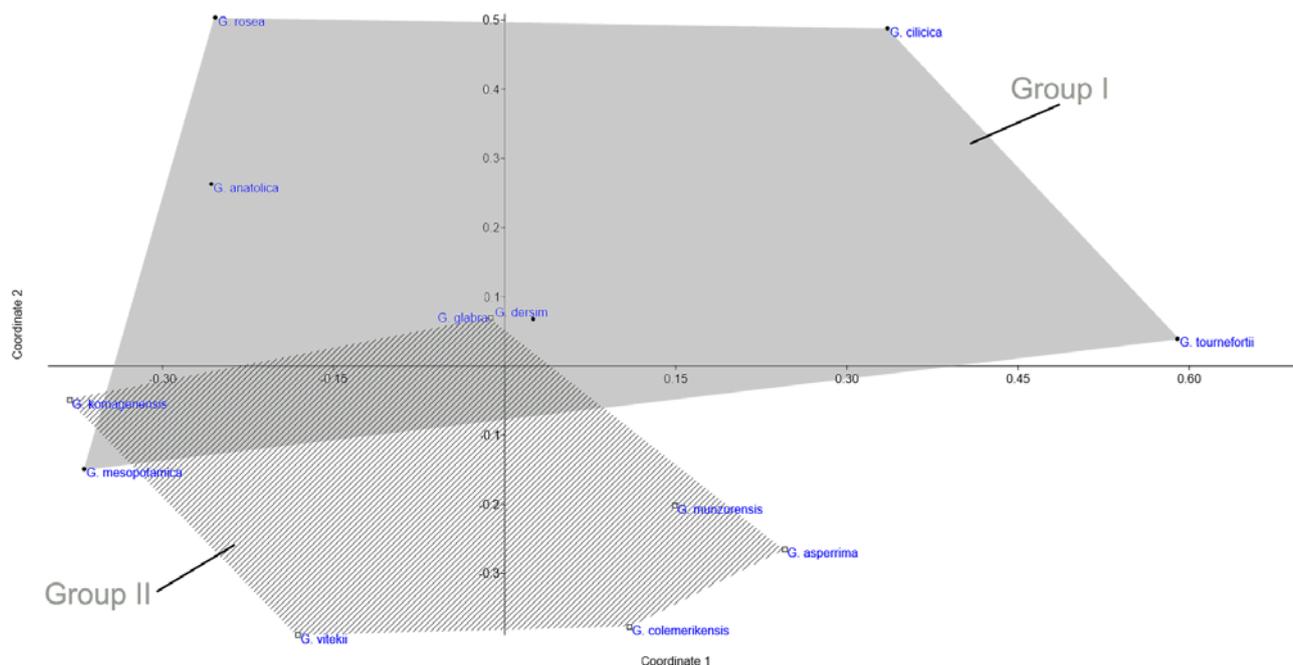


Fig. 5. PCoA analysis based on six quantitative karyological parameters of investigated taxa.

The chromosomes with secondary constriction ranged from 2 to 6 in number. The total haploid chromosome length (THL) varied from 24.97 μm (*G. asperima*) to 42.56 μm (*G. rosea*).

Karyotypes of the analysed species exhibit a predominance of metacentric chromosomes. However, one or two submetacentric chromosomes were detected in each taxon. Due to the prevalence of metacentric pairs and to the absence of strong differences between smaller and larger chromosomes, asymmetry indices were in general low. However, some species show a tendency to have karyotypes distinct on asymmetry grounds:

Gundelia rosea, with relatively high intrachromosomal (MCA) and *G. tournefortii* with low intrachromosomal asymmetry, also, *G. vitekii* with high interchromosomal (CVCL) and *G. anatolica* low interchromosomal asymmetry.

DISCUSSION

Karyotype data for all taxa are reported for the first time in the present study with the exception *G. tournefortii* and *G. rosea*. The present investigation on *Gundelia* supports earlier data about $2n = 2x = 18$ (Waisel 1962; Al-Taey and Hossain 1984; Ghaffari and Chariat-Panahi 1985; Nersesyan and Nazarova 1989; Ghukasyan and Janjughazyan 2015). The article by Nersesyan and Nazarova (1989) is the most detailed work

published on the karyology of *Gundelia tournefortii*. Three chromosome types were also seen in that study. However, according to our results, type B chromosome was not observed in *Gundelia tournefortii* chromosomes. However, earlier works accepted only one species in the genus, and this could be the cause of this difference.

According to Trautvetter (1876), flower number within the partial synflorescences differs between different populations of *Gundelia*. Moreover, the partial synflorescences in the middle part of the synflorescence are formed by 3 to 8 flowers according to the articles published in recent years. These flowers have been defined as “cephaloid flowers” in the following sections of the article. The taxa examined in this article were evaluated according to cephaloid flowers, and two groups have been recognised. Group I consists of mainly 3(-5) cephaloid flowers; Group II consists of mainly 6 or more cephaloid flowers (Table 3). In addition to this, according to chromosome types (A-C), taxa are divided into two main groups too. In agreement with our results, these groups are quite overlapping. Namely, type B chromosomes were present in all the taxa of Group I and no type B chromosomes was observed in the taxa of Group II with the exception of *G. mesopotamica* and *G. anatolica*. These species differ from others by having 6-8 cephaloid flowered and type B chromosomes. Also, *G. anatolica* is the only species in which there are no type C chromosomes. According to Tarikahya Hacıoğlu and Fırat (2017), *G.*

anatolica is a derived species. This difference in chromosomal morphology supports this argument.

According to PCoA analysis, the species belonging to the same group tend to cluster together substantially (Figure 5). The presence of type A and type B chromosomes does not show variation at intraspecific level, these chromosome types were observed in all metaphase stages. However, type A chromosomes showed some infraspecific variation.

In conclusion, this study illustrated that two different groups can be distinguished, according to chromosome morphology. Therefore, the chromosome types can be used, in addition to other characters, for species identification and classification in the genus *Gundelia*.

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