INTRODUCTION

Doronicum L. (Asteraceae: Senecioneae) comprises 26 species and 4 subspecies naturally growing in open or forest habitats from sea level up to 5000 m height in a geographic region extending from Europe and North Africa to Asia (Álvarez Fernández, 2003). The members of the genus are characterized by perennial rhizomatous herbs with alternate simple leaves, one to several radiate yellow-flowered capitula comprising an involucral of herbaceous phyllaries arranged in 2 or 3 rows, and cylindrical to obovate-cylindrical shaped cypsela with 10 longitudinal ribs (Álvarez Fernández et al., 2001). The first world-wide monograph of the genus Doronicum was carried out by Cavillier (1907, 1911). In recent years, more comprehensive taxonomic revisions of Doronicum which contributed to the systematics of the genus, have been performed on the basis of morphological and
molecular data (Álvarez Fernández & Nieto Feliner, 1999; Álvarez Fernández et al., 2001; Álvarez Fernández, 2003). Up to now, also many karyological (Lindqvist, 1950; Skalinska, 1950; Baksay, 1956; Contandriopoulos, 1957; Favarger & Küpfer, 1968; Lovka et al., 1972; Kuzmanov & Ancev 1973; Löve & Kjellqvis, 1974; Garbari et al., 1980; Van Loon, 1980; Belaeva & Siplivinsky, 1981; Van Loon & Oudemans, 1982; Kuzmanov & Georgieva, 1983; Strid & Franzén, 1983; Davliandize, 1985; Strid & Anderson, 1985; Chacón, 1987; Lippert & Heubl, 1988; Tasenkevitch et al., 1989; Vir Jee & Kachroo, 1989; Baltisberger, 1991; Ruiz de Clavijo, 1993; Pachschwöll et al., 2015) and chemical (Paolini et al., 2007; Lazarević et al., 2009; Bharti et al., 2012; Syed et al., 2014) studies including several Doronicum taxa have been reported on the genus.

The first taxonomic revision of Turkish Doronicum was performed by Edmondson (1975), who recognized 13 species and two subspecies. Since then, Álvarez Fernández (2003) has prepared the latest worldwide revision of Doronicum including several nomenclature and taxonomic clarifications, and listed 30 taxa, 10 of which also distributed in Turkey with approximately 30% endemism rate (D. cacaliifolium Boiss. & Heldr., D. haussknechtii Cavill. and D. reticulatum Boiss.). In the present paper, we preferred to use the plant names accepted in the taxonomic treatment of Álvarez Fernández (2003) for the studied taxa. Doronicum grows in various habitats as damp meadows, streamside, shady or rocky places in Juniperus L. scrub, Abies Mill. dominated woodland, and alpine and subalpine forest margins at the altitude of 50-2900 m in Turkey (Edmondson, 1975). The species of subsection Macrophylla Cavill. forming the majority of Doronicum species in Turkey, was considered to be a systematically difficult taxonomic group in which indumentum characters have great importance for distinguishing the taxa (Edmondson, 1975). There have been reported some chemical (Akpinar et al., 2009), ethnobotanical (Ugurlu & Secmen, 2007; Ari et al., 2015) and molecular (Kadıoğlu, 2013) studies including only a few Turkish Doronicum taxa.

Chromosome number data are valuable today in systematics. Although the documentation of chromosome number is a priority for the conservation of the world’s plant genetic resources, to date, chromosome number data of only approximately 25% of flowering plants have been determined (Stace, 2000; Garbari et al., 2012). Despite the great floristic richness of Turkey, only 15% of the vascular plant taxa have had their chromosome number investigated (Vladimirov et al., 2015). Very few counts have been reported in the literature previously for the genus Doronicum. The present study aims to report the chromosome number of seven Turkish representatives of Doronicum, two of which are endemic to Turkey. Our study will contribute to the knowledge of the chromosome number of the genus.

**MATERIAL AND METHOD**

**Sampling:** Locality information of 12 representatives of seven Turkish Doronicum taxa used for chromosome counting is given in Table 1, and the distribution of the examined taxa are shown in Figure 1 using Turkey Grid System (Davis, 1965). All specimens were first processed using the standard herbarium techniques given by Woodland (1997). They were identified using the Flora of Turkey and The east Aegean Islands (Edmondson, 1975), and stored at the Herbarium of Karadeniz Technical University, Department of Biology (KTUB).

![Figure 1](image-url). Distribution map of the examined taxa according to grid square system of Davis (1965) (Refer to Table 1 for explanation of accession no).
Table 1. Locality information of the examined specimens.

| No | Taxa                                      | Locality                        | Date         | Voucher number |
|----|-------------------------------------------|----------------------------------|--------------|----------------|
| 1  | *Doronicum haussknechti* Cavill           | A7 Giresun: Karagöl, 2620 m      | 02 viii 2002 | Umdu 23        |
| 2  | *Doronicum dolichotrichium* Cavill        | A7 Artvin: Ananc, Kaniş Plateau, 2200 m | 02 viii 2003 | Umdu 47        |
| 3  | *Doronicum macrophyllum* Fisch. subsp. *macrophyllum* | A7 Gümüşhane: Artabel Lakes Nature Park, 2200-2500 m | 24 vii 2003 | Umdu 91        |
| 4  | *Doronicum macrophyllum* Cavill           | A7 Gümüşhane: Akça Village, 1800 m | 02 viii 2002 | Umdu 26        |
| 5  | *Doronicum haussknechti* Cavill          | A7 Gümüşhane: Artabel Lakes Nature Park, 2000-2900 m | 06 viii 2003 | Umdu 83        |
| 6  | *Doronicum haussknechti* Cavill          | A7 Gümüşhane: Artabel Lakes Nature Park, 24 vii 2004 | 24 viii 2004 | Umdu 97        |
| 7  | *Doronicum maximum* Boiss. & A. Huet      | A7 Giresun: Balaban Mountain, 2700 m | 02 viii 2002 | Umdu 24        |
| 8  | *Doronicum oblongifolium* DC.            | A9 Artvin: Ananc, Küçüksu Mountain, 2700 m | 03 vii 2004 | Umdu 113       |
| 9  | *Doronicum orientale* Hoffm.             | A7 Gümüşhane: Kızılağaç Plateau, 1900 m | 05 vii 2002 | Umdu 21        |
| 10 | *Doronicum orientale* Hoffm.             | A7 Trabzon: K.T.U. Campus, 50 m  | 30 ii 2002   | Umdu 11        |
| 11 | *Doronicum reticulatum* Boiss.           | A7 Gümüşhane: Artabel Lakes Nature Park, 2800 m | 27 vii 2002 | Umdu 31        |

Cytological analyses: Actively growing root tips were used for somatic chromosome counts. At first, the roots of the living plants were cleaned of soil particles, and then 1–1.5 cm long root tips were cut off and pre-treated with 0.5 % colchicine for 3 hours in the field. Then the samples were treated to the fixation process using the Carnoy solution (3:1 absolute alcohol: glacial acetic acid) for at least 24 hours at +4°C. Fixed root tips were transferred to 70 % alcohol and stored at +4 °C until analyses. Afterwards, the root tips were hydrolyzed with 1 N HCl for 12 minutes at 60°C and stained with 2 % aceto-orcein for 24 hours at room temperature. Stained root tips were squashed in a drop of 45 % acetic acid, and the preparations were mounted in entellan to obtain permanent slides (Jones & Rickards, 1991; Elçi, 1994; Martin et al., 2012). The best metaphase plates, including at least ten well-spread cells, were photographed with Olympus BX51 microscope with a digital camera attachment. Also, the chromosomes were extracted from the permanent slides and counted individually by using both enlarged photographs (10 × 100) and drawings.

RESULTS

Chromosome counts of 12 accessions belonging to seven taxa of *Doronicum* from Turkey were presented in Figures 2-3. The somatic chromosome numbers were determined as 2n=2x=60 for all the examined taxa. To the best of our knowledge, these are the first chromosome counts for *D. haussknechti*, *D. dolichotrichum*, *D. maximum* and *D. reticulatum*.

*Doronicum haussknechti* Cavill. Syn: *Doronicum tobeyi* J.R.Edmondson: This species is one of the Turkish endemics growing in streams at an altitude of 2600 m in B6 and A7 squares (Edmondson, 1975; Umdu, 2005). IUCN threat category of *D. haussknechti* was considered as Near Threatened (NT) by Ekim et al., (2000). It proved to be 2n=2x=60 (Figure 2a-b) from one Turkish population.

*Doronicum dolichotrichum* Cavill. Syn: *D. hakkiaricum* Edmondson, *D. hyrcanum* Widd. & Rech. fil.: This species is a Euxine element which was described from Caucasia. It is distributed in mountain flushes, lush alpine meadows and streamside at the altitude of 2000-2900 m in A7, A9 and C9 squares (Edmondson, 1975; Umdu, 2005). It proved to be 2n=2x=60 (Figure 2c-d) from two Turkish accessions.

Figure 2. Somatic metaphases; a-b. *D. haussknechti* (Umdu 23, 2n=60), c-d. *D. dolichotrichum* (Umdu 47, 2n=60), e-f. *D. macrophyllum* subsp. *macrophyllum* (Umdu 97, 2n=60), g-h. *D. maximum* (Umdu 24, 2n=60) (a, c, e. photo, b, d, f. drawing, bar: 10 µm).
**Doronicum maximum** Boiss. & A.Huet:

This taxon is the most widespread species of subsect. *Macrophylla* Cavill in Turkey growing on the streamside and moist rocky places along with watercourses at the altitude of 2000-2700 m (Edmondson, 1975; Umdu, 2005). The somatic chromosome number was determined as 2n=2x=60 (Figure 2g-h) from one Turkish accession.

**Doronicum oblongifolium** DC.:

This species, one of the Caucasus originated *Doronicum* members, grows in open moist rocky places along with watercourses at the altitude of 2000-2700 m in the northeast of Anatolia (A9 square) (Edmondson, 1975; Umdu, 2005). The present study provided the diploid chromosome number of 2n=2x=60 for *D. oblongifolium* (Figure 3a-b) from one Turkish accession.

**Doronicum orientale** Hoffm.:

*D. orientale* is the most widespread species of the genus in Turkey which is largely restricted to the western half of the country in shady places in the forest and scrub at the altitude of 50-2000 m (Edmondson, 1975; Umdu, 2005). The somatic chromosome number of this species was determined as 2n=2x=60 (Figure 3c-d) from two Turkish accessions.

**Doronicum reticulatum** Boiss. Syn: *D. bithynicum* Edmondson, *D. bithynicum* subsp. *bithynicum*, *D. thirkei* Sch. Bip. ex Boiss.

This species, endemic to Turkey, is a E. Medit. element growing on rocky slopes and *Juniperus* scrub at the altitude of 1450-2800 m in A2(A), A3, A7, B2 and B3 squares (Edmondson, 1975; Umdu, 2005). IUCN threat category of *D. reticulatum* was considered as Vulnerable (VU) by Ekim et al., (2000). The somatic chromosome number was counted as 2n=2x=60 (Figure 3e-f) from one Turkish accession.

**DISCUSSION**

Bremer (1994) reported that the basic chromosome number of the genus *Doronicum* is x=30, which is characteristic for the “cacalioid” group in Senecionaceae. However, previous authors suggested x=10 for the genus (Fernandes & Queirós, 1971; Majoyský & Murín, 1987). The most recent chromosome counts related to many *Doronicum* taxa providing multiples of 30 (2n=60) supported the basic number of x=30 for the genus and indicated that polyploidy is common within the genus (Álvarez Fernández, 2003). The chromosome number was reported as 2n=60 for *D. glaciale* (Wulfen) Nym. and *D. grandiflorum* Lam. (Pachschwöll et al., 2015), 2n=120 for *D. plantagineum* L., (Löve & Kjellqvist, 1974; Ruiz de Clavijo, 1993) and *D. stiriacum* (Vill.) Dalla Torre (Murin, 1978; Pachschwöll et al., 2015) while some taxa showed both ploidy levels (e.g., *D. carpatum* Boiss. & Reuter ex Willk. & Lange 2n=60, 120 (Chacón, 1987), *D. clusii* (All.) Tausch 2n=60, 120 (Tasenkevitch et al., 1989; Huber & Baltisberger, 1992; Pachschwöll et al. 2015), *D. altaicum* Pall. 2n=30, 60 (Zhukova, 1967; Stepanov, 1994), *D. macrophyllum* 2n=30, 60 (Zhukova, 1967; Davlianidze, 1984), *D. oblongifolium* 2n=40, 60 (Zhukova, 1967; Davlianidze, 1985). In the present study, the somatic chromosome number of 2n=2x=60 was counted for the investigated seven taxa of *Doronicum* (Asteraceae) from Turkey. Our results are in agreement with the previous counts in the literature. To our knowledge, these results are new to science for *D. dolichotrichum*, a Euxine element (Figure 2c-d), *D. maximum*, the most widespread *Doronicum* in Turkey (Figure 2g-h), and the two Turkish endemic taxa, *D. haussknechtii* (Figure 2a-b) and *D. reticulatum* (Figure 3e-f). Most of the species investigated in this paper are distributed at alpine regions. Polyploidy and hybridization are considered among the major factors affecting high-mountain biodiversity for Asteraceae and also in *Doronicum* (Pachschwöll et al., 2015; Álvarez Fernández, 2003). Polyploid species are thought to be common in alpine environments due to their potential for increased adaptability to extreme conditions (Pachschwöll et al., 2015). However, the phenomenon stating the significant effect of polyploidization on patterns and diversification rates is still controversial (Otto & Whitton, 2000). Besides, Mas de Xaxars et al. (2016) hypothesized that compared to polyploidy, dysploidy could be more affective in evolutionary processes in high mountain *Artemisia*.

*D. macrophyllum* subsp. *macrophyllum* possesses 2n=2x=60 (Figure 2e-f). This is the first chromosome count for the taxon from a Turkish accession. Our result is consistent with the previous count reported by Zhukova (1967) in Russia. However, 2n=30 diploid chromosome number has been reported from Georgia for *D. macrophyllum* (Davlianidze, 1984). The geographical
distribution of the taxa has been considered among the main reasons for the ploidy level variation within the plant species (Morawetz, 1984). Ozcan et al. (2008) also reported that plant taxa originating from different geographical regions can have different chromosome numbers.

The present study provided the diploid chromosome number of 2n=60 for *D. oblongifolium* (Figure 3a-b) from a Turkish accession for the first time. Our result is in agreement with the counts by Sietozaroia (1967) and Zhukova (1967) from Russia. However, 2n=40 diploid chromosome number has been reported from Georgia for *D. oblongifolium* (Davlianidze, 1985). This is the only count that is inconsistent with 2n=30, and according to Álvarez Fernández (2003), it should be recounted. Our counting of 2n=60 for *D. orientale* (Figure 3c-d) is consistent with several previous counts reported for this species by Sietozaroia (1967) in Russia, Larsen & Laegaard (1971) in Sicilia, Strid & Anderson (1985) in Greek, Peruzzi et al., (2012) in Italy.

The chromosome numbers of seven *Doronicum* taxa are reported for the first time from Turkish accessions. Although no difference was determined among the examined species in terms of somatic chromosome number, the present paper has made an important contribution to the knowledge of the chromosome number of the genus *Doronicum*.

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