Karyological studies of *Clinopodium* L. (Sect. *Pseudomelissa*) and *Micromeria* Benth. s. str. (Lamiaceae) from Turkey

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Abstract — Using the squash method of preparation, somatic chromosome numbers were counted of *Clinopodium cilicicum*, *C. congestum*, *C. dolichodontum*, *C. serpyllifolium* subsp. *barbatum*, *C. serpyllifolium* subsp. *brachycalyx*, *C. serpyllifolium* subsp. *giresunicum*, *C. serpyllifolium* subsp. *serpyllifolium*, *Micromeria cremnophila* subsp. *anatolica*, *M. cristata* subsp. *phrygia*, *M. cristata* subsp. *orientalis*, *M. elliptica*, *M. graeca* subsp. *graeca*, *M. juliana*, *M. myrtifolia*, *M. nervosa* all of which grow naturally in Turkey. Chromosome numbers of 12 taxa are reported for the first time.

Key words: Chromosome number; Clinopodium; Micromeria; Turkey.

INTRODUCTION

The genus *Micromeria* Benth. s. str. is distributed from the Macaronesian-Mediterranean region to southeast Africa, India, and China, with species growing from the sea level to 4500 m of altitude (Bräuchler et al. 2008). Traditionally, *Micromeria* was considered to have four sections. Sect. Micromeria, Sect. Pseudomelissa, Sect. Pineolentia and Sect. Cymularia (HARLEY et al. 2004). The species of the section Pseudomelissa were transferred into the genus Clinopodium L. because the species of Pseudomelissa are similar to those of the genus Clinopodium in terms of having wide leaves and smooth leaf margins, and similar chromosome numbers. So the total number of the sections reduced to three. It is now accepted that the genus Micromeria has 54 species, 32 subspecies and 13 varieties (BRÄUCHLER et al. 2008).

The first revision of *Micromeria* s.l. species in Turkey was made by DAVIS *et al.* (1982), who

recognised 14 species (22 taxa). In the Flora of Turkey, the species were placed in three sections, Sect. *Micromeria* with seven species (12 taxa), Sect. *Cymularia* with one species, and Sect. *Pseudomelissa* with six species (nine taxa). After the transfer of the section *Pseudomelissa* from the genus *Micromeria* to *Clinopodium* by BRÄUCHLER *et al.* (2008), the genus is now represented by eight species in Turkey (DIRMENCI *et al.* 2010; ARABACI *et al.* 2010).

The closely related genera *Micromeria* s. str. Benth., *Clinopodium* L. s.l. (including *Acinos* Miller and *Calamintha* Miller) and *Cyclotrichium* (Boiss.) Manden. & Scheng. (Lamiaceae, Nepetoideae, Mentheae, Menthinae) have recently been revised on the bases of morphological, molecular, chemical, palynological, and cytological data and numerical taxonomy (DIRMENCI *et al.* 2010; Arabaci *et al.* 2010).

Micromeria s.l. has various somatic chromosome numbers of 2n = 20, 22, 26, 30, 48, 50 and 60 (Hedberg 1957; Morton 1962; Bjorkqvist et al. 1969; Gill 1971; Dahlgren et al. 1971; Cardona 1973; Hedberg and Hedberg 1977; Cardona and Contandriopoulos 1980, 1983; Fernandes and Leitão 1984; Bir and Saggoo 1981, 1985; Luque and Diaz Lifante 1991; Morales 1990a, b, 1991, 1992, 1993; Castro et al. 2007;

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Bräuchler *et al.* 2008). Similarly, *Clinopodium* s. str. has various somatic chromosome numbers of 2n = 18, 20, 22, 24 and 48 (Nilsson and Lassen 1971; Löve and Kjellqvist 1974; Ubera 1979; Fernandes and Leitão 1984; Morales 1990a, b 1994).

We hope by studying cytological features of *Clinopodium* Sect. *Pseudomelissa* and *Micromeria* s.str. a better understanding of these taxa relationships would be obtained in future.

MATERIALS AND METHODS

Seeds of the study taxa were collected from different localities of Turkey at fruiting times (Table 1). Collected specimens were deposited in Balikesir University, Education Faculty of Necatibey, and Herbarium of Department of Biology Education. Root tips were obtained by germinating mature seeds in a laboratory. The root tips were fixed in Carnov solution (3:1 absolute ethanol: glacial acetic acid) at +4°C overnight after pre-treatment in α-monobromonaphthalene at +4°C for 16 hours. After being hydrolyzed in 1N HCl, the root tips were stained with 2% aceto orcein. Preparations were made using the squash method. At least ten metaphase cells, ideal for counting somatic chromosome numbers, were used to determine chromosome numbers. Slides of these cells were placed in the investigation microscope (Olympus BX51), and then the photographs were transferred to the computer by camera attachment. Photographs of the chromosomes were taken from these slides by using Image Analysis System.

RESULTS AND DISCUSSION

16 taxa of the genus Clinopodium (Sect. Pseudomelissa) and Micromeria s.str. growing naturally in Turkey, Clinopodium cilicicum (Syn.: M. cilicica Hausskn. ex P.H. Davis), C. congestum (Syn.: M. congesta Boiss. & Hausskn. ex Boiss.), C. dolichodontum (Syn.: M. dolichodonta P.H. Davis), C. serpyllifolium subsp. barbatum (Syn.: M. fruticosa (L.) Druce subsp. barbata (Boiss. & Kotschy) P.H. Davis), C. serpyllifolium subsp. brachycalyx (Syn.: M. fruticosa (L.) Druce subsp. brachycalyx P.H. Davis), C. serpyllifolium subsp. giresunicum (Syn.: M. fruticosa (L.) Druce subsp. giresunica P.H. Davis), C. serpyllifolium subsp. serpyllifolium (Syn.: M. fruticosa (L.) Druce subsp. serpyllifolium (Syn.: M. fruticosa (L.) Druce subsp. serpyllifolia (Bieb.) P.H. Davis), Microme-

ria cremnophila subsp. amana, M. cremnophila subsp. anatolica, M. cristata subsp. phrygia, M. cristata subsp. orientalis, M. elliptica, M. graeca subsp. graeca, M. juliana, M. myrtifolia, M. nervosa were examined cytogenetically (Table 1, Figs. 1-16).

Somatic chromosome numbers of Clinopodium serpyllifolium subsp. barbatum, C. serpyllifolium subsp. brachycalyx, C. serpyllifolium subsp. giresunicum, C. serpyllifolium subsp. serpyllifolium, C. cilicicum, C. congestum, C. dolichodontum, Micromeria cremnophila subsp. amana, M. cremnophila subsp. anatolica, M. cristata subsp. phrygia, M. cristata subsp. orientalis and M. elliptica were counted for the first time.

Among studied taxa, chromosome numbers of some taxa were reported by different investigators at different times. For instance, chromosome number of Micromeria fruticosa was reported as 2n = 22 (Morales 1991). Having a chromosome number of 2n=22 in the world, Clinopodium serpyllifolium (Syn.: M. fruticosa) has four subspecies in Turkey. In this study, we determined somatic chromosome numbers of all of these subspecies. It is an interesting situation that the taxa of *C. serpyllifolium* subsp. brachycalyx, C. serpyllifolium subsp. giresunicum, C. serpyllifolium subsp. serpyllifolium have a chromosome number of 2n = 22 agreeing with the literature while the other subspecies Clinopodium serpyllifolium subsp. barbatum has a chromosome number of 2n = 16. At the end of the preparations made repeatedly, a new somatic chromosome number of 2n = 16 was observed for the subspecies C. serpyllifolium subsp. barbatum for the first time.

According to the literature, *Micromeria graeca* subsp. *graeca* has 2n=60 chromosomes (Uhrikova and Ferakova 1980; Morales 1990a, b). However, we observed diploid chromosome number of 2n=30 for the subspecies *M. graeca* subsp. *graeca* justifying the former results from polyploidy.

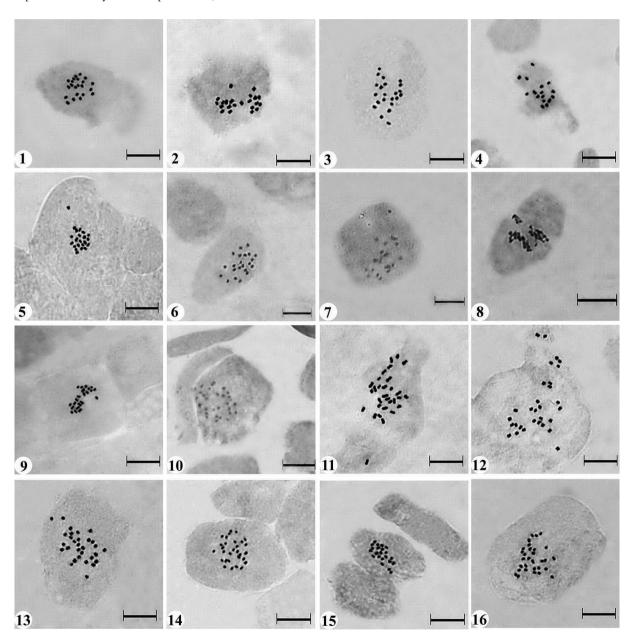
Micromeria juliana was examined cytogenetically by different researchers at various times, and it was reported that the species has a chromosome number of 2n=30 (Papes and Silic 1981; Markova 1989; Fernandes and Leitão 1984). In our study, chromosome number counted for the species M. juliana is in agreement with previous studies.

In a study performed by DIAZ LIFANTE *et al.* (1992), it was reported that the species *Micromeria nervosa* has a chromosome number of 2n=30. Our study agrees with the literature in

terms of karyological data obtained for *M. nervosa* as in *M. juliana*.

The taxa of the genera Micromeria and Clinopodium, Micromeria biflora, M. filiformis subsp. cordata (Syn.: M. cordata), M. filiformis, M. fontanesii, M. graeca subsp. graeca, M. inodora, M. juliana, M. nervosa, M. sphaciotica, M. imbricata var. imbricata (Syn.: M. punctata) Clinopodium serpyllifolium (Syn.: M. fruticosa), C. capitellatum (Syn.: M. capitellata), C. dalmaticum

(Syn.: M. dalmatica), and C. thymifolium (Syn.: M. thymifolia), were studied cytogenetically so far (VILLA 1978; Uhrikova and Ferakova 1980; Papes and Silic 1981; Bir and Saggoo 1981, 1984, 1985; Cardona and Contandriopoulos 1980, 1983; Saggoo 1983; Fernandes and Leitão 1984; Gill 1984; Montmollin 1986; Markova 1989; Morales 1990a, b 1991; Diaz Lifante et al. 1992; Khatoon and Ali 1993; Vogt and Oberprieler 1994).



Figs. 1-16 — Metaphase chromosomes of Clinopodium and Micromeria taxa. (1) C. cilicicum. (2) C. congestum. (3) C. dolichodontum. (4) C. serpyllifolium subsp. barbatum. (5) C. serpyllifolium subsp. brachycalyx. (6) C. serpyllifolium subsp. giresunicum. (7) C. serpyllifolium subsp. serpyllifolium. (8) M. cremnophila subsp. amana. (9) M. cremnophila subsp. anatolica. (10) M. cristata subsp. phrygia. (11) M. cristata subsp. orientalis. (12) M. elliptica. (13) M. graeca subsp. graeca. (14) M. juliana. (15) M. myrtifolia. (16) M. nervosa. Bar = 10 μm.

Table 1. Localities and collector numbers of studied Clinopodium and Micromeria taxa.

Taxon	Locality	Collector no	Chromosome counts
Clinopodium cilicicum (Hausskn. ex P.H. Davis) Bräuchler & Heubl.	C5 Icel: Gozne, 1200 m, 07.08.2007	T. Dirmenci (3486) & T. Arabaci	2n=22
C. congestum (Boiss. & Hausskn. ex Boiss.) Kuntze	C6 Gaziantep: Dülükbaba, Rock Tombs, 08.08.2007	T. Dirmenci (3500) & T. Arabaci	2n=22
C. dolichodontum (P.H. Davis) Bräuchler & Heubl.	C4 Icel: Between Gulnar and Ermenek, Gunesli Village Road, burned cedar forest, 13.08.2005	T. Dirmenci (3091-a) & T. Arabaci	2n=22
C. serpyllifolium (M. Bieb.) Kuntze subsp. barbatum (P.H. Davis) Bräuchler	C6 Hatay: Yayladag, east of Keldag, 1200 m, 13.08.2005	T. Dirmenci (3085) & T.Arabaci	2n=16
C. serpyllifolium (M. Bieb.) Kuntze subsp. brachyadyx (P.H. Davis) Bräuchler	C6 Hatay: East of Iskenderun, Highway, 50. km, 13.08.2005	T. Dirmenci (3087) & T. Arabaci	2n=22
C. serpyllifolium (M. Bieb.) Kuntze subsp. giresunicum (P.H. Davis) Bräuchler	A7 Giresun: Between Dereli and Sebinkarahisar, Halil Rıfat Pasa Tunnel, 1025 m, Pınarlı Village 5 km south, 04.08 2008	T. Dirmenci (3666) & Akcicek	2n=22
C. serpyllifolium (M. Bieb.) Kuntze subsp. serpyllifolium	A9: Artvin: Artvin-Savsat road, 15. km, 415 m, 02.09.2008	T. Dirmenci (3645) & Akcicek	2n=22
M. crennophila Boiss. & Heldr. subsp. amana (Reich, fil.) P.H., Davis	C5 Adana: Pozantı, Alpu Village, Karinca Mount, Radar surrounding, 1650 m, 07.07 2007	T. Dirmenci (3450) & Akcicek	2n=30
M. crennophila Boiss. & Heldr. subsp. anatolica P.H. Davis	B6 Malatya: Between Malatya and Darende, Develi Village 1 km east, 4795 ft, 10.08 2007	T. Dirmenci (3504) &T. Arabaci	2n=22
M. cristata (Hampe) Griseb. subsp. phrygia P.H. Davis	B2 Kutahya: Between Kutahya and Gediz, 18. km, rocky slopes, 12.09.2008	T. Dirmenci (3665) & Akcicek	2n=30
M. cristata (Hampe) Griseb. subsp. orientalis P.H. Davis	B6 Malatya: Between Malatya and Darende, Develi Village 1 km east, 4795 ft, 10.08 2008	T. Dirmenci (3505) & T. Arabaci	2n=30
M. elliptica C. Koch	A9 Artvin: Artvin-Ardanuç road, 1-2. km, 400 m, east of The River of Coruh, rocky places, 27.06.2008	T. Dirmenci (3610) & Akcicek	2n=30
M. graeca (L.) Bentham ex Reichb. subsp. graeca	A1 (E) Istanbul: Halkalı, opposite to train station, rocky places, 08.06.2008	T. Dirmenci (3681) & Akcicek	2n=30
M. juliana (L.) Benth. ex Reichb.	B1 Balıkesir: Kazdagı, Evkayasi surrounding, 1300-1600 m, 04.07.2006	T. Dirmenci 3215	2n=30
M. myrtifolia Boiss. & Hohen.	C1 Mugla: Datca, Knidos Ruins, 30-200 m, 19.05.2006	T. Dirmenci (3111) & B. Yildiz	2n=22
M. nervosa (Desf.) Benth.	C2 Mu la: Datça, Knidos, rocky limestone slopes, 30-200 m, 19.05.2006	T. Dirmenci (3112) & B. Yildiz	2n=30

In a revision study on *Micromeria* in the Arabian Peninsula and tropical regions of South Africa, Ryding (2007) reported that various collections of two different varieties of *M. imbricata* have a chromosome number of 2n=30 according to previous cytological studies (Hedberg 1957; Morton 1962; Hedberg and Hedberg 1977; Morales 1990a, b). Ryding also reported that chromosome numbers of many taxa of the genus are the same, i.e. 2n=30 (Gill 1971; Bir and Saggoo 1981, 1985; Morales 1993). Of the taxa of *Micromeria* s. str. in our project, some taxa were determined as having somatic chromosome number of 2n=30.

Various different chromosome numbers have previously been reported for *Micromeria inodora*, i.e. 2n=26, 30, 48, 50 and 60 (CARDONA 1973; CARDONA and CONTANDRIOPOULOS 1983; MORALES 1990a,b, 1993; BRÄUCHLER *et al.* 2008). Similarly, *Micromeria filiformis* has two different chromosome numbers of 2n=30 and 60 (DAHLGREN *et al.* 1971; CARDONA and CONTANDRIOPOULOS 1980; MORALES 1990a, b).

MORALES (1992) reported that the most frequent chromosome number in Micromeria is 2n=30. Morales (1992) also showed that one population of M. graeca has a chromosome number of 2n=20 and five populations of the species have 2n=60 chromosomes while three populations of M. capitellata have 2n = 50, and M. inodora has 2n = 26 and 48 chromosomes. Morales (1992) also counted chromosome numbers of species in the section *Pseudomelissa*, counting 2n=20 chromosomes for Clinopodium dalmaticum and C. thymifolium, and 2n=22 chromosomes for Clinopodium serpyllifolium. In a chemical, palynological and karyological study on the species of *Microme*ria growing in the Iberian and Balearic Islands, the chromosome number of Clinopodium serpyllifolium was reported as 2n=22 for the first time MORALES (1991). In that publication, it was also reported that in karyological studies conducted previously, the species of M. filiformis have 2n=30and 60, M. graeca 2n=20, M. inodora 2n=26 and 48, M. juliana 2n=30 chromosomes (Bjorkovist et al. 1969; Dahlgren et al. 1971; Cardona 1973; CARDONA and CONTANDRIOPOULOS 1980, 1983; Fernandes and Leitáo 1984).

In a revision of the genus *Micromeria*, diploid chromosome numbers were counted only for the species M. *imbricata* reporting various numbers of 2n=20, 26, 30, 48 and 60 as in many other taxa of the genus (Bräuchler *et al.* 2008).

In a different karyological study on the genus *Micromeria*, the chromosome numbers of the sections *Micromeria* and *Clinopodium* (Sect. *Pseudo*-

melissa) were reported as 2n = 20, 26, 30, 48, 50,60 and 2n = 20, 22, and 30, respectively (MORALES 1993). Of the studied taxa, only the species M. nervosa and M. juliana have a chromosome number of 2n=30 agreeing with the literature while the other taxa differ from the literature in terms of chromosome number. Considering the fact that there can be variations of chromosome numbers even within the same taxon of the genus *Microme*ria, these disagreements in chromosome numbers with the literature are not surprising. For instance, M. graeca has 2n=20 and 60 chromosomes in the literature while the chromosome number of M. graeca subsp. graeca was cited as 2n=30 in the Flora of Turkey. Actually, it is not surprising that the subspecies has triploid chromosome number (2n=3x=30) considering the basic chromosome number of x = 10, and the number given in the literature confirms that the taxon shows hexaploidy (Morales 1993). Thus, the existence of polyploidy in this taxon is obvious. Similarly, M. cremnophila was cited in the literature as having a diploid chromosome number of 2n=30 (Morales 1993). The somatic chromosome number of M. cremnophila subsp. amana is 2n=30 being in agreement with the literature while that of *M. cremnophila* subsp. anatolica is 2n=22 differing from the literature. The chromosome number of M. myrtifolia was reported as 2n=30 in the literature while it was counted as 2n=22 in our study. This difference results from locality difference as in many other species of the genus.

Represented by five subspecies in Flora of Turkey, M. cristata was found having a chromosome number of 2n=30 (Morales 1993). Morales (1993) reported that the most frequent chromosome number in M. cristata subsp orientalis and M. cristata subsp. phrygia were counted as 2n=30 as while three other subspecies, i.e. M. cristata subsp. carminea, M. cristata subsp. cristata, M. cristata subsp. xylorrhiza, could not be germinated during our project, presumably because of dormancy. Consequently, root tips could not be obtained even though many methods were tried out to break the dormancy such as cold shock, warm shock, red light, dark-light media, various temperature periods etc. The same situation was observed in the species Clinopodium caricum (Syn.: M. carica) and C. molle (Syn.: M. mollis) so the chromosome numbers of these species could not be determined.

Clinopodium serpyllifolium (Syn.: M. fruticosa) has a diploid chromosome number of 2n=22 in the literature (MORALES 1993). In our project, the diploid chromosome numbers of its subspecies were

found to be 2n=22 as in the literature, except for the subspecies C. serpyllifolium subsp. barbatum whose somatic chromosome number was found to be 2n = 16 which is the first report for the genus Clinopodium s.l. Actually, Morales (1993) stated the chromosome number of M. inodora as 2n = 48, marking it with a question mark. Assuming that MORALES' result for the species M. inodora is true, it could be possible to state that the basic chromosome number of the genus is x = 8. Fernandes and LEITÁO (1984) gave basic chromosome numbers of x=5, 10 and 15 for the taxa of *Micromeria* s.l. According to karyological data obtained from our study, the fact that the chromosome number of C. serpyllifolium subsp. barbatum is 2n=16 implies an extra basic chromosome number of x=8 for the genus. This situation is important in the light of assessing the genus cytogenetically. Our study provides an insight into eliminating the question marks (M. inodora 2n = 48) in previous studies.

Clinopodium alpinum (L.) Kuntze (Syn.: Acinos alpinus (L.) Moench, C. acinos (L.) Kuntze (Syn.: Acinos arvensis (Lam.) Dandy) and C. graveolens subsp. rotundifolium (Pers.) Govaerts (Syn.: Acinos rotundifolius Pers.), were reported having diploid a chromosome number of 2n=18 (LÖVE and Kiellovist 1974; Ubera 1979; Fernandes and LEITÃO 1984; MORALES 1990a, b). In our study, the taxa of *Clinopodium* Sect. *Pseudomelissa* have 2n=16 and 22 chromosomes. It is usual finding different chromosome numbers for genera. In our study, the somatic chromosome number of 2n = 18was not observed for the taxa of *Clinopodium*.

The taxa of *Clinopodium nepeta* subsp. glandulosum (Req.) Govaerts (Syn.: Calamintha ascendens Jordan), C. menthifolium subsp. ascendens (Jord.) Govaerts (Svn.: Calamintha sylvatica subsp. ascendens (Jordan) P.W. Ball) and C. nepeta (L.) Kuntze (Syn.: Calamintha nepeta (L.) Savi subsp. nepeta (L.) Savi were reported having two different chromosome numbers of 2n = 24 and 48(Nilsson and Lassen 1971: Fernandes and Leitão 1984; Morales 1994).

In a cytological study on the taxa *Clinopodium* vulgare subsp. arundanum (Boiss.) Nyman and C. vulgare L. subsp. vulgare L., the chromosome numbers were reported as 2n=20 for both taxa (FERNANDES and LEITÃO 1984). However, diploid chromosome number of the taxa of Clinopodium is found to be 2n = 16 and 22.

In a previous study on the species Satureja innota (Pau) G. López, S. montana L. and S. obovata La G. chromosome numbers of these species were determined as 2n = 30 (LÖVE AND KJELLQVIST 1974; LÓPEZ GONZÁLEZ 1982). The genus Micromeria

s.str. also has taxa which have chromosome number of 2n=30 (M. nervosa, M. juliana, M. graeca subsp. graeca, M. cremnophila subsp. amana, M. elliptica, M. cristata subsp. phrygia and M. cristata subsp. *orientalis*). This is significant due to the fact that some taxa of the genus Micromeria were situated in the genus *Satureja* previously.

In general, the genera of the family Lamiaceae close to Micromeria, i.e. Acinos, Calamintha, Clinopodium, Cyclotrichium, Satureja and Melissa, differ from each other cytogenetically. In terms of somatic chromosome numbers, for instance, Acinos has 2n = 18, Calamintha 2n = 24, 46 and 48, Cyclotrichium 2n=16, Clinopodium 2n=18, 20, 22, 24, 36 and 48, Micromeria 2n=20, 22, 26, 30, 48, 50 and 60, Satureja 2n=30, Melissa 2n=32, Mentha 2n = 18, 20, 24, 36, 40, 48, 50, 72, 96, 120 chromosomes according to the literature (NILSSON and Lassen 1971; Cardona 1973; Löve and Kiellovist 1974; HARLEY and BRIGHTON 1977; UBERA 1979; LOPEZ 1982; CARDONA and CONTANDRIOPOULOS 1983; FERNANDES and LEITÃO 1984; LUQUE and LIFANTE 1991; MORALES 1990a,b 1991, 1994; CHAMBERS and HUMMER 1994; BRÄUCHLER et al. 2008; DIRMENCI et al. 2010).

By determining chromosome numbers of the taxa of *Clinopodium* and *Micromeria*, this study highlights the way for future studies.

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