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The Karyotype of Three *Limonium* Miller Species in the Family of Plumbaginaceae Conducted Using Image Analysis System

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Abstract: In this research, a karyological analysis on three naturally growing *Limonium* Miller species were made using Image Analysis System. The species studied are; *Limonium iconicum* (Boiss. and Heldr.) O. Kuntze, *L. lilacinum* (Boiss. and Bal.) Wagenitz and *L. globuliferum* (Boiss. and Heldr.) O. Kuntze. The chromosome numbers are determined as 2n = 34 in *L. iconicum*, as 2n = 36, in *L. lilacinum* and as 2n = 18 in *L. globuliferum*. The chromosome lengths in these taxa varied between 1.44 to 6.10 µm. The karyotype formula of the species studied were; in *L. iconicum* 10m+5sm+2T, in *L. lilacinum* 7m+10sm+1T and in *L. globuliferum* 4m+5sm. The ideograms of each species were arranged according to the decrease in their length.

Key words: Chromosome number, karyomorphology, Limonium, Plumbaginaceae

INTRODUCTION

Plumbaginaceae is a cosmopolitan family distributed widely in the temperate zones of the Northern Hemisphere. It prefers arid or saline, often coastal, environments (Kubitzki, 1993; Lledó *et al.*, 2005).

Limonium Mill. genus is represented with approximately 400 taxa and 12 sections throughout the world (Febles and Pérez-Rodríguez, 2004). Limonium is represented with 21 taxa (five sections) in Turkey (Bokhari and Edmondson, 1982; Yildirimli and Koca, 2006).

Limonium species (L. iconicum and L. lilacinum) are labeled locally by the Turkish people as eşek kulağı and deve kulağı. Whereas the fresh leaves of this species are eaten by animals, the dried florescences are used as drog.

Previous studies on the chromosome numbers of the Limonium genus determined the chromosome number as 2n = 12, 14, 16, 17, 18, 24, 25, 26, 27, 28, 32, 33, 34, 35, 36,42, 43, 51 and 54. The studies have proven that this genus has four different basic chromosome number (x = 6, 7, 8and 9) (Michaelis, 1964; Febles, 1989; Borgen, 1969; Van Loon, 1974; Papatsou and Phitos, 1975; Erben, 1978, 1979, 1993; Brullo and Pavone, 1981; Artelari and Kamari, 1986; Goldblatt, 1988; Artelari et al., 1992; Ardevol Gonzales et al., 1993; Arrigoni and Diana, 1993; Sáez et al., 1998; Rosselló et al., 1998; Goldblatt and Johnson, 2000; Palacios et al., 2000; Lledó et al., 2003; 2005). The main objectives of this study are to report new counts for three species of Limonium collected in Turkey and to analyses in detail the karyotypes of these three Turkish species using Image Analysis System.

MATERIALS AND METHODS

Seed material was collected in Turkey in 2005 (Table 1). Vouchers have been deposited at the herbaria of Afyonkarahisar Kocatepe University, Department of Biology.

Mitotic chromosome preparations were prepared from root meristems obtained from germinating seeds. Root tips were pretreated for 16 h in α-monobromonaphthalene at 4°C, fixed in 3:1 absolute alcohol-glacial acetic acid, then the root tips were hydrolyzed with 1 N HCl for 3 min at 60°C overnight and stained with 2% aceto-orcein for 2 h at room temperature. Stained root tips were squashed with a drop of 45% acetic acid and permanent slides were made by mounting with Depex. We examined slides under Olympus BX-50 Photomicroscope using an oil immersion objective (100 X). Photographs were taken using the same microscope. The ideogram was taken only of five well spread metaphase plates and measurements were made on enlarged micrographs. The classification of chromosomes, the length of long and short arm, arm ratio, centromeric index and relative chromosomal length were measured using Software Image Analyses (BsPro200) loaded on a personal computer. The classification of chromosomes into median (m), submedian (sm), subterminal (st) and

Table 1: The localities of studied specimens in Limonium

	Chromosome				
Taxon	No. (2n)	Locality	Voucher		
Limonium iconicum	34	Afyonkarahisar	Kala 1531		
Limonium lilacinum	36	Afyonkarahisar	Kala 1309		
Limonium globuliferum	18	Afyonkarahisar	Kala 1530		

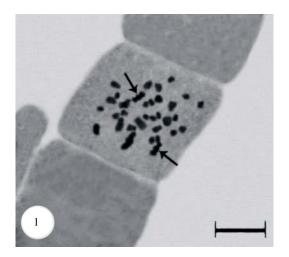
terminal point (T) was based on the analysis of metaphase chromosomes (Levan *et al.*, 1964). Ideograms of these taxa were arranged in decreasing length.

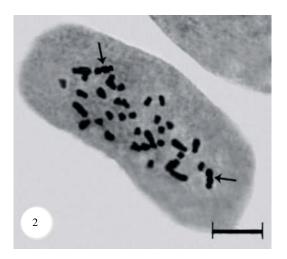
RESULTS AND DISCUSSION

The karyomorphological features reported here have not been reported in previous studies on the species of Limonium iconicum, L. lilacinum and L. globuliferum. The karyological data of all species are given in Table 2 and Fig. 1-3. The number of somatic chromosomes is 2n = 34 in Limonium iconicum, 2n = 36 in Limonium lilacinum and 2n = 18 in Limonium globuliferum. The basic chromosome number is x = 9, 17 and 18. The karyotype formulas are different in these three Limonium species. One pair of satellite in metaphase chromosomes is identified in Limonium iconicum and Limonium lilacinum. In this research no polyploidy is observed.

Section: Limonium: Limonium iconicum (Boiss. and Heldr.) O. Kuntze is found in Afyonkarahisar: around the salty area in Heybeli hot spring, at the altitude of 1000 m. on 25.07.2006, Kala 1531. It had a somatic chromosome number of 2n = 34 (Fig. 1). The Karyotype formula, chromosome morphology total, arm ratio and centromeric index are shown in Table 2. At mitotic metaphase phase, ten pairs of median (m), five pairs of submedian (sm) and two pairs of terminal point (T) chromosomes are observed. The chromosome lengths varied between 1.44 to $4.67 \, \mu m$. The total haploid chromosome length is measured as $42.51 \, \mu m$. One pair of satellite in metaphase chromosomes is identified. The ideogram of this species, endemic to Turkey, is given in Fig. 4.

Section: Sphaerostachys Boiss: Limonium lilacimum (Boiss. and Bal.) Wag. is found in Afyonkarahisar: Çay, in the salty grass areas in the surroundings of Lake Eber, at the altitude of 950 m. on the 20.07.2005, Kala 1309. 2n = 36 is the somatic chromosome number of this species (Fig. 2). The karyotype formula, chromosome morphology, arm ratio and centromeric index are summarized in Table 2. At the mitotic metaphase phase seven pairs of median (m), ten pairs of submedian (sm) and one pair of terminal point (T) chromosomes are observed. The chromosome lengths are between 1.53 to 2.76 μ m. The total haploid chromosome length is measured as 48.23 μ m. One pair of satellite in metaphase chromosomes is identified in this species endemic to Turkey. The ideogram is given in Fig. 4.





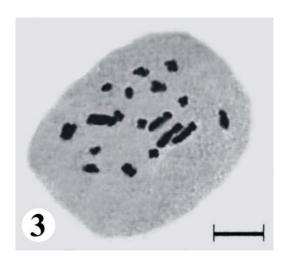


Fig. 1-3: (1) Limonium iconicum (2n = 34), (2). L. lilacinum (2n = 36) and (3). L. globuliferum (2n = 18). Scale Bar: $10 \mu m$

Table 2: Karyotype details in taxa of Limonium

Taxon	Chromosome No. (2n)	Chromosome sizes (µm)	AR	CI	THC (µm)	M	SM	T	X
Limonium iconicum*	34	1.44-4.67	1.96	1.95	42.51	10	5	2	17
Limonium lilacinum*	36	1.53-2.76	1.92	1.90	48.23	7	10	1	18
Limonium globuliferum	18	1.86-6.10	1.87	3.76	29.61	4	5	-	9

AR: arm ratio; CI: Centromeric index; THC: Total length of haploid complement; M: Median; SM: Submedian; T: Terminal point; X: Basic chromosome number, * one pair of satellites in the chromosome pairs is marked with an asterisk

Section: Sphaerostachys Boiss: Limonium globuliferum (Boiss. and Heldr.) O. Kuntze has been located in Afyonkarahisar: around the salty area of Heybeli hot spring at the altitude of 1000 m, on the 25.07.2006, Kala 1530. The somatic chromosome number of this species is 2n = 18 (Fig. 3). The karyotype formula, chromosome morphology, arm ratio and centromeric index of this species are shown in Table 2. At the mitotic metaphase stage four pairs of median (m), five pairs of submedian (sm) chromosomes are observed. The chromosome length varied from 1.86 to 6. 10 μm. Total haploid chromosome length is measured as 29.61 μm. The ideogram of this species is given in Fig. 4.

The results of the present study are the first identifying the chromosome numbers and morphologies of the species *Limonium iconicum*, *L. lilacinum* and *L. globuliferum*. The diploid chromosome numbers are determined as 2n = 34 for *L. iconicum*, as 2n = 36 for *L. lilacinum* and as 2n = 18 for *L. globuliferum*. The chromosome numbers identified is in line with previous studies on specimens from different localities (Erben, 1978, 1979, 1993; Brullo and Pavone, 1981; Artelari and Kamari, 1986; Goldblatt, 1988; Artelari *et al.*, 1992; Ardevol Gonzales *et al.*, 1993; Sáez *et al.*, 1998; Rosselló *et al.*, 2003, 2005). Table 3 shows previous counts in some taxa of *Limonium*.

Limonium genus diverges into two sections according to Turkish flora enlistments (Limonium and Sphaerostachys). The species in the present study have proven three different somatic chromosome and basic chromosome numbers.

L. iconicum species is endemic to Turkey. The diploid chromosome number is 2n = 34 and the basic chromosome number is defined as x = 17. This taxon has the largest arm ratio (1.96) and the length of the smallest chromosome number is (1.44 μ m). The karyotype formula of this species is 10m+5sm+2T. In metaphase chromosomes a pair of satellite is determined.

L. lilacinum species is also endemic to Turkey. 2n = 36 is diploid chromosome number of this species and the basic chromosome number is x = 18. It has the largest haploid chromosome length (48.23 μ m) of this species; but at the same time it also has the smallest centromeric index (1.90). The karyotype formula is 7m+10sm+1T. A pair of satellite is determined in the metaphase chromosomes of this species.

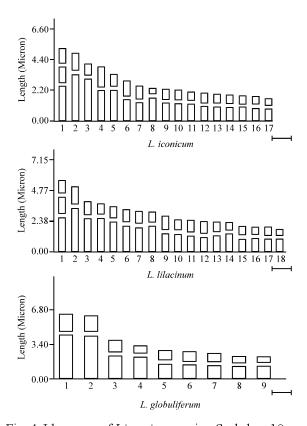


Fig. 4: Ideograms of *Limonium* species. Scale bar: 10 μm

In the Karyological literature review on *Limonium* genus taxa no satellite has been reported. However, in the present karyological study, a double pair of satellite chromosomes is determined in the mitotic metaphase chromosomes of *L. iconicum* and *L. lilacinum* species.

Contrary to *L. lilacinum* and *L. iconicum*, *L. globuliferum* species is not endemic to Turkey. It has a diploid chromosome number of 2n = 18. The basic chromosome number is x = 9 and has the largest centromeric index (3.76) and chromosome length $(6.10 \, \mu m)$ among the species of the present study. This taxon; nevertheless, has the smallest arm ratio (1.87) and total haploid chromosome length $(29.61 \, \mu m)$. The Karyotype formula of this taxon is 4m + 5 sm and there are not any terminal point metaphase chromosomes contrary to the other species.

Regarding this genus in the literature, the chromosome number of only four taxa is reported on the Turkish flora (Papatsou and Phitos, 1975; Erben, 1978;

Davis et al., 1988; Artelari et al., 1992; Güner, 2000). Cytological studies and their results on the Limonium taxa are given in Table 3. Erben (1978, 1979) noted that within section Limonium, diploid species show two basic chromosome numbers (n = 8 and n = 9) and postulated that the complement of the polyploidy arose through several combinations, involving reduced and unreduced gametes. Hence, triploid (2n = 24, 25, 26 and 27) and tetraploid (2n = 34, 35 and 36) taxa combine genomes of the two basic cytotypes (Palacios et al., 2000).

Myriolepis section, which found in Limonium genus, separated from Limonium genus and constituted a new genus (Lledó et al., 2003). According to the literature, L. diffusum and L. ferulaceum, previously in section Myriolepis, the diploid chromosome numbers were defined as 2n = 16 (Erben, 1978, 1979). However the basic chromosome number of Limonium subgenus (e.g., L. cymuliferum) were defined as x = 8 and x = 9. L. ferulaceum species' chromosome are more or less equal and consists of median metaphase chromosomes, but in L. diffusum species, the chromosome lengths are not equal. However, L. sinuatum (subgenus Pteroclados) has the same chromosome number and their karyotypes consist completely of subterminal chromosome pairs and are declared to be of equal size. In the same literature, Siphonantha and Polyarthrion sections have diploid chromosome number 2n = 18 (Lledó et al., 2003). In our research only in Limonium globuliferum out of the three Limonium species diploid chromosome number is observed as 2n = 18. Hence, Siphonantha and Polyarthrion sections' somatic chromosome numbers are compatible with the literature. The karyotype formula of Limonium species differs with the literature. This fact may stem from diversity in the locality.

In the karyological studies on Pteroclados section, endemic to Canary Islands, nine species and one subspecies of Limonium genus in the Nobiles subsection, all taxa are reported to have 2n = 14 somatic chromosome numbers. The basic chromosome number was reported as x = 7 (Febles and Pérez-Rodríguez, 2004). The karyotype formulas are reported generally as 7 m chromosome pairs (except L. bourgeaui and L. puberulum). L. bourgeaui and L. puberulum karyotype formulas are mentioned as 6m+1sm. In this study, the somatic chromosome numbers of Limonium species diverge from the above-mentioned literature in regard to the number and morphology of the chromosomes.

Erben and Arán introduced *Limonium mateoi* species to literature in 2005 and defined its somatic chromosome number as 2n = 18 in line with our finding on the chromosome number of *L. globuliferum*.

The chromosome numbers 16 plant taxa endemic the North Cyprus was defined in 2006 by Yıldız and Gücel. Limonium albidum (Guss.) Pignatti subsp. cyprium

Table 3: Previous counts in som Taxon	2n	Authors
L. graecum var. hyssopifolium	42	Papatsou and Phitos (1975)
L. sinuatum	16	Erben (1978, 1979)
L. virgatum	27	Erben (1978, 1979)
L. diffusum	16	Erben (1978, 1979)
L. ferulaceum	16	Erben 1978, 1979)
L. cymuliferum	16	Erben (1978, 1979)
L. belli difôlium	18	Artelari et al. (1992)
L. narbonense	36	Palacios et al. (2000)
L. vulgare	36	Palacios et al. (2000)
L. camposanum L. gymnesicum	27 27	Palacios et al. (2000) Palacios et al. (2000)
L. gynnesicum L. girardianum	26	Palacios et al. (2000)
L. delicatulum	25	Palacios et al. (2000)
L. cavanillesii	27	Palacios et al. (2000)
L. anguste-bracteatum	26	Palacios et al. (2000)
L. rigualii	27	Palacios et al. (2000)
L. cossonianum	16	Palacios et al. (2000)
L. furfuraceum	18	Palacios et al. (2000), Lledó et al. (2005)
L. tenuicaule	18	Palacios et al. (2000)
L. minutum	18	Palacios et al. (2000)
L. dichotomum	18	Palacios et al. (2000)
L. caesium	18	Palacios et al. (2000)
L. globatum L. echioides	12 18	Palacios et al. (2000) Palacios et al. (2000)
L. lobatum	12	Erben (1979)
L. arborescens	14	Michaelis (1964)
L. bourgeaui	14	Borgen (1970), Ortega and Navarro (1977)
L. brassicifolium subsp.	14	Michaelis (1964), Borgen (1970)
brassicifolium -		
L. brassicifolium subsp.	14	Larsen (1960)
macropterum	1.4	Lauren 1058 (1060) Barren (1070)
L. fruticans	14	Larsen 1958 (1960), Borgen (1970), Gagnieu <i>et al.</i> (1973)
L. imbricatum	14	Febles (1989)
L. macrophyllum	14	Larsen (1958, 1960), Borgen (1970)
L. perezii	14	Borgen (1970)
L. puberulum	14	Larsen (1958, 1960), Michaelis (1964)
•		Van Loon (1974)
L. spectabile	14	Michaelis (1964)
L. sventenii	14	Borgen (1970), Febles (1989)
L. pectinatum	12	Larsen (1958, 1960), Borgen (1969)
L. papillatum	12	Borgen (1970)
L. ovalifolium subsp.	24	Borgen (1970)
canariense L. tuberculatum	32	Michaelis (1964), Borgen (1970)
L. thiniense	26	Lledó et al. (2005)
L. echioides	18	Lledó et al. (2005)
L. cossonianum	16	Lledó et al. (2005)
L. estevei	16	Lledó et al. (2005)
L. carthaginense	18	Lledó et al. (2005)
L. leonardi-llorensii	26	Sáez et al. (1998)
L. marisolii	27	Sáez et al. (1998)
L. calcarae	18	Brullo and Pavone (1981)
L. syracusanum	18	Brullo and Pavone (1981)
L. bocconei	18	Brullo and Pavone (1981)
L. temuculum	18	Brullo and Pavone (1981)
L. lopadusanum	18	Brullo and Pavone (1981)
L. panormitanum	18	Brullo and Pavone (1981)
L. minutiflorum	26	Brullo and Pavone (1981)
L. cosyrense	27	Brullo and Pavone (1981)
L. pignattii	27	Brullo and Pavone (1981)
L. lojaconi	32	Brullo and Pavone (1981)
L. intermedium	32	Brullo and Pavone (1981)
L. ionicum	35	Brullo and Pavone (1981)
L. serotinum	36	Brullo and Pavone (1981)
L. mazarae	36	Brullo and Pavone (1981)
L. secundiranium	36	Brullo and Pavone (1981)
L. hyblaeum	36	Brullo and Pavone (1981) Present study
L. lilacinum	36	

34 Present study

Present study

L. iconicum

L. globuliferum

Meikle is among these taxa. The somatic chromosome number of L. albidum is 2n = 18 just like L. globuliferum in the present study (Yidiz and Gucel, 2006).

These findings of the present study are in regard to the somatic chromosome number of the species studies inline with the literature. However, it diverges from previous literature in regard to chromosome morphology and chromosome lengths. This shows that there are cytological differences to be determined regarding genus *Limonium*. It further will shed light to similar studies on the karyology of the same genus.

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REFERENCES

- Ardevol-Gonzales, J.F., L. Borgen and P.L. Pérez De Paz, 1993. Checklist of chromosome numbers counted in Canarian vascular plants. Sommerfeltia, 18: 51-59.
- Arrigoni, P.V. and S. Diana, 1993. Contribution á la connaissance du genre *Limonium* en Corse. Candollea, 48: 631-677.
- Artelari, R. and G. Kamari, 1986. A karyological study of ten *Limonium* species (Plumbaginaceae) endemic in the Ionian area, Greece. Willdenowia, 15: 497-513.
- Artelari, R. and G. Kamari et al., 1992. In: Mediterranean chromosome number reports-2. Flora Mediterranea, 2: 229-232.
- Bokhari, M. and J.R. Edmondson, 1982. Limonium Miller. In: Davis, P.H. (Ed.). Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh, 7: 465-476.
- Borgen, L., 1969. Chromosome numbers of vascular plants from the Canary Islands, with special reference to the occurrence of polyploidy. Nytt. Mag. Bot., 16: 81-121.
- Borgen, L., 1970. Chromosome numbers of Macaronesian flowering plants. Nytt. Mag. Bot., 17: 145-161.
- Brullo, S. and P. Pavone, 1981. Chromosome numbers in the *Sicilian* species of *Limonium* Miller (Plumbaginaceae). Actas III Congr. Optima. Anales Jard. Bot. Madrid., 37: 535-555.
- Davis, P.H., R.R. Mill and K. Tan, 1988. Flora of Turkey and East Aegean Islands (Supplement) Vol. 10, Edinburgh University Press, Edinburgh.

- Erben, M., 1978. Die gattung *Limonium* in südwest mediterranean Raum. Mitt. Bot. Staatssamml. München., 14: 361-631.
- Erben, M., 1979. Karyotype differentiation and its consequences in Mediterranean *Limonium*. Webbia, 34: 409-417.
- Erben, M., 1993. *Limonium* Mill. (nom. cons.). En Castroviejo colaboradores Flora Ibérica. Plantas vasculares de la Peninsula Ibérica a Islas Baleares. Vol. 3. Real Jardin Botánica de Madrid. CSIC. Fareso, Madrid, pp. 2-143.
- Febles, R., 1989. Estudios en la Flora Macaronésica: Algunos números de cromosomas. VI. Bot. Macaronésica, 17: 57-76.
- Febles, R. and E.Y. Pérez-Rodríguez, 2004. Análisis cariotípico de *Limonium* Mill. Sección *Pteroclados* Boiss. subsección *Nobiles* Boiss. (Plumbaginaceae). Bot. Macaronésica, 25: 79-94.
- Gagnieu, A., R. Linder and V. Voggenreiter, 1973. Caryotypes de la Flore Insularia de Tenerife. Monog. Biol. Canar., 4: 126-133.
- Goldblatt, P., 1988. Index to plants chromosome numbers 1984-85. Monographs in Systematic Botany. Vol. 23. Missouri Botanical Garden St. Louis.
- Goldblatt, P. and D.E. Johnson, 2000. Index to plants chromosome numbers 1996-97. Monographs in Systematic Botany. Vol. 81. Missouri Botanical Garden St. Louis.
- Güner, A., 2000. Limonium Miller. In: Flora of Turkey and the East Aegean Islands, Güner, A., N. Özhatay, T. Ekim and K.H.C. Başer (Eds.). Vol. 11. Edinburgh University Press, Edinburgh.
- Kubitzki, K., 1993. Plumbaginaceae. In: The Families and Genera of Vascular Plants, Kubitzki, K., J.G. Rohwer and V. Bittrich (Eds.). Springer, Berlin. Germany, 2: 523-530.
- Larsen, K., 1958. Preliminary note on the cytology of the endemic canarian element. Bot. Tidsskr., 54: 167-169.
- Larsen, K., 1960. Cytological and experimental studies on the flowering plants of the Canary Islands. Biol. Skr. Dan. Vid. Selsk., 11: 1-60.
- Levan, A., K. Fredga and A.A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220.
- Lledó, M.D., M. Erben and M.B. Crespo, 2003. *Myriolepis*, a new genus segregated from *Limonium* (Plumbaginaceae). Taxon, 52: 67-73.
- Lledó, M.D., M.B. Crespo, M.F. Fay and M.W. Chase, 2005. Molecular phylogenetics of *Limonium* and related genera (Plumbaginaceae): Biogeographical and systematic implications. Am. J. Bot., 92: 1189-1198.

- Michaelis, G., 1964. Chromosomenzahlen einiger kanarischer endemismen. Planta, 62: 194.
- Ortega, J. and B. Navarro, 1977. Estudios en la Flora Macaronésica: Algunos números de cromosomas. Bot. Macaronésica, 4: 69-76.
- Palacios, C., J.A. Rosselló and F. González-Candelas, 2000. Study of the evolutionary relationships among *Limonium* species (Plumbaginaceae) using nuclear and cytoplasmic molecular markers. Mol. Phylogenet. Evol., 14: 232-249.
- Papatsou, S. and D. Phitos, 1975. Two new taxa from the Eastern Aegean. Notes R.B.G. Edinb., 34: 203-204.
- Rosselló, J.A., L. Sáez and A.C. Carvalho, 1998. *Limonium carvalhoi* (Plumbaginaceae), a new endemic species from the Balearic Islands. Anales Jard. Bot. Madrid, 56: 23-31.

- Sáez, L., A.C. Carvalho and J.A. Rosselló, 1998. Limonium marisolii L. Llorens (Plumbaginaceae) Revisited. Anales Jard. Bot. Madrid, 56: 33-41.
- Van Loon, J.C., 1974. A cytological investigation of Flowering plants from the Canary Islands. Acta Bot. Neerl., 23: 113-124.
- Yidiz, K. and S. Gucel, 2006. Chromosome numbers of 16 endemic plant taxa from Northern Cyprus. Turk. J. Bot., 30: 181-192.
- Yildirimli, S. and A.D. Koca, 2006. A new species Limonium adilgunerii Yildirimli and A. Dogru-Koca (Plumbaginaceae) from edge of Tuzgolu, Inner Anatolia, Turkey. Ot. Sist. Bot. Dergisi, 13: 11-16.