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Karyology of Plants Growing on Serpentines in Bulgaria

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Abstract — The karyotypes of species from genera *Aethionema, Thlaspi*, *Arenaria, Petrorhagia* and *Silene* growing on serpentines in Bulgaria are studied. The chromosome numbers and the karyotype morphology of the serpentine plants are illustrated and compared with data of previously investigated populations of these species growing on non-serpentine terrains. The chromosome number for the newly described species *Aethionema rhodopaeum* is presented from the second subpopulation. Chromosome data for the species *Thlaspi apterum* are provided for the first time. First records based on Bulgarian material are given for the following species: *Thlaspi ochroleuicum, Arenaria procera* ssp. *procera, Silene fabarioides,* and *Silene spergulifolia.*

Key words: Bulgaria, chromosome numbers, karyology, serpentine.

INTRODUCTION

The serpentines are characterized by their chemical composition – high content of Fe, Mg, Cr, Co and Ni, and low concentrations of Si, Ca, K, P and Mo which affect the flora and vegetation growing on it. When chemical properties of substrate are arrayed discontinuously, opportunities for colonization by different species as well as factors leading to speciation can occur (Kruckeberg 1986). In allover the world serpentines are famous with an unusual flora more or less rich of local endemic species, i.e. serpentine endemics (Brooks 1987; Baker *et al.* 1992; Robertis and Proctor 1992). Two categories of endemics are proposed by Stebbins and Major (1965) incorporating the age of the endemics, its systematic position, and cytological data (chromosome number and ploidal level), paleoendemics and neoendemics respectively. A number of authors (Kruckeberg and Rabinowitz 1985; Brooks 1987; Kruckeberg 1992; Mayer *et al.* 1994) reported these kinds of endemics as characteristic for serpentines from different parts of the world.

Still little is known about the edaphically restricted species (local endemics) and their role for the study of plant speciation. Rajakaruna (2004) and Brady *et al.* (2005) pointed out that the study of factors contributing to the evolution of edaphically endemic species can shed light on the relationship between adaptation and reproductive isolation. Any time, this process can be understood after well known karyotype morphology and with advent of novel genetic approaches such as the study of quantitative trail loci (QTLs). Today it is widely recognized that the process of differentiation and evolution of plant taxa often reflects in changes of chromosome number and morphology, and such phenomena as polyploidy, aneuploidy and chromosomal rearrangements are closely related to speciation (Greilhuber and Ehrendorfer 1988). In that reason, additional information about karyology of plants growing on serpentine and non-serpentine substrate is needed. Not much work has been devoted to study karyotaxonomy of the rich Balkan serpentine flora (Constantinidis *et al.* 2002; Kamari *et al.* 2003; Pavlova and Tosheva 2003; 2004).

This paper aims to investigate the chromosome number and karyotype morphology of taxa from Caryophyllaceae and Brassicaceae, most of which are growing predominantly or restricted to the serpentines in Bulgaria thus to help in taking taxonomic decisions and elucidate evolutionary problems.

MATERIALS AND METHODS

The karyological study was conducted on seed material collected from different plant species dis-

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tributed on serpentine terrains in the Rhodopes Mts. (Fig. 1). Most of the investigated plants grow predominantly or exclusively on serpentines. The voucher specimens are deposited in the Herbarium of Sofia University "St. Kliment Ohridski", Department of Botany (SO). List of the taxa investigated with provenance, chromosome numbers found and summarized karyotype content for all taxa exception of *Aethionema rhodopaeum* and *Thlaspi ochroleucum* are shown in Table 1.

The karyotypes are investigated on metaphase plates prepared from root meristem of germinating seeds, treated in hot hydrolysis and stained in chematoxilin after Gomory (PEARSE 1960). Where possible, the chromosomal type is determined after the centromere index I^c= s/s+l, according to

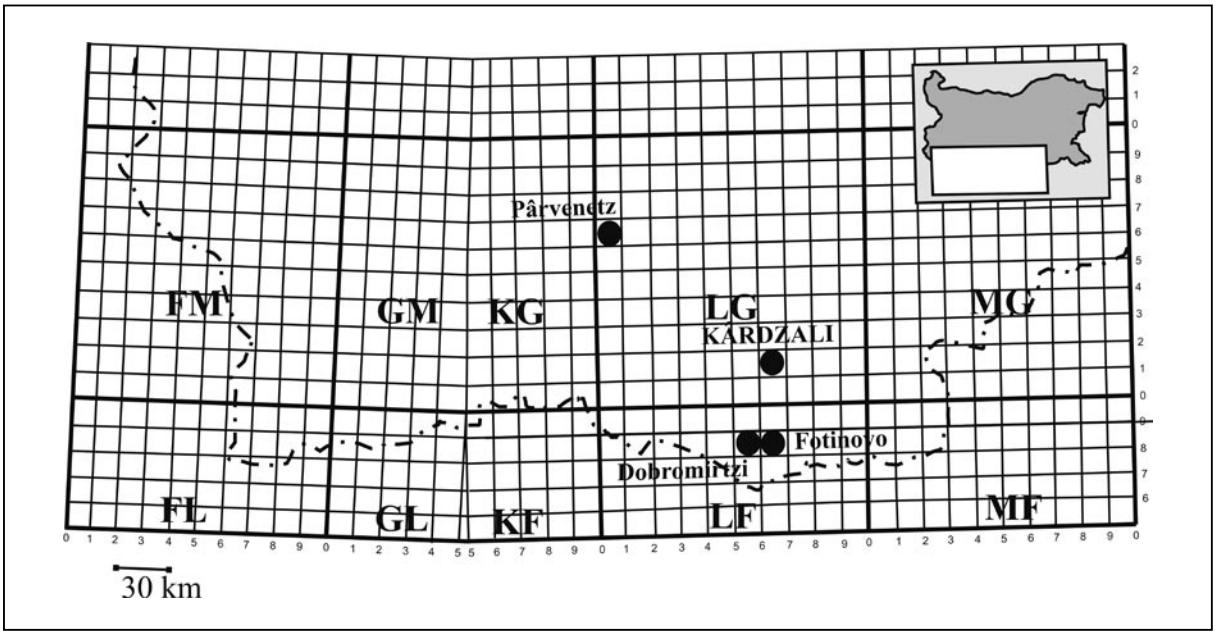

Fig. 1 — UTM Grid map of Bulgaria (1: 1 500000) with localities of the studied material.

Table 1 — List of the taxa investigated with provenance, chromosome number found and karyotype content: * first report; ** first record based on Bulgarian material.

Taxon	Provenance and Voucher N°	2n	M	M^{sat}	SM	SM^{sat}	I	Isat	X^{max}:X^{min}
Aethionema rhodopaeum	Rhodope Mts., near Dobromirtzi village, SO - 103915	24**							1.66:1
Thlaspi apterum	Rhodope Mts., near Fotinovo village, SO - 104577	28*	18	2	6	2			2.27: 1
Thlaspi ochroleuicum	Rhodope Mts. near town Kardzali, SO - 104595	42**							3:1
Arenaria procera ssp. *procera*	Rhodope Mts., near village Parvenetz, SO - 102451	22*	12	8	2				2.37: 1
Petrorhagia velutina	Rhodope Mts., near Fotinovo village, SO - 102678	30	20		8	2			2.3: 1
Silene conica	Rhodope Mts., near village Parvenetz, SO - 103305	20	2	2	2		2	12	1.43:1
Silene fabarioides	Rhodope Mts., near Dobromirtzi village, SO - 103439	24**	8		8	4		4	1.78:1
Silene spergulifolia	Rhodope Mts., near Fotinovo village, SO -102485	24**	16		8				2:1

the classification proposed by GRIF and AGAPOVA (1986). The measurements of short (s) and long arm (l) was performed on microscope at magnification ×2000. Karyotype asymmetry deduced from the ratio between the short arms of the chromosomes and their total length was expressed as total form percent (TF%).

The nomenclature of the taxa follows GREUTER *et al.* (1986), TUTIN *et al.* (1968 – '80; 1993), and KOZUHAROV (1992). Investigated taxa are listed in alphabetical order.

RESULTS AND DISCUSSION

Brassicaceae

Aethionema rhodopaeum D. Pavlova - 2n = 24 (Fig. 2 A)

Voucher material - Eastern Rhodope Mts.: Near Dobromirtzi village, due east of Zlatograd. Left on the main road on the top of the scree slope planted with pines. Alt. 389 m; 41° 23.053' N; 25° 12.096' E; UTM Grid – LF 58; 07 June 2005, D. Pavlova 103 915 (SO).
Distribution - Bulgarian endemic found in the Eastern Rhodopes Mts. on serpentine rocky terrains at 300 – 450 m a.s.l. (PAVLOVA 2007). The species has a local distribution. Presently, this plant is known only from two serpentine localities: southwards of Goljamo Kamenjane village (locus classicus) and southeastwards of Dobromirtzi village. This plant should be considered as an obligate endemic serpentinophyte.
Karyotype - The tetraploid chromosome number 2n = 24 is established. The karyotype is symmetrical, consisting of closed in size chromosomes of metacentric and submetacentric type. The longest chromosome pair is of metacentric type 1.6 μm long, but the shortest 1μm long is of unclear type. The ratio between the length of the longest and the shortest chromosome (X^{max}: X^{min}) is 1.66: 1.

This is the second population studied from serpentines in Bulgaria. The data provided by PAVLOVA (2007) for the locus classicus subpopulation are confirmed. No differences in the chromosome number and karyotype morphology among the two investigated populations were established.
Aethionema rhodopaeum is related to the *A. saxatile* group in the sense of TUTIN *et al.* (1993). The smaller dimensions of the chromosomes and the chromosome number link *A. rhodopaeum* to other taxa of *A. saxatile* group. The lack of information about the relation of the studied taxa within the complex (WARWICK and AL-SHEHBAZ 2006) and the serpentine substrate does not allow any comparisons. In most cases only chromosome counts were provided.

Thlaspi apterum Velen. - 2n = 28 (Fig. 2 B)

Voucher material - Eastern Rhodope Mts.: near Fotinovo village, about 1 km down a forest track to a place above an oxbow of the Varbica River, on a rocky serpentine hill slope. Alt. 425 ± 20 m; 41° 21.800' N; 25° 18.628' E; UTM Grid – LF 68; 06 June 2005, D. Pavlova 104 577 (SO).
Distribution - Bulgarian endemic (GREUTER *et al.* 1986). MEYER (2006) reported this plant (sub *Noccaea aptera*) for Albania, Bosnia, Montenegro, Macedonia, Serbia and European Turkey and for the following floristic regions in Bulgaria: West frontier mountains, Znepole region, Rila Mts., Vitosha Mts., Sredna Gora Mts., Sofia region, Stara planina Mts. This plant characteristic for the serpentine terrains in the Eastern Rhodopes Mts. forms large populations. According to REEVES (pers. comm.) this species from the Eastern Rhodopes Mts. accumulates Ni in anomalously high concentrations.
Karyotype - The chromosome number established is 2n = 28. The karyotype is symmetrical (TF 68.7%), consisting of 2n = 18m + 2m-SAT + 6sm + 2sm-SAT = 28 chromosomes, varying in size from 1.5 μm to 0.66 μm. The satellites are small, located on the short arms of the submetacentric chromosome pair. In all metaphase plates one metacentric chromosome pair shows unstable centromere and as a result the arms are quite separated one from another. The ratio between the length of the longest and the shortest chromosome (X^{max}: X^{min}) is 2.27: 1.

The karyotype morphology of the species is reported for the first time.

Thlaspi ochroleucum Boiss. & Heldr. - 2n = 42 (Fig. 2 C)

Voucher material - Eastern Rhodope Mts.: near Kardzhali (about 4 km in southwestern direction from the town), on a serpentine hillslope above the Varbica River along the road to Dzebel. Alt. 275 m; 41° 33.821' N; 25° 22.932' E; UTM Grid – LG 61; 06 June 2005, D. Pavlova 104 595 (SO).
Distribution - Balkan subendemic (TUTIN *et al.* 1993; ARTELARI 2002). As ARTELARI (2002) pointed out this species belongs to *T. praecox* group, a polyploidy complex in which at least 12 poorly defined species described from Southeast Europe are present. The distribution of this plant in Bulgaria is in the following floristic regions: Stara planina Mts., Slavjanka Mts. and Rhodope Mts. (KOZUHAROV ed. 1992).

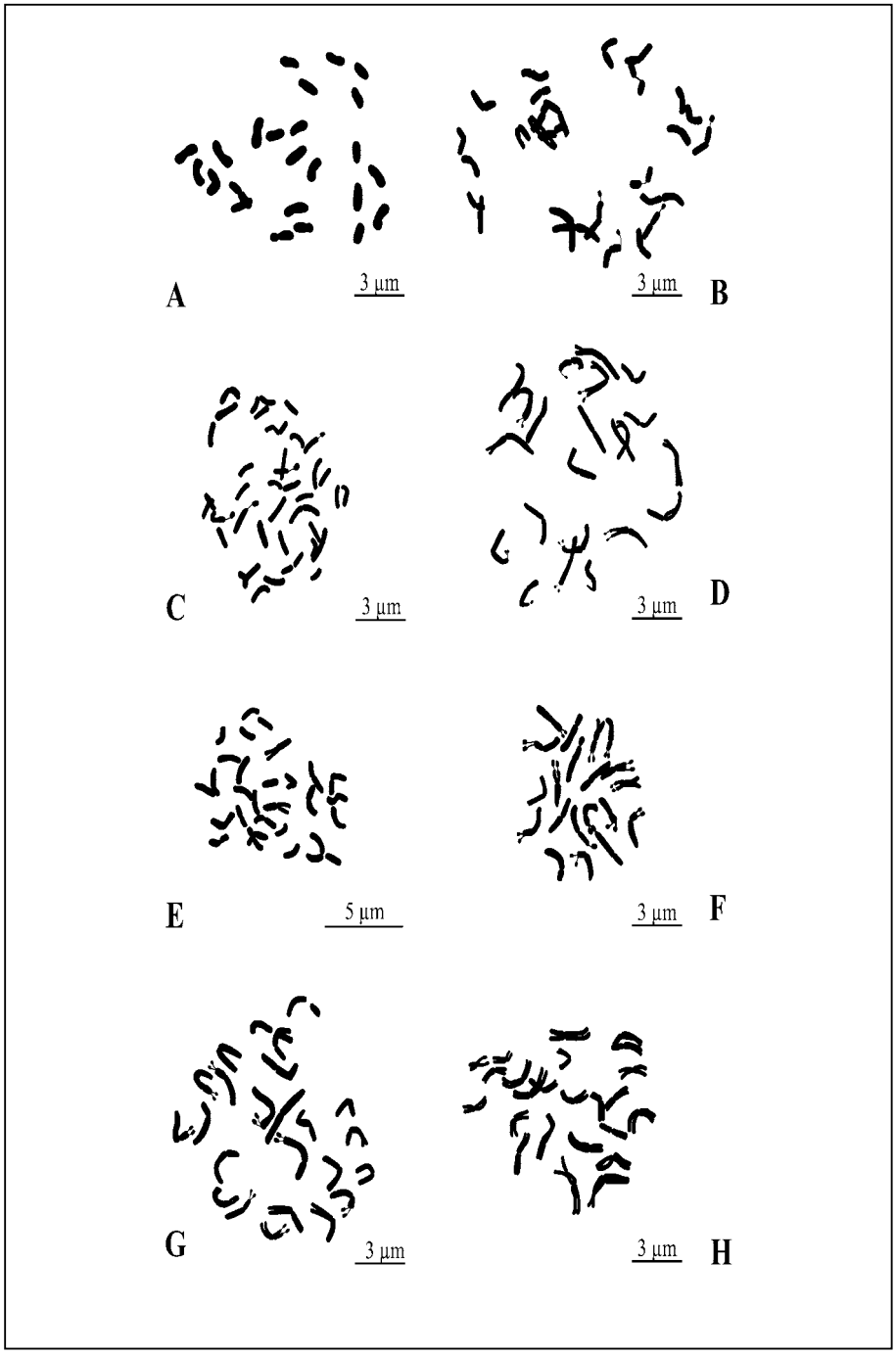

Fig. 2 — Karyotypes of the investigated species. A: *Aethionema rhodopaeum*; B: *Thlaspi apterum*; C: *T. ochroleucum*; D: *Arenaria procera* ssp. *procera*; E: *Petrorhagia velutina*; F: *Silene conica*; G: *S. flavescens*; H: *S. spergulifolia.*

Karyotype - The hexaploid chromosome number 2n = 42 is presented here for the first time. The diploid number 2n = 14 for a population from Greece (ARTELARI 2002) was not confirmed.

The karyotype consists of small in size chromosomes with unclear morphology and does not allow an unambiguous classification of the chromosomes. Two pairs of chromosomes have ball-shaped satellites. The chromosome size varies from 1.2 to 0.4 µm. The ratio between the length of the longest and the shortest chromosome (X^{max}:X^{min}) is 3: 1.

This is first report for this plant based on material growing on serpentines in Bulgaria.

Caryophyllaceae

Arenaria procera Spreng. ssp. procera - 2n = 22 (Fig. 2 D)

Voucher material - Central Rhodope Mts.: Near Parvenetz village, serpentine scree slope on the east side of the road, about 2 km south of Parvenetz village. Alt. 360 m; 42° 03.948' N; 24° 39.472' E; UTM Grid – LG 06; 24 June 2002, D. Pavlova 102 451 (SO).
Distribution - This plant is found in the Thracian plain and Tundza hilly region on dry grassy places up to 100 m a.s.l. (KOZUHAROV ed. 1992). Our finding in the Central Rhodope Mts. on serpentine rocky places ca. 300 m a.s.l. contributes to the chorology and ecology of this taxon.
Karyotype - The chromosome numbers 2n = 22, 44, 110 are reported by TUTIN *et al.* (1993), GOLDBLATT and JOHNSON (1994). The karyotype is symmetrical (TF 79.5%) consisting of metacentric and submetacentric chromosomes. The karyotype formula is 2n = 12m + 8m-SAT + 2sm = 22. The chromosome length in the metaphase plates studied varies between 2.8 μm and 1.18 μm. The ratio X^{max}: X^{min} is 2.37: 1.

Petrorhagia velutina (Guss.) P.W. Ball & Heywood - 2n = 30 (Fig. 2 E)

Voucher material - Eastern Rhodope Mts.: Near Fotinovo village, on south-facing rocky gully slope; ultramafic rocks and soils with sparse *Alyssum murale*. Alt. 421 ± 20 m; 41° 22.531' N; 25° 19.165' E; UTM Grid – LF 68; 05 June 2005, D. Pavlova 102 678 (SO).
Distribution - The distribution range covers the Mediterranean, the Caucasus Mts. and Southwestern Asia. This species is found in Bulgaria up to 1000 m a.s.l. in the following floristic regions: Black Sea coast, Eastern Stara planina Mts., Struma valley, Slavjanka Mts., Thracian plain, Tundza hilly region and Strandza Mts. (KOZUHAROV 1992).
Karyotype - The chromosome number 2n = 30 confirms previous counts (GOLDBLATT and JOHNSON 1991; 1994; 1998; 2000 for references).

Non-serpentine populations of this taxon from Bulgaria (Struma valley and Slavjanka Mts.) were previously investigated by PETROVA (1995) who reported a chromosome number 2n = 30. The karyotype established is symmetrical, consisting of six pairs of metacentric and nine pairs of submetacentric chromosomes, one of them with satellites.

The investigated serpentine population has also a symmetrical karyotype (TF 74.3%). It consists of 2n = 2x = 20m + 8sm + 2sm-SAT = 30 chromosomes. The ratio X^{max}: X^{min} is 2.3: 1. No differences were found between this population and the data provided by PAVLOVA and TOSHEVA (2004).

This count and the karyotype morphology are the second report for populations growing on serpentine rock.

Silene conica L. - 2n = 20 (Fig. 2 F)

Voucher material - Central Rhodope Mts.: Near Parvenetz village, serpentine scree slope on the west side of the road, about 2 km south of Parvenetz village. Alt. c. 350 m; 42° 03' N; 24° 39' E; UTM Grid – LG 06; 06 June 2003, D. Pavlova 103 305 (SO).
Distribution - The distribution range covers Northwestern Africa, Mediterranean and warm-temperate Europe, eastwards through Anatolia, Caucasus and Northern Iran to Northeastern Afghanistan (GREUTER 1997).
This annual plant is widely distributed on dry grassy, sandy and rocky places in Bulgaria, mostly on limestone, but also on siliceous and serpentine substrates.
Karyotype - The established chromosome number 2n = 20 agrees with several chromosome reports on *S. conica* from various European countries (FEDOROV 1969; VACHOVA and FERAKOVA 1978; van LOON and SNELDERS 1979; DVORAK and DADAKOVA 1984; BALTISBERGER *et al.* 1993; PETROVA 1995) and with the species from Sect. *Conomorpha*. Some authors report 2n=24 (MOORE 1973; GOLDBLATT 1985) for species from this section which is a common chromosome number of other sections within *Silene*. Van LOON and SETTEN (1982) report 2n=20 and 24 for *Silene conica* from Bulgaria.

The karyotype formula is 2n = 2m + 2m-SAT + 2sm + 2I + 12I-SAT = 20. The karyotype is asymmetrical (TF 31.25%), consisting of metacentric submetacentric and intercentric chromosomes. Most of the chromosomes with satellites are of intercentric type and the satellites are attached to the short arm of the chromosomes. The longest chromosomes are 1.65 μm while the shortest are 1.15 μm. The ratio X^{max}: X^{min} is 1.43:1.
S. conica is hardly distinguishable from the morphologically close annual species *S. subconica* Friv. which also grows on serpentine terrains in the Central Rhodope Mts. The investigated karyotypes of the two species from the same locality are clearly distinguishable. The karyotype of *S. subconica* is symmetrical, consisting mainly of metacentric and submetacentric chromosomes

and a small number of chromosomes with satellites (PAVLOVA and TOSHEVA 2003).

The karyotype of the species growing on serpentines is asymmetrical in comparison with the data published by PETROVA (1995) from non-serpentine population. The chromosomal formula of the population is given as 2n = 2x = 4m + 4m-SAT + 12sm. It is obvious that the evolutionary trend in the investigated population of *S. conica* is connected with chromosomal rearrangements as a result of probable adaptation to the edaphic conditions of the serpentine rock.

This count is the first report from populations distributed on serpentines in Bulgaria.

Silene fabarioifes Hausskn. - 2n = 20 (Fig. 2 G)

Voucher material - Eastern Rhodope Mts.: Near Dobromirtzi village, due east of Zlatograd. Left on the main road; on the top of the scree slope planted with pines. Alt. 389 m; 41° 23' N; 25° 12' E; UTM Grid – LF 58; 07 June 2003, D. Pavlova 103 439 (SO).

Distribution - Balkan endemic (ASSYOV *et al.* 2006). The localities in Bulgaria are included in the following floristic regions: Znepole, Vitosha Mts., Slavjanka Mts., Rhodope Mts. and Thracian plain, mainly on calcareous rocky places (KOZUHAROV 1992). This plant was also found on serpentines in the Eastern Rhodope Mts. (PAVLOVA *et al.* 2004) but not confined to it as pointed out by MELZHEIMER (1980) and CONSTANTINIDIS *et al.* (2002).

Karyotype - It is asymmetrical (TF 37.9%), consisting of 2n = 2x = 8m + 8sm + 4sm-SAT + 4I-SAT = 24 chromosomes. The longest chromosomes (2.64 μm) are of metacentric type but the shortest chromosomes (1.65 μm) are of submetacentric type. Two of the submetacentric chromosomes have satellites attached to the short and to the long arms. Two more chromosome pairs have satellites connected to the short arms but they are from intercentric type. The ratio X^{max}: X^{min} is 1.78:1.

The chromosome number 2n = 24 confirms previous counts of CONSTANTINIDIS *et al.* (2002) for populations from Greece, Nomos Ioanininon. Two of the investigated populations differ by a greater number of metacentric chromosomes in Greek population and by a number of pairs with satellites.

The chromosome number and karyotype morphology are reported for the first time from Bulgaria.

Silene spergulifolia Bieb. - 2n = 24 (Fig. 2 H)

Voucher material - Eastern Rhodope Mts.: Near Fotinovo village, on south-facing rocky gully slope; ultramafic rocks. Alt. 421 ± 20 m; 41° 22.531' N; 25° 19.165' E; UTM Grid – LF 68; 03 July 2003, D. Pavlova 102 485 (SO).

Distribution - This plant is distributed in Southeastern Europe and Turkey on different habitats (CHATER *et al.* 1993).

Karyotype - It is symmetrical (TF 75.4%) with chromosomes varying of length between 0.99 μm and 1.98 μm. The centromere index gives reasons to consider the chromosomes being of metacentric and submetacentric types. The karyotype consists of 2n = 2x = 16m + 8sm = 24 chromosomes. The ratio X^{max}: X^{min} is 2:1. Three chromosome pairs cannot be distinguished one from another, being of metacentric type and overlapping in size.

The chromosome number 2n = 2x = 24 confirms previous counts of GOUKASIAN and SAFARIAN (1990), GOUKASIAN and NERSERIAN (1992), NERSESIAN and GOUKASIAN (1995) and YILDIZ and CIRPICI (1996) for populations from Armenia and Turkey. The karyotypes of all investigated populations are symmetrical with the prevailance of metacentric and submetacentric chromosomes of small size.

The karyotype morphology of the species is reported for the first time for populations growing on serpentine rocks in Bulgaria.

CONCLUSIONS

1. The karyotypes for most of the investigated species are symmetrical, with the prevailance of metacentric and submetacentric chromosomes. The karyotypes of *Silene fabarioides* and *S. conica* are asymmetrical with metacentric, submetacentric and intercentric chromosomes.
2. The chromosomes of the investigated species from Brassicaceae are generally small in size, ranging between 0.4 and 1.6 μm. The ratio between the largest and the shortest chromosome in the karyotype varies from 1.66:1 in *Aethionema rhodopaeum* to 3:1 in *Thlaspi ochroleucum*. These two species show unclear morphology and a karyotype formula is impossible to be presented.
3. In all karyotypes were established chromosomes with satellites. The number of chromosome pairs with satellites varies from one (*Petrorhagia velutina*) to seven (*Silene conica*). Such a big number of chromosome pairs with satellites was found for the first time for this species.
4. The chromosome numbers established for Caryophyllaceae taxa are diploid, based on different basic chromosome numbers (x = 10, 12 for *Silene;* x = 11 for *Arenaria*; x = 15 for *Petrorhagia*)

and confirm data reported from other regions and non-serpentine areas.
5. All investigated species from Brassicaceae are polyploids. AL-SHEHBAZ *et al.* (2006) pointed out that polyploidy and aneuploidy had played an important role in the speciation processes in Brassicaceae. The lack of sufficient karyological information for the investigated taxa does not give us reason for comparisons and conclusions about the changes in ploidy level under the influence of the serpentine substrate.
6. The reaction of the plants from different taxonomical groups to the specific edaphic conditions of the serpentines is not identical. Despite that many of the species characteristic for serpentines, like representatives from *Silene*, are conservative in their chromosome number, they changed the karyotype morphology more often after chromosomal rearrangements.

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