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Karyotypes and genome size of *Onosma* species from northern limits of the genus in Carpathians

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Abstract — Karyology and genome size of four species of the genus *Onosma* from northern border of the area of the genus in Slovakia were studied. Different chromosome number is characteristic for each of the studied representatives. *O. tornensis* (2n=14) has 7 pairs of homologous S (short) chromosomes 1.62 – 2.94 µm long, 5 pairs are metacentric and 2 pairs are submetacentric; nuclear DNA content: 1.56 pg/2C. *O. visianii* (2n=18) has 9 pairs of homologous chromosomes 1.91 – 3.18 µm long, 3 pairs are metacentric, 4 pairs submetacentric and 2 pairs are subacrocentric (one pair bears satellites); nuclear DNA content 4.12 pg/2C. *O. arenaria* (2n=20) has 6 pairs of homologous metacentric L (long) chromosomes 3.22 – 4.33 µm long and 8 unpaired S chromosomes 1.07 – 2.36 µm long, 7 of these chromosomes are metacentric and 1 submetacentric; nuclear DNA content 5.15 pg/2C. *O. pseudoarenaria* (2n=26) has 6 pairs of homologous metacentric L chromosomes 3.36 – 5.03 µm long and 7 pairs of homologous metacentric S chromosomes 1.07 – 2.36 µm long and nuclear DNA content 5.74 pg/2C. Conspicuous difference in chromosome structure of particular species and in their DNA content is further discussed in the paper.

Key words: *Boraginaceae*, cytometry, genome size, karyotypes, *Onosma.*

INTRODUCTION

The genus *Onosma* L. comprises ca. 150 species distributed mainly in the East and the Central Asia and in the Mediterranean area (Ball 1972; Willis 1973; Meusel *et al.* 1978). Traditionally (although incorrectly from the point of view of current nomenclature), Boissier (1879) divided the genus into three sections: *Asterotricha* Boiss., *Haplotricha* Boiss. and *Heterotricha* Boiss. Riedl (1962) separated two sections: *Onosma* and *Protonosma* Popov. Groups *Haplotricha* and *Heterotricha* sensu Boissier are included into *Onosma* subsect. *Onosma* and group *Asterotricha* into independent subsection *Onosma* subsect. *Asterotricha* (Boiss.) Gürke (Riedl 1962). Karyology is in line with the division of the genus *Onosma* in Europe into three Boissier's groups, these probably reflected evolution of the genus in European part of its area. Ancient taxa of the groups *Asterotricha* and *Haplotricha* are probably ancestors of the hybridogenous group *Heterotricha* (Teppner 1971 and 1972; Vouillamoz 2001).

In *Haplotricha*, the basic chromosome number, x=6, known from diploid cytotype 2n=12 (e.g., *O. setosa* Ledeb., *O. fastigiata* (Braun-Blanq.) Lacaita) and tetraploid cytotype 2n=24 (e.g., *O. fastigiata*), has prevailed, the basic chromosome number x=9 (e.g., *O. visianii* Clementi, *O. graeca* Boiss.) is less frequent (Teppner 1991). In both basic chromosome numbers, the chromosomes are represented by "*O. setosa* type" (Teppner 1991) - long chromosomes, which were seen in the prophase of mitosis. Basic chromosome numbers x=7 (e.g., *O. simplicissima* L., *O. frutescens* Lam.) and x=10 (e.g., *O. polyphylla* Ledeb.) are rare and less studied (Teppner 1971; Tissot-Daguette 1979; Popova and Zemskova 1990).

In the group *Asterotricha* there are two basic chromosome numbers, x=7 prevails, another one is x=11 (*O. stellulata* Waldst. et Kit. and relatives, Teppner 1971). Diploid chromosome number 2n=14 is known in the most of the taxa in Europe, polyploid cytotypes (2n=20, 21, 26, 27, 28, 30, 32, 38, 42, 43, 44, 50, 51) occur more often in southern Balkan and Asia Minor. Probably many of them, euploids or aneuploids, have got allopolyploid origin (e.g., *O. sanguinolenta* Vatke – 2n=30=14 + 16, *O. alborosea* Fisch et C. A. Mey. – 2n=44=30 + 14, Teppner 1980). The chromosomes of the representatives of *Asterotricha* group are short and

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were seen in the end of prophase or in the beginning of metaphase of mitose – chromosomes of "*O. echioides* type" (TEPPNER 1974 and 1991).

Hybridogenous group *Heterotricha* comprises 2 groups of taxa: "*O. arenaria* group" with 2n=20, 12L + 8S and "*O. pseudoarenaria* group" with 2n=26, 12L + 14S. According to TEPPNER (1971) L chromosomes (L-long) correspond to "*O. setosa* type" and S chromosomes to "*O. echioides* type". This affirmation is based on their length and their "behaviour" (which stadium of prophase they are visible in). "*O. pseudoarenaria* group" has originated probably by allotetraploidisation of some taxa from *Haplotricha* group (12-chromosome cytotype, *O. fastigiata* for example) and from *Asterotricha* group (14-chromosome cytotype, *O. echioides* L. for example). "*O. arenaria* group" with specific 8S chromosomes has originated in backcrossing of representatives of "*O. pseudoarenaria* group" and some taxa from *Haplotricha* group with 12 chromosomes (TEPPNER 1971 and 1972; VOUILLAMOZ 2001). These hypotheses were supported by molecular analyses performed on material from western Alps and adjacent area (VOUILLAMOZ 2001). Chromosome numbers in *Heterotricha* group, 2n=20 and 2n=26 seem to be stable, but the exceptions in "*O. arenaria* group", 2n=12, 2n=24-26, 2n=18, 2n=19, 2n=22 (nonspecific proportion of L and S chromosomes), and in "*O. pseudoarenaria* group", 2n=27, 2n=28, 2n=12L + 16S, were also found (VOUILLAMOZ 2001, karyological survey). Unusual chromosome numbers in *O. helvetica* (in the sence of Teppner) are probably erroneous for different reasons (TEPPNER 1974; PERUZZI *et al.* 2004; information provided to them by TEPPNER).

In eastern Asia at the borders between Asia and Europe, taxa with basic chromosome number x=8 occur (e.g., *O. microcarpa* STEV., POPOVA and ZEMSKOVA 1985; *O. sericea* Willd., TISSOT-DAGUETTE 1972 and TEPPNER 1974). Their relationships with the mentioned european three groups are unclear, furthermore representatives are from entirely different groups (TEPPNER 1974; POPOVA and ZEMSKOVA 1985). Some species with x=8 can represent one of parents of hybrid species like *O. sanguinolenta* or *O. alboroseum* (TEPPNER 1974 and 1980, see part devoted to *Asterotricha*). This basic chromosome number is probably more frequent (TEPPNER 1974).

TEPPNER provided many important data on chromosome morphology in his several papers, but only few of them are usable for comparison with recent investigations (e.g., TEPPNER 1974 and 1991). Comprehensive studies were then given by LUQUE (1990), KAMARI *et al.* (1996) CONSTANTINIDIS *et al.* (2002), PERUZZI *et al.* (2004) and COPPI *et al.* (2006). Another karyological studies in the genus *Onosma* with comments to chromosome morphology are given by FAVARGER (1971), TEPPNER (1971; 1980; 1981; 1988 and 1996), TISSOT-DAGUETTE (1972) and POPOVA and ZEMSKOVA (1985 and 1990; Tab. 1).

Genome size of plants has been estimated for over 50 years (BENNETT and LEITCH 2005a). Studies of genome size of plants have been important in several science disciplines including molecular biology, systematics and ecology (BENNETT *et al.* 2000). However only C-values of 4427 species of angiosperms were pooled in an electronic form – the Angiosperm DNA C-values database (BENNETT and LEITCH 2005b). From this database it is evident, that only small number of data was published for the family Boraginaceae. Besides "lower popularity" of the family among scientists, the main reason of low number of C- value data may be the problems in nuclei isolation and staining. SUDA (2004) reffered that samples prepared from leaf tissue of representatives of Boraginaceae, some plants of the genus *Echium* L., yielded hardly any fluorescence signal, but measuring of young seedlings took off this problem. The reason of these problems is not clear, but the results could be made worse by the presence of secondary metabolites in Boraginaceae. For illustration, the presence of inhibitors of phenolic nature can strongly affect measurement results (GREILHUBER *et al.* 2007; DOLEŽEL *et al.* 2007). Therefore it is not surprising that genome size data in the genus *Onosma* have not been published yet.

Karyological data for Slovak representatives of the genus *Onosma* were brought by several authors (BAKSAY 1957; MÁJOVSKÝ *et al.* 1970; TEPPNER 1971; MÁJOVSKÝ *et al.* 1978 and TISSOT-DAGUETTE 1979). The survey of these data was published in MÁJOVSKÝ *et al.* (1987). Few recent data were given by LETZ *et al.* (1999), MÁJOVSKÝ *et al.* (2000) and MÁRTONFI *et al.* (2002) (Tab. 2.). Actually all of the published data were summarized in MARHOLD *et al.* (2007).

Four morphologically different species with different chromosome numbers can be distinguished in Slovakia: *O. arenaria* Waldst. et Kit. (2n=20), *O. pseudoarenaria* Schur. (2n=26), *O. visianii* Clementi (2n=18), *O. tornensis* Jáv. (2n=14) (HOLUB and KMEŤOVÁ 1993). This conception of four taxa is employed also in the presented study. These taxa are considered critically endangered in Slovakia, even one of them, stenoendemic species *O. tornensis*, which is known only from few localities in Slovenský kras karst, is included in Bern Convention on the Conservation of European Wildlife and Natural Habitats (BERN1) (FERÁKO-

VÁ *et al.* 2001). Slovak populations of four species of the genus *Onosma* represent nothern border of its area in the Central Europe. The limits of the areas are usually represented by places where the occurrence of the species is strongly limited by climatic as well as anthropic factors. This is also the case of the genus *Onosma* in Slovakia. In the past climatic changes during glacials and interglacials limited occurrence of many european organisms to refugias known in the Southern Europe. Actually the Central Europe represents one of the regions where previously isolated populations have got secondary contacts, which have led to hybrid speciation. One of the major groups of the genus *Onosma*, *Heterotricha*, has originated probably by this way. Genus *Onosma* at the northern border could be considered as a suitable model for the study of evolutionary history.

The aims of the presented study are: (i) to characterize karyological variation in the species of the genus *Onosma*, which are distributed on the northern limits of the occurrence of the genus and (ii) to complete these data with genome size data, which have not been published so far.

MATERIALS AND METHODS

Plant material - Plants for karyological and flow cytometry analysis from the genus *Onosma* were collected in 10 localities in Slovakia (for details and number of samples see Appendix). GPS co-ordinates of each locality were determined using GPS instrument Geko 101 (Garmin). The collection of the samples was carried on the basis of exception from nature conservation of Ministry of Environment of the Slovak Republic no. 1210/481/05-5.1, because the taxa of the genus *Onosma* occuring in Slovakia are considered critically endangered (FERÁKOVÁ *et al.* 2001). This is also the reason, why relatively small population samples were collected and voucher specimens for flow cytometry consist only from small parts of the plants (few leaves and/or part of inflorescence). Vouchers are deposited in KO herbarium. Collected plant material for karyological analysis was cultivated on experimental plots in the Botanical Garden of P. J. Šafárik University in Košice (Slovakia) and plant material for flow cytometry (few leaves) was preserved and kept in cold conditions (for maximum of 2 days) up to the time of analysis.

Karyological analysis - For karyological analyses root meristems of potted plants were used. For a pretreatment, the root tips were placed in cold water (0-1°C) for 16-18 hours, then transferred to 0.002 M aquaeous solution of 8-hydroxyquinoline at the temperature of 1-2°C for 6-8 hours. Then the root tips were fixed in acetic ethanol (glacial acetic acid and 96% ethanol in the ratio 1: 3), hydrolyzed for 5 minutes in 1N HCl at 60°C. The meristems were squashed using cellophane technique (MURÍN 1960) and stained in 10% Giemsa stain solution in distilled water. The slides were then washed in distilled water, dried and observed in a drop of immersion oil. The best methaphase plates were selected for calculation of karyotype characteristics. Photographs of these metaphase plates were taken and the chromosomes were measured. For the chromosome identification and comparison the following characteristics were used: absolute chromosome length, relative chromosome length (the ratio of the length of particular chromosome to the sum of lengths of all chromosomes in the metaphase plate studied), arm index (the ratio of the length of longer to shorter arms) and centromeric index (ratio of the length of shorter arm to the length of chromosome). The classification of chromosomes is according to LEVAN *et al.* (1964).

Flow cytometry - The samples for flow cytometry analysis were prepared from leaves of *Onosma* plants by a two-step procedure, consisting of separate nuclear isolation and staining steps, using propidium iodide as DNA intercalator (OTTO 1990; DOLEŽEL and GÖHDE 1995). Relative and absolute DNA contents were measured on Becton-Dickinson flow cytometer (Becton Dickinson, San Jose, CA, USA) in the Laboratory of Flow cytometry at the Institute of Biological and Ecological Sciences of P. J. Šafárik University in Košice (Slovakia). To keep offered maximum differencies between standard and sample (SUDA 2004) we used two internal reference standards: *Glycine max* 'Polanka' (2C DNA content = 2.37 pg) and *Pisum sativum* 'Ctirad' (2C DNA content = 8.76 pg). Seeds of standards were provided by J. Doležel (Olomouc). Using the same protocol, samples and reference standards were isolated and stained separately before being mixed and analysed, which was referred to as the pseudo-internal standardization (NOIROT *et al.* 2005; GREILHUBER *et al.* 2007). Approximately 1 cm^2 of young leaf was chopped with a new razor blade (in Petri dish) in 2 ml of cold OttoI buffer and this suspension was filtered through 42 µm nylon mesh and centrifugated. Supernatant was removed and pellet (ca 100 µl) was resuspended in 100 µl of fresh Otto I buffer. After 30 min of incubation at room temperature, fluorochrome solution was added. It consists of Otto II buffer, RNAse, propidium iodide and β-mercaptoethanol. After 10 min in-

Tab. 1 — Survey of karyological data in the genus *Onosma*, which bring at least some of chromosome characteristics. Chromosome classification was, where this was possible, united according to LEVAN et al. (1964). Additionally, the abbreviation hb is used for designation of further not specified heterobrachiale chromosomes. Karyotype formulas are either taken over from the author or prepared on the basis of published data. L and S mark (if the author gives this datum) long or short chromosomes, respectively. When the formulas could not be drawn up, verbal description is given. The taxa are arranged according to increasing chromosome number.

Taxon	Locality	Chromosome number	Karyotype formula of chromosme description	Length of chromosomes (μm)	Reference
O. bubanii Stroh	Spain, Huesca, Vilas del Turbón	12	metacentric and submetacentric		LUQUE 1990
O. fastigiata (Braun-Blanq.) Lacaita	West France	12	metacentric and submetacentric, 4 sat		TISSOT-DAGUETTE 1972
O. tricerosperma subsp. *hispanica* (Degen et Hervier) P. W. Ball	Spain, Cuenca, between Cañaveras and Villaconejo de Trabague	12	2M + 6m + 2sm + 2sm^{sat}	3.58 – 4.88	LUQUE 1990
O. tricerosperma subsp. *granatensis* (Debeaux et Degen) Stroh	Spain, Sierra Nevada	12	2M + 6m + 2sm + 2sm^{sat}	3.71 – 4.94	LUQUE 1990
O. echioides (L.) L.	Italy, Tuscany, Pomarance	14	8m + 4sm + 2sm^{sat}	2.2 (mean)	COPPI *et al.* 2006
O. echioides var. *veronensis* Lacaita	Italy, Sega near Verona	14	10m + 2sm + 2sm^{sat}		TEPPNER 1974
O. echioides (L.) L.	Italy, Celano - road to Ovindoli	14S	2 heterobrachiale-sat, 12 with unspecified centromere position		TEPPNER 1971
O. elegantissima Rech. f. et Goulimy	Greece, Mt. Vourinos	14	10m + 2m^{sat} + 2m/sm^{sat}		CONSTANTINIDIS *et al.* 2002
O. erecta Sibth. et Sm. subsp. *erecta*	Kreta, Peloponnes	14	8m + 2m^{sat} + 2hb + 2hb^{sat}		TEPPNER 1988
O. inexspectata Teppner	Turkey, C6 Adana, col of Nurdağ	14	10m + 2m^{sat} + 2sm^{sat}		TEPPNER 1974
O. mattirolii Bald.	Albania, Tomorr mountain	14	10m + 4sat (for last 4 centromere position not given)		TEPPNER 1996
O. simplicissima L.	Caucasus mountains, more localities	14	12m + 2m^{sat}	1.78 – 3.03	POPOVA and ZEMSKOVA 1990
O. sorgeri Teppner var. *sorgeri*	Turkey, B6 Sivas Gök Pinar	14 and 14 + 2B	2m^{sat} + 4sm/st^{sat} + 8 with unspecified centromere position		TEPPNER 1980
O. stridii Teppner	Greece, Mt Kallidromon	14	10m + 2m^{sat} + 2m/sm^{sat}		CONSTANTINIDIS *et al.* 2002
O. tornensis Jáv.	Slovakia, Turňa nad Bodvou, Turniansky hradný vrch hill	14	10Sm + 4Ssm	1.62 – 2.94	this study
O. bourgaei Boiss.	4 localities given from Turkey	16	4m + 8sm/st + 4^{sat} (for last 4 centromere position not given)		TEPPNER 1996
O. gigantea	Israel	16	10m + 4sm^{sat} + 2st^{sat}		TEPPNER 1974
O. microcarpa Steven	Caucasus mountains, more localities	16	14m + 2m^{sat}	I-II pairs 5.0 – 5.1 III-VII pairs 3.5 – 4.2 VIII pair 2.7	POPOVA and ZEMSKOVA 1985
O. sericea Willd.	Armenia, Jerevan	16	8m/sm + 6st + 2st^{sat}		TISSOT-DAGUETTE 1972
O. sericea Willd.	Armenia, Jerevan	16	4m + 6sm + 6st^{sat}		TEPPNER 1974

O. sericea Willd.	Armenia, Jerevan	16	4m + 6sm + 6st^{sat}		TEPPNER 1974
O. troodi Kotschy	Cyprus, Mt. Troodos	16	6m + 4sm + 6st	2.8 (mean)	COPPI *et al.* 2006
O. graeca Boiss.	Greece, more localities	18	6m + 6sm + 2st + 4st^{sat} or 8m + 6sm + 2st^{sat} + 2t^{sat}		TEPPNER 1991
O. visianii Clementi	more localities given from central Europe and West Balkan peninsula	18L	4m + 8hb + 4hb^{sat} + 2^{sat} (for last 2 centromere position not given)		TEPPNER 1971
O. visianii Clementi	more localities given from Europe	18	4m + 6sm + 4st + 4st^{sat} or 8m + 2sm + 4st + 4st^{sat}		TEPPNER 1991
O. visianii Clementi	Slovakia, Turňa nad Bodvou, Turniansky hradný vrch hill	18	6m + 8sm + 2st + 2st^{sat}	1.91 – 3.18	this study
O. arenaria Waldst. et Kit.	Slovakia, Slovenský kras karst, Jablonov nad Turňou, Kukudičová skala hill	12L+8S	12Lm + 7Sm + 1Ssm	L 3.22 – 4.33 S 1.07 – 2.36	this study
O. arenaria subsp. *pennina* Braun-Blanq.	Switzerland	12L + 8S	10Lm/sm + 2Lm/sm^{sat} + 6Sm + 2Shb		TEPPNER 1971
O. leptantha Heldr.	2 localities given from Greece, Peloponnisos	22	4m + 4hb + 14 with unspecified centromere position		TEPPNER 1981
O. pygmaea Riedl	Greece, Grevena distr., village of Kranea	22 + 0-1B	10m + 6hb + 2sm^{sat} + 4hb^{sat}		TEPPNER 1981
O. stellulata Waldst. et Kit.	2 localities given from Croatia and Bosnia and Herzegovina	22	10m + 6hb + 2sm^{sat} + 4hb^{sat}		TEPPNER 1971
O. stellulata Waldst. et Kit.	Croatia, Velebit mountains, Mt. Crnopac	22	10m + 6hb + 2sm^{sat} + 4hb^{sat}		TEPPNER 1981
O. fastigiata (Braun-Blanq.) Lacaita	West France	24	metacentric and submetacentric, 6 sat		TISSOT-DAGUETTE 1972
O. tricerosperma Lag. subsp. *tricerosperma*	Spain, Albacete - Villapalacios	24	4M + 12m + 4sm + 4sm^{sat}	3.21 – 4.70	LUQUE 1990
O. helvetica (A. DC.) Boiss.	2 localities given from France and Italy	12L + 16S	no data	L 6 – 7 S 1 – 2	FAVARGER 1971
O. helvetica (A. DC.) Boiss. subsp. *helvetica*	Switzerland, Martigny - La Bâtiaz	12L + 14S	10Lm/sm + 2Lm/sm^{sat} + 10Sm + 2St + 2St^{sat}		TEPPNER 1971
O. helvetica ssp. lucana (Lacaita) Peruzzi, Aquaro et Cesca	Italy, Calabria - Paludi	12L + 14S + 0-2B	12Lm + 12Sm + 2Sm^{sat}	L 6.02 – 8.16 S 2.77 – 4.89	PERUZZI *et al.* 2004
O. pseudoarenaria Schur	Slovakia, Zemplínske vrchy hills, Ladmovce - Dlhá hora hill	12L+14S	12Lm + 14Sm	L 3.36 – 5.03 S 1.39 – 2.67	this study
O. caucasica Levin	North Caucasus	28	26m/sm + 2m/sm^{sat}		TEPPNER 1971
O. caucasica Levin	Caucasus mountains, more localities	28	26m + 2m^{sat}	I-IV pairs 4.0 – 4.5 (V pair not given) VI-XI pairs 2.6 – 3.4 XII-XIV pairs 2.0 – 2.3	POPOVA and ZEMSKOVA 1985
O. alborosea Fisch. et Mey.	Turkey, Içel, Anamur-Mersin; also unspecified material from botanic garden in Karlsruhe	43 (from botanic garden), 44	24m/sm + 13-14hb + 6-7hb^{sat}		TEPPNER 1974
O. kaheirei Teppner	Greece, Attika, Imitos (=Hymettos) SE of Athens	50 + max 8B	metacentric and heterobrachiale sat		TEPPNER 1988
O. kaheirei Teppner	Greece, Sterea Ellas, Nomos Attikis, Mt. Pateras	50, 51	metacentric and submetacentric, 0-4sat	1.8 – 3.5	KAMARI *et al.* 1996

Tab. 2 — Chromosome numbers given for the genus *Onosma* in Slovakia.

Species	Chromosome numbers	Reference	Locality phytogeographical district in brackets
O. arenaria	2n = 20	MÁRTONFI *et al.* (2002)	Kukudičova skala hill (Slovenský kras)
O. pseudoarenaria	2n = 26	MÁJOVSKÝ *et al.* (1978)	Burda hills (Burda)
	2n = 26	MÁRTONFI *et al.* (2002)	village of Ladmovce (Východoslovenská nížina)
O. tornensis	2n = 14	BAKSAY (1957)	village of Turňa nad Bodvou (Slovenský kras)
	2n = 14 & 28*	MÁJOVSKÝ *et al.* (1970)	village of Turňa nad Bodvou (Slovenský kras)
	2n = 14, n = 7	TEPPNER (1971)	village of Turňa nad Bodvou (Slovenský kras)
	2n = 14	TISSOT-DAGUETTE (1979)	„Czechia" [Czechoslovakia]
	2n = 14, n = 7	TISSOT-DAGUETTE (1979)	Slovakia
	2n = 14	TISSOT-DAGUETTE (1979)	village of Turňa nad Bodvou (Slovenský kras)
O. visianii	2n = 18	LETZ *et al.* (1999)	village of Turňa nad Bodvou (Slovenský kras)
	2n = 18	LETZ *et al.* (1999)	village of Drienovec (Slovenský kras)
	2n = 18	MÁJOVSKÝ *et al.* (2000)	village of Turňa nad Bodvou (Slovenský kras)
	2n = 18	MÁJOVSKÝ *et al.* (2000)	village of Domica (Slovenský kras)

* – doubtful datum marked by original authors as „polysomaty" which refers to endopolyploidy.

cubation at 4°C, each sample and standard were mixed and measured. 5000 or 10 000 nuclei were analysed for each sample using a BD CellQuest Pro Software (Becton Dickinson, San Jose, CA, USA). The estimation of DNA amount of samples was based on value of the G1 peak means: DNA amount of sample = DNA amount of used standard × [(sample G_1 peak mean)/(standard G1 peak mean)]. Statistics of measured data was performed using Statgraphics v. 15.0.10 software.

RESULTS AND DISCUSSION

The results of the study of 4 species of the genus *Onosma* from Carpathian region confirmed known chromosome numbers for the species studied. New results are represented by karyotype studies of these species (Tab. 3, Fig. 1) which allowed to prepare karyotypic formulas. Chromosomes of the studied species are 1.07 µm to 5.03 µm long. In accordance with accustomed designation, chromosomes of three taxa are evaluated as long (L) and short (S), chromosomes of *O. visianii* are not included in these categories (for the reasons given later in the discussion). For particular species, karyotypic formulas are the following: *O. tornensis* (2n=14S): 10Sm + 4Ssm; *O. visianii* (2n=18): 6m + 8sm + 2st + 2st^{sat}; *O. arenaria* (2n=12L + 8S, S chromosomes unpaired): 12Lm + 7Sm + 1Ssm and *O. pseudoarenaria* (2n=12L + 14S): 12Lm + 14Sm.

For *O. tornensis* (group *Asterotricha*) this is the first published karyotype. It can be compared with the karyotypes known in the group of 14 chromosome taxa (see Tab. 1). To sum up, chromosomes of these taxa are usually metacentric in the number 8-14, further ones are submetacentric or they can bear satellites (cf., TEPPNER 1974; 1988 and 1996; POPOVA and ZEMSKOVA 1990; CONSTANTINIDIS *et al.* 2002 and COPPI *et al.* 2006). These data, including our results and many others which brought only chromosome counts, confirm probably common occurrence of this group of diploid taxa. As far as the length of the measured chromosomes is concerned, POPOVA and ZEMSKOVA (1990) give the values 1.78 – 3.03 µm for *O. simplicissima* (which is, however, *Haplotricha* group) and COPPI *et al.* (2006) give mean value of 2.2 µm for *O. echioides*. Our results are similar for *O. tornensis*: 1.62 – 2.94 µm, 2.12 µm mean value.

O. visianii studied in this work belongs to the group *Haplotricha.* As it is given in the formula, chromosomes of this species are metacentric, submetacentric and acrocentric (also with satellite). They are not classified with the above categories L and S. Their absolute length (1.91 µm - 3.18 µm) suggests their classification with the group of short chromosomes, however, as indicated by TEPPNER (1991) they are visible in earlier stadium of prophase of mitosis. This led him to classification of these chromosomes into the group of long chromosomes of the type "*O. setosa*". In the figures of mitotic metaphases of *O. visianii* published by TEPPNER (1991), chromosomes are, however, longer in some cases (very early metaphase?) and shorter, resembling thus visually the metaphases observed in our work, in the others. Similarly, in the ideograms of two metaphases observed by TEPPNER (1991), the absolute chromosome length calculated with the employment of the scales giv-

Tab. 3 — Chromosome characteristics of studied *Onosma* species. N – ordinal number of chromosome, D – length of chromosome in μm; RD – relative chromosome length; RI – arm index; CI – centromeric index; T – chromosome type: m – metacentric, sm – submetametric, st – subacrocentric, sat - satelite).

	N	D	RD	RI	CI	T
Onosma arenaria	1	4.33	0.0723	1.53	39.5	m
	2	4.15	0.0692	1.35	42.6	m
1-6 are pairs of homologous L chromosomes, 7-14 unpaired S chromosomes	3	3.90	0.0651	1.20	45.5	m
	4	3.82	0.0637	1.08	48.1	m
	5	3.70	0.0617	1.10	47.6	m
	6	3.22	0.0537	1.16	46.3	m
	7	2.36	0.0394	1.94	34.0	sm
	8	1.95	0.0325	1.21	45.2	m
	9	1.84	0.0307	1.38	42.0	m
	10	1.76	0.0294	1.22	45.0	m
	11	1.71	0.0285	1.20	45.5	m
	12	1.52	0.0254	1.32	43.7	m
	13	1.48	0.0247	1.29	43.1	m
	14	1.07	0.0179	1.15	46.5	m
Onosma pseudoarenaria	1	5.03	0.0656	1.44	41.0	m
	2	4.56	0.0595	1.27	44.1	m
1-6 are pairs of homologous L-chromosomes, 7-13 are pairs of homologous S-chromosomes	3	4.04	0.0527	1.27	44.1	m
	4	3.89	0.0507	1.27	44.0	m
	5	3.64	0.0475	1.32	43.1	m
	6	3.36	0.0438	1.17	46.1	m
	7	2.67	0.0348	1.28	43.9	m
	8	2.42	0.0316	1.22	44.9	m
	9	2.10	0.0274	1.19	45.8	m
	10	1.85	0.0241	1.26	44.1	m
	11	1.77	0.0231	1.49	40.2	m
	12	1.62	0.0211	1.42	41.5	m
	13	1.39	0.0130	1.44	41.0	m
Onosma tornensis	1	2.94	0.0989	1.24	44.6	m
	2	2.53	0.0851	1.13	46.9	m
1-7 are pairs of homologous S chromosomes	3	2.23	0.0750	1.27	44.0	m
	4	2.02	0.0680	1.10	47.6	m
	5	1.90	0.0639	1.44	41.0	m
	6	1.62	0.0545	1.74	36.6	sm
	7	1.62	0.0545	1.80	35.7	sm
Onosma visianii	1	3.18	0.0722	1.31	43.2	m
	2	2.98	0.0677	1.33	42.7	m
1-9 are pairs of homologous chromosomes	3	2.71	0.0615	2.38	29.4	sm
	4	2.42	0.0550	1.62	38.9	m
	5	2.40	0.0545	1.83	34.3	sm
	6	2.20	0.0500	5.32	15.7	st
	7	2.13	0.0484	2.40	29.3	sm
	8	2.09	0.0475	2.20	32.0	sm
	9	1.91	0.0434	3.60	22.7	st, sat

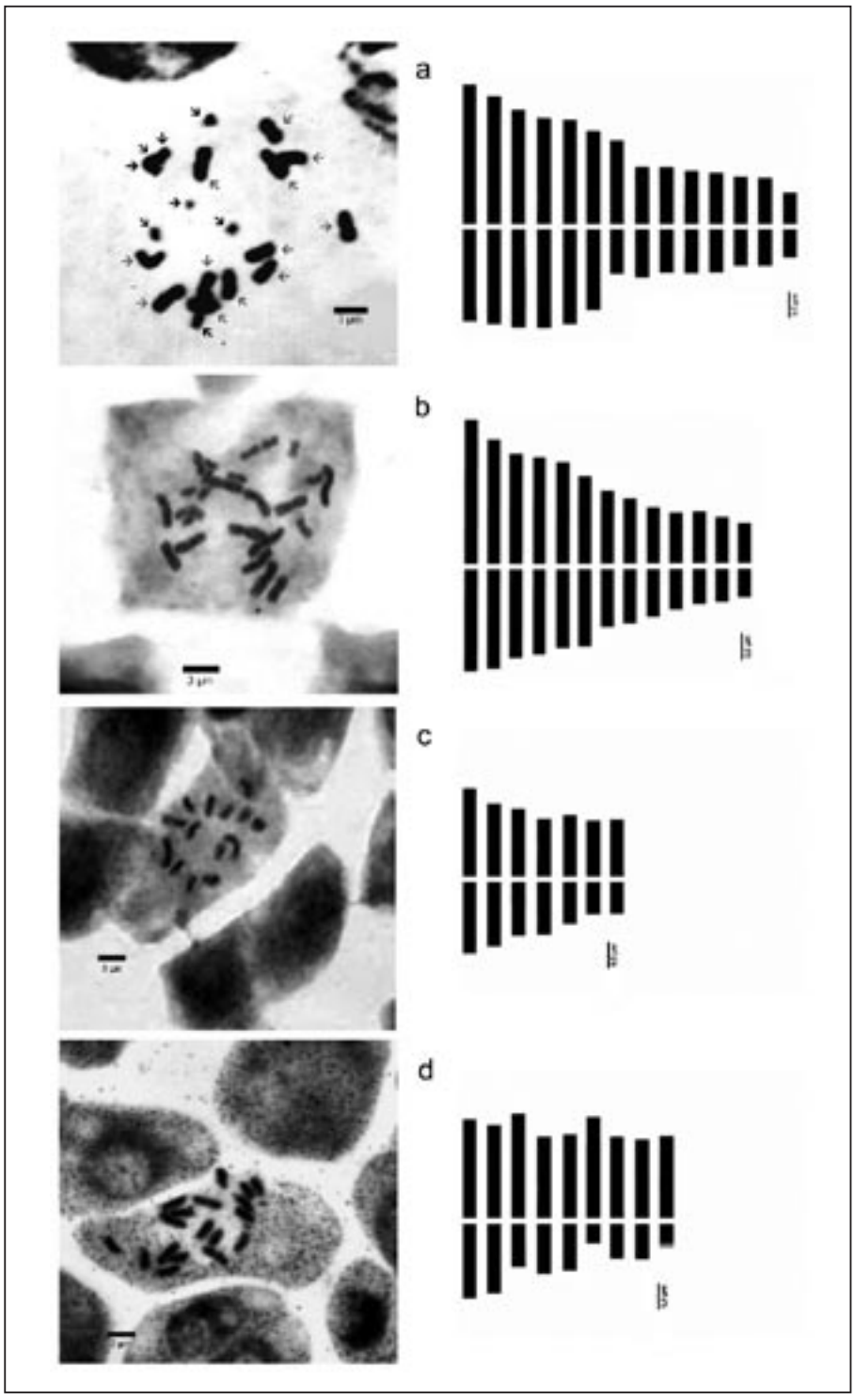

Fig. 1 — Microphotographs of c-metaphase (scale bar 3 μm) and ideograms (scale bar 0.5 μm) of the *Onosma* species studied: **a** – *O. arenaria* 2n=12L + 8S, 6 pairs of homologous L chromosomes and 8 unpaired S chromosomes (gray arrows – L chromosomes, black arrows – S chromosomes); **b** – *O. pseudoarenaria* 2n=12L + 14S, 6 pairs of homologous L chromosomes and 7 pairs of homologous S chromosomes; **c** – *O. tornensis* 2n=14S, 7 pairs of homologous S chromosomes; **d** – *O. visianii* 2n=18, 9 pairs of homologous chromosomes.

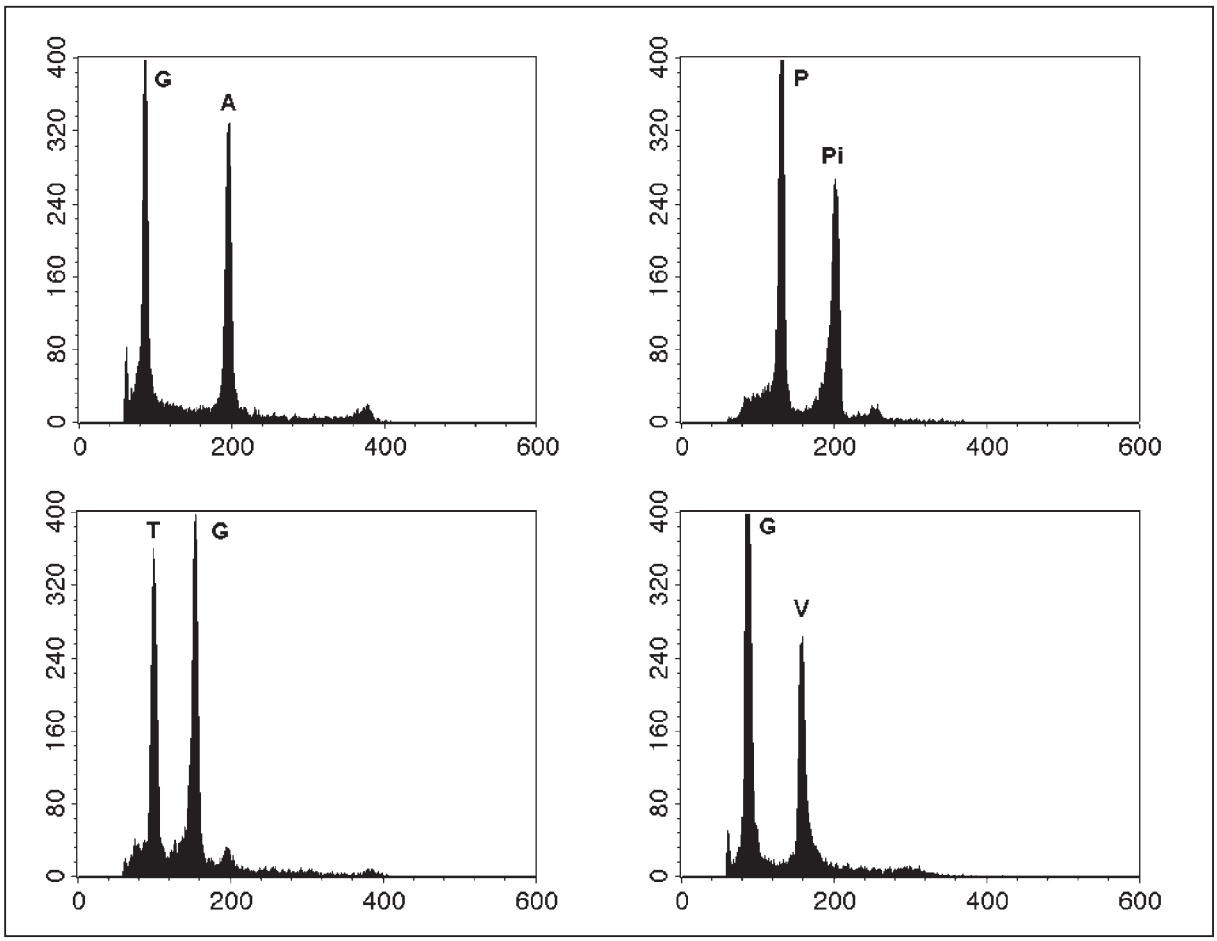

Fig. 2 — Flow cytometric histograms of relative fluorescence intensity (propidium iodide) obtained after simultaneous analysis of nuclei of reference standard (G = *Glycine max* 'Polanka', 2C = 2.37 pg DNA or PI = *Pisum sativum* 'Ctirad', 2C = 8.76 pg DNA) and *Onosma* samples studied, A = *O. arenaria*, P = *O. pseudoarenaria*, T = *O. tornensis*, V = *O. visianii*.

en in the figures are 2.27 μm – 3.15 μm and 1.90 μm – 3.05 μm. These results agree with our data. Karyotype formulas for *O. visianii* prepared on the basis of Teppner's data (TEPPNER 1971 and 1991) similarly point out heterogeneity of chromosome set (see Tab. 1) and the presence of metacentric, submetacentric and acrocentric chromosomes.

There are also few detailed karyological data for further two species: *O. arenaria* and *O. pseudoarenaria* from the group *Heterotricha*. Our results confirmed the presence of long and short chromosomes in expected numbers. Despite the fact that these species are probably of hybridogenous origin (TEPPNER 1971 and 1991; VOUILLAMOZ 2001), they are characteristic mainly by chromosomes with centromere position in median region of chromosomes. For the species *O. arenaria* there are only data by TEPPNER (1971) concerning *O. arenaria* subsp. *pennina* Braun-Blanq. from Switzerland – this subspecies has L chromosomes metacentric or submetacentric, only two short chromosomes are given by TEPPNER (1971) as heterobrachiale. Our data for O. *arenaria* subsp. *arenaria* differ only little and the length of chromosomes of *O. arenaria* have not been published so far. Karyotype data for *O. pseudoarenaria* presented here are published for the first time. The same chromosome number 2n=12L + 14S (together with the data on karyotype) was given by TEPPNER (1971) for *O. helvetica* subsp. *helvetica* from Switzerland and by PERUZZI *et al.* (2004) for *O. helvetica* subsp. *lucana* (Lacaita) Peruzzi, Aquaro et Cesca from southern Italy, however, there are substantial differences between the karyotypes of these taxa (see Tab. 1). Opposite to our results, study on the latter gives data of evidently longer chromosomes. Longer

Tab. 4 — Genome size analysis of *Onosma* species studied. The values are given as mean and standard deviation of the mean (SD) of the nuclear DNA content (pg/2C) and as a mean of the 1C genome size in Mbp. Chromosome abbreviations: S – short chromosomes, L – long chromosomes. Letters *a-d* followed after nuclear DNA content mean values indicate statistically significant differences between groups according to the Tukey's pairwise comparison test at $p \leq 0.05$ (Significance in ANOVA test: $p < 0.001$).

Taxon	Chromosome number	No. of samples	Nuclear DNA content (pg/2C)	SD	CV (%)	1C genome size (Mbp)*
O. tornensis	14S	5	1.56*a*	0.039	2.54	762
O. visianii	18	7	4.12*b*	0.241	5.87	2014
O. arenaria	12L+8S	9	5.15*c*	0.251	4.88	2518
O. pseudoarenaria	12L+14S	11	5.74*d*	0.247	4.32	2806

* 1 pg DNA = 978 Mbp according to Doležel et al. (2003)

chromosomes of *O. helvetica* were recorded also by FAVARGER (1971) in his earlier study. Moreover, data of TEPPNER (1971) indicate the presence of metacentric, submetacentric and also subacrocentric chromosomes within the chromosome set of *O. helvetica* subsp. *helvetica*. Compared with that, our data and the data of PERUZZI *et al.* (2004) coincide with fact, that bimodal chromosome sets of two different taxa consist of metacentric chromosomes only. Furthermore, data for both subspecies of *O. helvetica* indicate the presence of chromosomes with satellite, our data for *O. pseudoarenaria* do not. Finally, PERUZZI *et al.* (2004) recorded B-chromosomes, however, their presence is spread among taxa of *Heterotricha* (cf. VOUILLAMOZ 2001, karyological survey). Evident differencies between karyotypes of *O. helvetica* and *O. pseudoarenaria* can be considered in connection with hypotheses about their origin. The neighbour-joining analysis of RAPD performed by VOUILLAMOZ (2001) provided the results to discriminate between two distinct origins for the group of taxa with 2n=12L + 8S in Switzerland. VOUILLAMOZ (1999-2000) suggested polytopic origin for the taxa with 2n=12L + 14S based on karyological and morphological characters. It is possible that different results compared here prove polyphyletic origin of different taxa of *Heterotricha*.

No data on genome size of the studied species have been published so far. Despite the fact that karyological data for the studied species of the genus *Onosma* give long and short chromosomes we can state an increase of genome size related to increasing chromosome number (Tab. 4). 1C DNA values between 0.78 - 2.87 pg, which were measured in this work, point out relatively small genome in comparison with all angiosperms, where they range 0.065 – 127.40 pg, average value 6.30 pg (LEITCH and BENNET 2007). The evaluation of the obtained data shows statistically significant difference in DNA content of particular studied species of the genus *Onosma* (ANOVA and Tukey's pairwise comparison test). Discrimination between the species *O. arenaria* a *O. pseudoarenaria* is, however, on the basis of single data not sufficiently reliable, 2C DNA content varied between 4.77 pg and 5.39 pg for particular samples of *O. arenaria*, and between 5.32 pg and 6.18 pg for *O. pseudoarenaria.* With regards to these results and difficult determination of the two species on the basis of morphology, chromosome number and length remains henceforward the only reliable marker for their determinaton.

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Appendix

Origin of plant material used in karyotype study and study of genome size of species of the genus *Onosma* in Slovakia. Collectors: AS - Alžbeta Szabóová, EK - Emília Karasová, HR - Helena Rosinová, JB - János Bogoly, LM - L'uboš Majeský, PM - Pavol Mártonfi, VK - Vladislav Kolarčik, the numbers in brackets indicate the number of samples for flow cytometry.

Onosma arenaria Waldst. & Kit.

1. East Slovakia, Slovenský kras karst, Jablonov nad Turňou, Kukudičova skala hill, N: 48°36´11´´, E: 20°40´03´´, 508m; karyotype: 24. 5. 2001, PM, EK; cytometry: 13.7.2007, VK, EK (4).

2. West Slovakia, Belianske kopce hills near the town of Štúrovo, Vršok hill, N: 47°49´11´´, E: 18°39´25´´, 198m; cytometry: 25.7.2005, VK, LM (3).

3. West Slovakia, Nitra, National Nature Reserve Lupka, N: 48°20´14´´, E: 18°04´32´´, 245m; cytometry: 3.8.2005, VK, LM, HR (2).

Onosma pseudoarenaria Schur

4. East Slovakia, Zemplínske vrchy hills, Ladmovce - Dlhá hora hill, N: 48°25´46´´, E: 21°46´28´´, 202m; karyotype: 18. 7. 2001, PM, JB; cytometry: 6.7.2005, VK, JB (6).

5. West Slovakia, Čenkov near the town of Štúrovo, Čenkovský les forest, N: 47°46´56´´, E: 18°31´36´´, 111m; cytometry: 25.7.2005, VK, LM, AS (4).

6. West Slovakia, Kováčovské kopce hills near the town of Štúrovo, N: 47°49´27´´, E: 18°46´38´´, 200m; cytometry: 26.7.2005, VK (1).

Onosma tornensis Jáv.

7. East Slovakia, Turňa nad Bodvou, Turniansky hradný vrch hill, N: 48°36´41´´, E: 20°52´15´´, 301m; karyotype: 24. 5. 2001, EK; cytometry: 13.7.2005, VK, EK (5).

Onosma visianii Clementi

8. East Slovakia, Turňa nad Bodvou, Turniansky hradný vrch hill, N: 48°36´41´´, E: 20°52´15´´, 301m; karyotype: 14. 9. 2007, VK; cytometry: 13.7.2005, VK, EK (3).

9. East Slovakia, Slovenský kras karst, Jablonov nad Turňou, Kukudičová skala hill, N: 48°36´11´´, E: 20°40´03´´, 508m; cytometry: 13.7.2007, VK, EK (1). 10. West Slovakia, Považský Inovec hills, Tematínske kopce hills - Borovište hill, N: 48°40´15´´, E: 17°54´15´´, 361m; cytometry: 2.8.2005, VK (4).