

Chromosome numbers of selected woody species from the Bulgarian flora

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Abstract. The chromosome numbers of 15 woody species from the Bulgarian flora are reported: *Acer platanoides*, *A. pseudoplatanus*, *Ailanthus altissima*, *Alnus glutinosa*, *Carpinus betulus*, *Cornus mas*, *C. sanguinea*, *Coronilla emerus* subsp. *emeroides*, *Euonymus latifolia*, *Fraxinus ornus*, *Humulus lupulus*, *Periploca graeca*, *Quercus dalechampii*, *Sambucus nigra* and *Viburnum opulus*. The results for 12 species confirm previously reported counts from Bulgaria and elsewhere, whereas the chromosome numbers of *Ailanthus altissima* ($2n = 64$), *Alnus glutinosa* ($2n = 28$) and *Cornus mas* ($2n = 18$) are reported for the first time from Bulgarian accessions. New chromosome count of $2n = 14$ has been established for *Coronilla emerus* subsp. *emeroides* from Bulgarian accession.

Key words: Bulgarian flora, karyology, shrubs, trees

Introduction

More than 360 species of the Bulgarian flora are trees, shrubs or semi-shrubs (Kožuharov & al. 1988; personal data). Richest in species are *Rosaceae*, *Fabaceae*, *Salicaceae*, *Fagaceae*, *Ericaceae*, *Pinaceae*, *Aceraceae*. The intraspecific variation has been studied in more details only in economically important species.

So far about 15% of the Bulgarian phanerophytic flora has been studied karyologically. A higher number of species has been investigated from the families *Caprifoliaceae* (Česchmedžiev 1983; Česhmedžiev 1994), *Cistaceae* (Markova 1975), *Fabaceae* (Kozuharov & al. 1972; Kuzmanov 1978), *Oleaceae* (Markova & Goranova 1987; Anchev 1993), and *Rosaceae* (Markova 1968; Petrova & Stoyanova 1998). At the same time, karyological information is lacking on the families *Aceraceae*, *Betulaceae*, *Cupressaceae*, *Ericaceae*, *Fagaceae*, *Pinaceae*, *Rhamnaceae*, *Salicaceae*, *Tiliaceae*, *Ulmaceae*, or is based solely on single species for the Bulgarian territory. In general, the available infor-

mation is not sufficient for revealing the intraspecific variation and the distribution of different cytotypes on the territory of Bulgaria.

Material and methods

Plant material (seeds, fruits and live specimens) was collected from natural accessions in Bulgaria. The plants have been cultivated in the experimental glasshouse of the Institute of Botany (BAS). The karyological studies have been conducted at the Cytotaxonomic Laboratory of the same institute. Voucher specimens have been deposited in SOM.

Root-tips were pretreated with 0.01% colchicine for 60–90 min, fixed in ethanol:glacial acetic acid (3:1) for at least 2 h at room temperature, or for 24 h in refrigerator, and stored in 96% ethanol until required. Hydrolyzation was conducted in 1N HCl at 60°C for 20–40 min. Then root-tips were transferred in HCl:ethyl ether (1:1) for 10–20 min at 60°C, washed

in distilled water and stained with haematoxylin after Gomori (Melander & Wingstrand 1953) for 45–90 min at 60 °C. Finally root-tips were squashed in 45 % acetic acid and mounted in Euparal.

Results and discussion

Acer platanoides L.

$2n = 26$ (Fig. 1)

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720–800 m, 27.04.2004, coll. D. Ivanova & V. Vladimirov (no. 204-28).

The reported result confirms the data of Goranova & al. (in press) from a Bulgarian accession and of other authors from elsewhere (see references in Goldblatt 1981, 1988; Goldblatt & Johnson 1991, 1994, 2000).

Acer pseudoplatanus L.

$2n = 52$ (Fig. 2)

Valley of Strouma River: N-NW of Gabrene village, Petrich district, 41° 22' 18" N, 22° 57' 54" E, 317 m, 26.04.2004, coll. D. Ivanova, D. Dimitrova & V. Vladimirov (VV 04-38).

The chromosome number reported in the present paper coincides with the results of Ivanova & al. (2005) and Goranova & al. (in press) from Bulgarian accessions, and of other authors from abroad (see in Goldblatt 1981, 1984; Goldblatt & Johnson 1991, 1998, 2000).

Ailanthus altissima (Mill.) Swingle

$2n = 64$ (Fig. 3)

Valley of Strouma River: along the road Sandanski – Petrich, 117 m, 41° 28' 16" N, 23° 15' 15" E, 26.04.2004, coll. D. Ivanova, D. Dimitrova & V. Vladimirov (no. 204-17).

This is the first count from a Bulgarian accession. The result confirms the data published by Murín (1978) from Slovakian localities. Chromosome number of $2n = 80$ has been published by Desai (see reference in Fedorov 1969).

Alnus glutinosa (L.) Gaertn.

$2n = 28$ (Fig. 4)

Valley of Strouma River: N-NW of Gabrene village, Petrich district, 41° 22' 18" N, 22° 57' 54" E, 317 m, 26.04.2004, coll. D. Ivanova, D. Dimitrova & V. Vladimirov (VV 04-44).

This is the first record of a chromosome number of *A. glutinosa* from Bulgaria. Our count coincides with the data reported by many authors from elsewhere (see in Fedorov 1969; Goldblatt 1981, 1985, 1988; Goldblatt & Johnson 1990, 1991, 1994, 2000, 2003). A chromosome number of $2n = 56$ is given in Fedorov (1969).

Carpinus betulus L.

$2n = 64$ (Fig. 5)

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720–800 m, 27.04.2004, coll. D. Ivanova & V. Vladimirov (no. 204-31).

The result confirms earlier reports from accessions in Bulgaria (Petrova & al. in press) and elsewhere (see in Goldblatt 1981; Goldblatt & Johnson 2000).

Cornus mas L.

$2n = 18$ (Fig. 6)

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720–800 m, 27.04.2004, coll. D. Ivanova & V. Vladimirov (no. 204-30).

Our count is the first one reported for *C. mas* from Bulgarian accession. The same number has been reported previously by several authors (see in Fedorov 1969; Goldblatt 1984, 1985; Goldblatt & Johnson 2000). Chromosome number of $2n = 54$ has also been published (see in Fedorov 1969).

Cornus sanguinea L.

$02n = 22$ (Fig. 7)

Valley of Strouma River: N-NW of Gabrene village, Petrich district, 41° 22' 18" N, 22° 57' 54" E, 317 m, 26.04.2004, coll. D. Ivanova, D. Dimitrova & V. Vladimirov (VV 04-51, VV 04-52).

The chromosome number $2n = 22$ is in accordance with previously reported data from Bulgaria (Česhmedziev 1994; Petrova & al. in press) and other countries (see in Fedorov 1969; Goldblatt 1984, 1988; Goldblatt & Johnson 1991, 1994, 1996, 1998, 2000).

Coronilla emerus subsp. *emeroides* (Boiss. & Sprun.) Hayek

$2n = 14$ (Fig. 8)

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720–800 m, 27.04.2004, coll. D. Ivanova & V. Vladimirov (no. 204-50).

Previous counts from different Bulgarian localities (Krusheva 1975) show a chromosome number $2n = 16$. Our result coincides with the data reported by Šopova & Sekovski (1989) from Macedonia. Same results are given by Májovský & Uhríková (1985) and Verlaque & al. (1987) for *C. emerus* s.l.

***Euonymus latifolia* (L.) Mill.**

$2n = 64$ (Fig. 9)

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720-800 m, 27.04.2004, coll. *D. Ivanova* & *V. Vladimirov* (no. 204-44).

This chromosome number has already been reported from a Bulgarian accession (Goranova & al. in press). It confirms the data from elsewhere (see in Goldblatt 1988; Goldblatt & Johnson 1991).

***Fraxinus ornus* L.**

$2n = 46$ (Fig. 10)

Valley of Strouma River: N-NW of Gabrene village, Petrich district, 41° 22' 18" N, 22° 57' 54" E, 317 m, 26.04.2004, coll. *D. Ivanova*, *D. Dimitrova* & *V. Vladimirov* (VV 04-50).

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720-800 m, 27.04.2004, coll. *D. Ivanova* & *V. Vladimirov* (no. 204-32).

Our counts are the second report of the same chromosome number based on plant material from Bulgaria and confirm the earlier studies from elsewhere (see references in Ivanova & al. 2005).

***Humulus lupulus* L.**

$2n = 20$ (Fig. 11)

Valley of Strouma River: N-NW of Gabrene village, Petrich district, 41° 22' 18" N, 22° 57' 54" E, 317 m, 26.04.2004, coll. *D. Ivanova*, *D. Dimitrova* & *V. Vladimirov* (VV 04-54).

Our finding of $2n = 20$ is in agreement with numerous reports for this taxon from Bulgaria (Česhmedziev 1994) and elsewhere (Moutschen & al. 1973, for other references see in Fedorov 1969; Goldblatt 1981, 1985, 1988; Goldblatt & Johnson 1990, 1998, 2000, 2003). Stepanov & Muratova (1995) reported also the number $2n = 40$, and Shephard & al. (2000) $2n = 21$.

***Periploca graeca* L.**

$2n = 22$ (Fig. 12)

Valley of Strouma River: opening in *Platanus orientalis*-forest, N of Kolarovo village, Petrich district, 250 m,

27.04.2004, coll. *D. Ivanova*, *D. Dimitrova* & *V. Vladimirov* (no. 204-73).

The chromosome number reported here confirms the data given by Česhmedziev (1994) from Bulgaria and Strid & Franzen (1981) from Greece.

***Quercus dalechampii* Ten.**

$2n = 24$ (Fig. 13)

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720-800 m, 27.04.2004, coll. *D. Ivanova* & *V. Vladimirov* (no. 204-38).

This chromosome count confirms the data of Goranova & al. (in press) from Bulgaria and of Murín (1974), Váchová (1978) and D'Emerico & al. (1995) from abroad.

***Sambucus nigra* L.**

$2n = 36$ (Fig. 14)

Mt Belasitsa: in a deciduous forest, along the trail from Belasitsa chalet to the Waterfall, 720-800 m, 27.04.2004, coll. *D. Ivanova* & *V. Vladimirov* (no. 204-29).

Our chromosome count confirms the data of many authors from different parts of Europe and elsewhere (see in Goldblatt 1981, 1984, 1985, 1988; Goldblatt & Johnson 1990, 1996, 2000, 2003). Sandhu & Mann (1988) have established two B-chromosomes ($n = 18 + 0-2B$). The intraspecific taxa *S. nigra* f. *luteo-variegata* (Weston) Schwer. and *S. nigra* f. *nigra* have been studied from Bulgarian accessions (Česchmedjiev 1976; Česchmedžiev 1983) and both have the same chromosome number $2n = 36$.

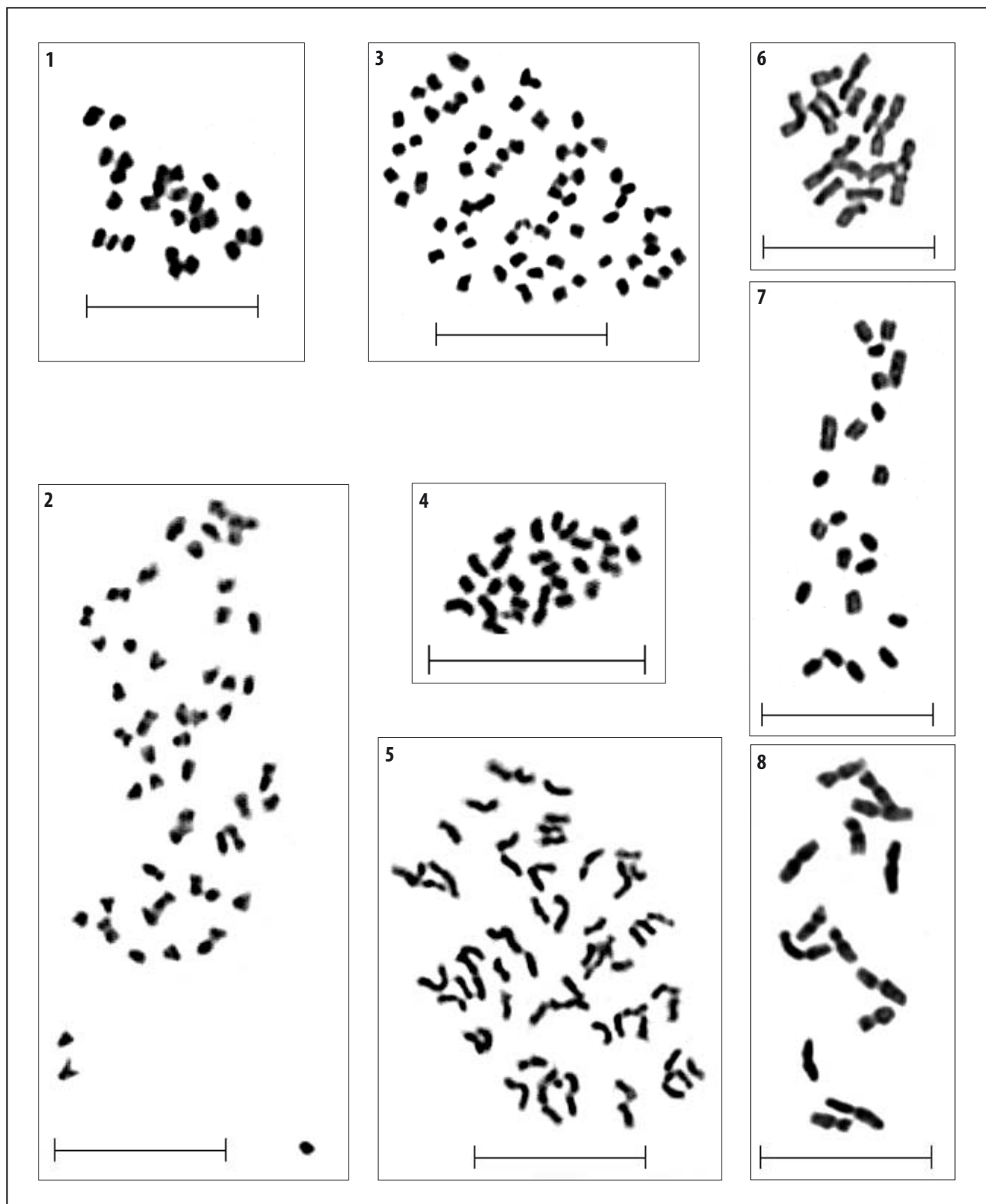
***Viburnum opulus* L.**

$2n = 18$ (Fig. 15)

Valley of Strouma River: opening in *Platanus orientalis*-forest, N of Kolarovo village, Petrich district, 250 m, 27.04.2004, coll. *D. Ivanova*, *D. Dimitrova* & *V. Vladimirov* (no. 204-64).

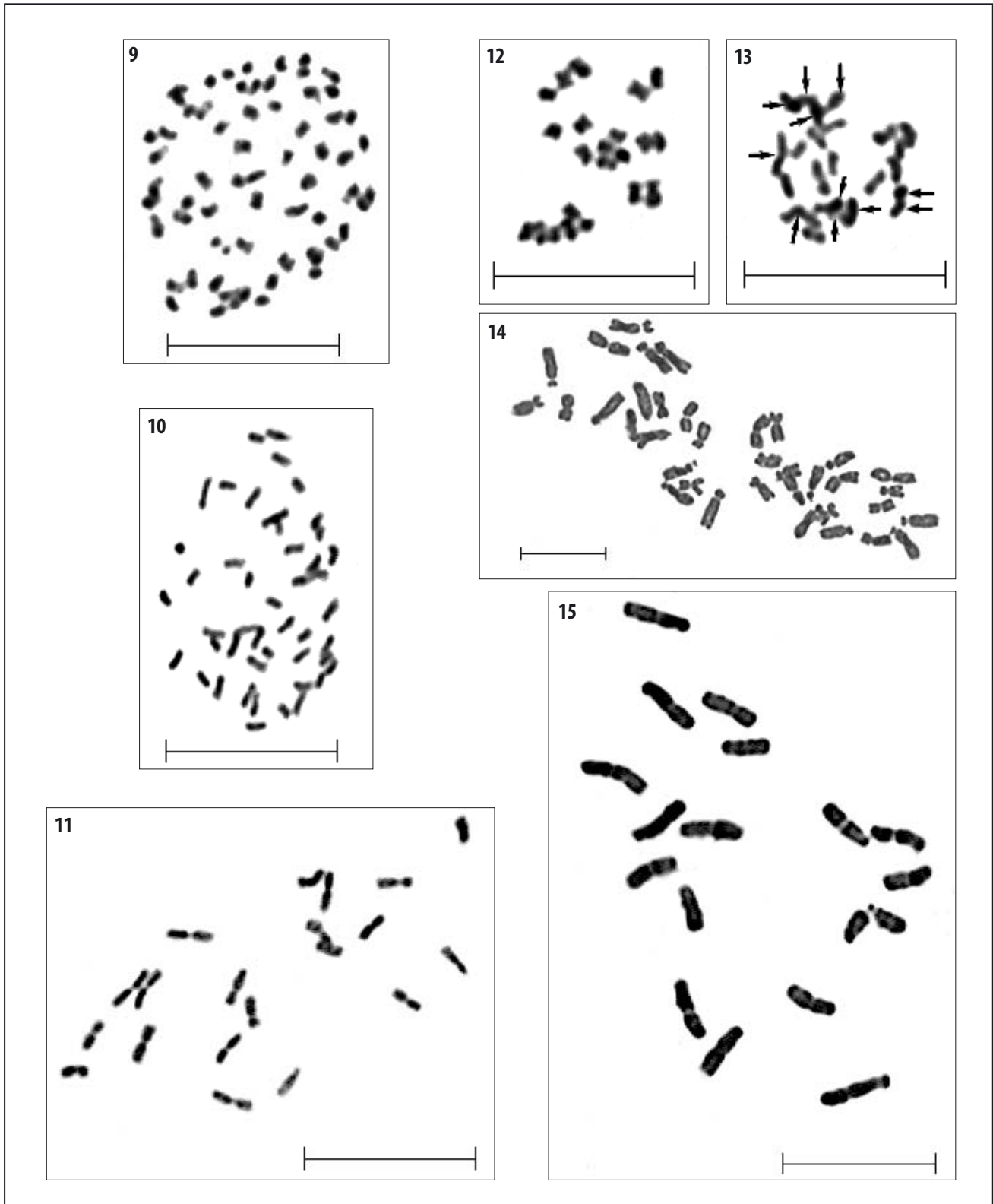
The chromosome count obtained by us confirms the earlier reports by Česhmedziev (1994) and Ivanova & al. (2005) from Bulgarian accessions, and other authors from elsewhere (see in Goldblatt 1981, 1984; Goldblatt & Johnson 1994, 1996, 2000).

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Figs 1–8. Microphotographs of metaphase plates of:

1, *Acer platanoides*, $2n=26$; 2, *Acer pseudoplatanus*, $2n=52$; 3, *Ailanthus altissima*, $2n=64$; 4, *Alnus glutinosa*, $2n=28$; 5, *Carpinus betulus*, $2n=64$; 6, *Cornus mas*, $2n=18$; 7, *Cornus sanguinea*, $2n=22$; 8, *Coronilla emerus* subsp. *emeroides*, $2n=14$. Scale bar – 10 μm .



Figs 9–15. Microphotographs of metaphase plates of: **9**, *Euonymus latifolia*, $2n=64$; **10**, *Fraxinus ornus*, $2n=46$; **11**, *Humulus lupulus*, $2n=20$; **12**, *Periploca graeca*, $2n=22$; **13**, *Quercus dalechampii*, $2n=24$; **14**, *Sambucus nigra*, $2n=36$; **15**, *Viburnum opulus*, $2n=18$. Scale bar – 10 μm .

References

- Anchev, M.E. 1993. Reports (217-227). – In: Kamari, G., Felber, F. & Garbari, F. (eds), Mediterranean chromosome number reports – 3. – Fl. Medit., **3**: 358-363.
- Česchmedjiev, I.V. 1976. Reports. – In: Löve, Á. (ed.), IOPB Chromosome number reports LIV. – Taxon, **25**(5/6): 642-643.
- Česchmedžiev, I. 1983. Cytotaxonomic investigation of the genus *Sambucus* L. in Bulgaria. – Fitologiya, **23**: 30-44.
- Česhmedziev, I. 1994. Reports (313-366). – In: Kamari, G., Felber, F. & Garbari, F. (eds), Mediterranean chromosome number reports – 4. – Fl. Medit., **4**: 269-279.
- D'Emerico, S., Bianco, P., Medagli, P. & Schivone, B. 1995. Karyotype analysis of *Quercus* spp. (*Fagaceae*). – Silvae Genet., **44**(2-3): 66-70.
- Fedorov, A.A. (ed.). 1969. Chromosome Numbers of Flowering Plants. Nauka, Leningrad.
- Goldblatt, P. (ed.). 1981. Index to plant chromosome numbers 1975-1978. – Monogr. Syst. Bot. Missouri Bot. Gard., **5**.
- Goldblatt, P. (ed.). 1984. Index to plant chromosome numbers 1979-1981. – Monogr. Syst. Bot. Missouri Bot. Gard., **8**.
- Goldblatt, P. (ed.). 1985. Index to plant chromosome numbers 1982-1983. – Monogr. Syst. Bot. Missouri Bot. Gard., **13**.
- Goldblatt, P. (ed.). 1988. Index to plant chromosome numbers 1984-1985. – Monogr. Syst. Bot. Missouri Bot. Gard., **23**.
- Goldblatt, P. & Johnson, D. E. (eds). 1990. Index to plant chromosome numbers 1986-1987. – Monogr. Syst. Bot. Missouri Bot. Gard., **30**.
- Goldblatt, P. & Johnson, D. E. (eds). 1991. Index to plant chromosome numbers 1988-1989. – Monogr. Syst. Bot. Missouri Bot. Gard., **40**.
- Goldblatt, P. & Johnson, D. E. (eds). 1994. Index to plant chromosome numbers 1990-1991. – Monogr. Syst. Bot. Missouri Bot. Gard., **51**.
- Goldblatt, P. & Johnson, D. E. (eds). 1996. Index to plant chromosome numbers 1992-1993. – Monogr. Syst. Bot. Missouri Bot. Gard., **58**.
- Goldblatt, P. & Johnson, D. E. (eds). 1998. Index to plant chromosome numbers 1994-1995. – Monogr. Syst. Bot. Missouri Bot. Gard., **69**.
- Goldblatt, P. & Johnson, D. E. (eds). 2000. Index to plant chromosome numbers 1996-1997. – Monogr. Syst. Bot. Missouri Bot. Gard., **81**.
- Goldblatt, P. & Johnson, D. E. (eds). 2003. Index to plant chromosome numbers 1998-2000. – Monogr. Syst. Bot. Missouri Bot. Gard., **94**.
- Goranova, V., Stanimirova, P. & Ančev, M. In press. Reports. – In: Kamari, G., Blanché, C. & Garbari, F. (eds), Mediterranean chromosome number reports – 16. – Fl. Medit., **16**.
- Ivanova, D., Stanimirova, P. & Vladimirov, V. 2005. Reports (1445-1456). – In: Kamari, G., Blanché, C. & Garbari, F. (eds), Mediterranean chromosome number reports – 15. – Fl. Medit., **15**: 719-728.
- Kožuharov, S., Ančev, M. & Petrova, A. 1988. The phanerophytic gene fund in Bulgarian flora and its conservation. – In: Velkov, D. (ed.), Proc. 10th Congr. Dendrol. 3-8.10.1988. Pp. 128-137. Jusuator, Sofia (in Russian).
- Kozuharov, S., Kuzmanov, B. & Markova, T. 1972. Reports. – In: Löve, Á. (ed.), IOPB Chromosome number reports XXXVI. – Taxon, **21**(2-3): 336-337.
- Krusheva, R. 1975. Reports. – In: Löve, Á. (ed.), IOPB Chromosome number reports L. – Taxon, **24**(5-6): 676-677.
- Kuzmanov, B. 1978. Cytotaxonomic investigation of Bulgarian leguminous plants. – In: Kožuharov, S.I. & Kuzmanov, B.A. (eds), Evolution of Flowering Plants and Florogenesis. *Fabaceae, Scrophulariaceae, Rubiaceae*. Vol. **1**, pp. 11-71. Publishing House Bulg. Acad. Sci., Sofia (in Bulgarian).
- Markova, M. 1975. Karyosystematische Untersuchungen an den *Cistaceae* Bulgariens. – Pl. Syst. Evol., **123**: 283-315.
- Markova, M.L. & Goranova, V.J. 1987. Chromosome numbers of some taxa of *Magnoliopsida*. I. – In: Kuzmanov, B. (ed.), Proc. 4th Natl. Conf. Bot. Vol. **1**, pp. 32-37. Sofia (in Bulgarian).
- Markowa, M. 1968. Chromosome number of four Bulgarian taxa of the genus *Rubus* L. – Izv. Bot. Inst. (Sofia), **18**: 75-77.
- Májovský, J. & Uhríková, A. 1985. Karyotaxonomisches Studium einiger Arten der slowakischen Flora V. – Acta Fac. Rerum Nat. Univ. Comen., Bot., **32**: 59-65.
- Melander, Y. & Wingstrand, K.G. 1953. Gomori's haematoxylin as a chromosome stain. – Stain Technol., **28**: 217.
- Moutschen, J., Moutschen, M. & Degraeve, N. 1973. Contribution à l'étude du déterminisme du sex chez les plantes dioïques. Particularités caryotypiques et meiotiques de *Humulus lupulus* et de *Humulus japonicus*. – Cellule, **69**: 311-339.
- Murín, A. 1974. Reports. – In: Májovský, J. & al. (eds), Index of chromosome numbers in the Slovakian flora. Part 4. – Acta Fac. Rerum Nat. Univ. Comen., Bot., **23**: 1-23.
- Murín, A. 1978. Reports. – In: Májovský, J. & al. (eds), Index of chromosome numbers in the Slovakian flora. Part 6. – Acta Fac. Rerum Nat. Univ. Comen., Bot., **26**: 1-42.
- Petrova, A. & Stoyanova, K. 1998. Reports (941-960). – In: Kamari, G., Felber, F. & Garbari, F. (eds), Mediterranean chromosome number reports – 8. – Fl. Medit., **8**: 251-262.
- Petrova, A., Zieliński, J. & Natcheva, R. In press. Reports. – In: Kamari, G., Blanché, C. & Garbari, F. (eds), Mediterranean chromosome number reports – 16. – Fl. Medit., **16**.
- Sandhu, P.S. & Mann, S.K. 1988. SOCGI plant chromosome number reports – VII. – J. Cytol. Genet., **23**: 219-228.
- Shephard, H.L., Parker, J.S., Darby, P. & Ainsworth, C.C. 2000. Sexual development and sex chromosomes in hop. – New Phytol., **148**: 397-411.
- Šopova, M. & Sekovski, Ž. 1989. Chromosome atlas of some Macedonian angiosperms. V. – Godišen Zborn. Prir.-Mat. Fak. Univ. Skopje, Biol., **39-40**: 353-365.
- Stepanov, N.V. & Muratova, E.N. 1995. Chromosome numbers of some taxa of higher plants on Krasnoyarsk territory. – Bot. Zhurn. (Moscow & Leningrad), **80**(6): 114-116 (in Russian).
- Strid, A. & Franzen, R. 1981. Reports. – In: Löve, Á. (ed.), Chromosome number reports LXXIII. – Taxon, **30**(4): 829-842.
- Váchová, M. 1978. Reports. – In: Májovský, J. & al. (eds), Index of chromosome numbers in the Slovakian flora. Part 6. – Acta Fac. Rerum Nat. Univ. Comen., Bot., **26**: 1-42.
- Verlaque, R., Seidenbinder, M. & Donadille, P. 1987. Recherches cytologiques sur la spéciation en région Méditerranéenne I: espèces à nombre chromosomique stable. – Biol. Écol. Médit., **10**: 273-289.