Karyological variation in the genus *Rubus*, subgenus *Rubus*: new data from the Czech Republic and synthesis of the current knowledge of European species

Karyologická proměnlivost rodu *Rubus*, podrodu *Rubus* – nové údaje z České republiky a syntéza současných znalostí z Evropy

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D e d i c a t e d t o t h e m e m o r y o f J o s e f H o l u b

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The polyploid agamic complex in the subgenus *Rubus* is one of the most intricate groups in the European flora. Sources of taxonomic complexity are polyploidy, facultative apomixis, both past and recent hybridization followed by segregation, and ecological plasticity. We present an updated overview of ploidy level/chromosome number in 228 representatives of the subgenus, which are native in Europe and for which there are karyological studies. The first section is focused on new data obtained in the Czech Republic during the last 14 years. These chromosome counts and determinations of ploidy level using flow cytometry mainly concern the 19 species recently described from Europe and 14 earlier described species that were newly recorded in the Czech Republic. The first karyological findings are presented for 27 species, comprising triploids (2n = 21, eight species), tetraploids (2n = 28, seventeen species), pentaploids (2n = 35, one species) and hexaploids (2n = 42, one species). *Rubus sendtneri* Progel is the first hexaploid species to be recorded in the Czech Republic. Currently known karyological characters of native European species are presented and discussed in the second section. This overview involves data adopted from the recently published Atlas Florae Europaeae, to which the new data we present in the first section was added. Ploidy level is known only for 30% of more than 750 species recognized in Europe. Tetraploids make up 80% of the karyologically examined species. Only three sexual diploid species are native to Europe. Triploids are mainly in the series *Discolores* and *Rubus* of the section *Rubus*, while they are unknown in the evolutionary derived section *Corylifolii*. Pentaploid species are rather less frequent in the subgenus *Rubus*, but occur more often in the section *Corylifolii* than in the section *Rubus*. The rare hexaploids occur only in the section *Corylifolii*. Several cytotypes within one species is rare in European brambles. Members of the section *Corylifolii* are considered to be hybrids and segregation products, which resulted from crosses between species of the section *Rubus* and tetraploid *R. caesius* of section *Caesii*. In this case the pattern in the variation in the ploidy level suggests that unreduced gametes had a role in genesis of the section *Corylifolii*. Taxonomy in *Rubus* is almost exclusively based on morphological characters of the respective taxa. An actualized karyological overview may increase our understanding of the processes that shaped the present structure of this polyploid complex, namely the origin of the recent hybrids and genesis of some of the apomictic species.

K e y w o r d s: chromosome numbers, Europe, facultative apomixis, karyological variation in sections and series, *Rubus*

Introduction

The subgenus *Rubus* is one of the polyploid agamic complexes belonging to one of the most complicated taxonomic groups of flowering plants in central Europe (Asker & Jerling 1992). The ploidy level in European species (the basic chromosome number x = 7) ranges from diploid to hexaploid and the tetraploids are the most widespread. In Europe, the rare diploids reproduce sexually, whereas the numerous polyploid species are facultative apomicts (Asker & Jerling 1992, Weber 1996, Thompson 1997, Kollmann et al. 2000). Sexuality in the polyploid species is highly variable (Šarhanová et al. 2012). Interspecific hybridization, both homoploid and heteroploid, is possible, as crossing experiments have shown (Nybom 1988). The taxa that hybridize can belong to distantly related species (Holub 1997). Some European species are stabilized apomicts, whereas others were formed (and are still being formed) by facultative hybridization between these polyploid taxa, followed by segregation (Weber 1996, Holub 1997). The segregants can restore sexuality (Nybom 1988), which increases the morphological instability in their progenies and thus complicates the identification of the parental species (Weber 1996, Holub 1997). Apomixis in brambles is transitional between apospory and diplospory (Asker & Jerling 1992). Although the embryo develops asexually, the endosperm develops from a fertilized central cell (pseudogamy); so, the fertilizing pollen is essential for the development of viable seed (Nybom 1986, Asker & Jerling 1992). Along with reproduction via seed, the species in the subgenus *Rubus* reproduce vegetatively by means of rooting of above-ground shoots (Asker & Jerling 1992) or by suckers produced by the roots (Weber 1995). The karyological knowledge of European brambles up to the mid 1990s is summarized by Thompson (1997). The karyological data for the whole of the genus *Rubus* throughout the world are also presented there. For 194 species of the subgenus *Rubus* in Europe, many of their chromosome numbers refer to plant material that originated from the British Isles, Scandinavia and the Netherlands. Thompson (1997), based on this overview and the concept of the nomenclature accepted at that time, indicates that around 74% of European species of bramble are tetraploid.

Current taxonomy of the subgenus *Rubus*, at least in Europe, is based on a new and more critical approach to the classification of the species (e.g. Holub 1993, 1995, Weber 1995, 1996, Kurtto et al. 2010). A stable morphological uniqueness of the respective biotype, occurring throughout a broader geographical range, is a pivotal requirement for recognition of separate species. Nevertheless, the richness of species, especially in northwestern and central Europe, is high. Initial rather poor knowledge about the variation in chromosome number in central-European species of the subgenus *Rubus* improved during the 1990s. At that time, the chromosome numbers were determined using conventional karyological techniques (Boratyńska 1994, 1995a, b, 1996, 1997, 1998, Iwatsubo et al. 1995, Krahulcová & Holub 1997, 1998a, b, c). Including both the native and introduced (and then naturalized) taxa in Europe, the cited papers include the chromosome numbers of altogether 119 species from central Europe, namely Poland, Czech Republic and Germany. Since the publication of these chromosome numbers, the number of species recognized in Europe has increased. The recently published Atlas Florae Europaeae (Kurtto et al. 2010) recognizes about 750 species of the subgenus *Rubus*, including those either introduced or cultivated.

Table 1. – List of new karyological data for the Czech Republic. Entry 2n refers to chromosome number, entry DNA ploidy level refers to the nuclear DNA content, which corresponds to the ploidy level determined using flow cytometry (terminology according to Suda et al. 2006). Categorization of species to series corresponds to Kurtto et al. (2010). The only species that was described later (*R. silvae-bohemicae*; Trávníček & Žíla 2011), was assigned to series *Micantes*, according to its original description. The table contains new karyological data except that for *R. constrictus*, *R. lusaticus*, *R. pedemontanus* and *R. hadracanthos*, for which the previously published data (Krahulcová & Holub 1997, 1998a, c) were confirmed. Symbols used: ■ recently described species (since 1996); ▲ species recorded in the Czech Republic during the last twenty years; *new data for this species; × new data for the Czech Republic.

Taxon	Number of localities	2n	DNA ploidy level	Reference to species description	Fig. in this paper
Sect. *Rubus*					
Subsect. *Rubus*					
Ser. *Rubus*					
R. ambrosius Trávn. et Oklej. ■	3		3x (2n ~ 21)*	Trávníček et Oklejewicz, Folia Geobot. 40: 422, 2005	2A
R. constrictus P. J. Müller et Lefèvre	1	28		P. J. Müller et Lefèvre, Jahresber. Pollichia 16-17: 79, 1859	
R. sorbicus H. E. Weber ▲	1		4x (2n ~ 28)*	H. E. Weber, Feddes Repert. 91: 3, 1980	
Ser. *Nessenses* H. E. Weber					
R. scissus W. C. R. Watson ▲	3		4x (2n ~ 28)*	W. C. R. Watson, J. Bot. 75: 162, 1937	
Subsect. *Hiemales* E. H. L. Krause					
Ser. *Discolores* (P. J. Müller) Focke					
R. austroslovacus Trávn. ■	3	21*		Trávníček, Preslia 77: 52, 2005	
R. flos-amygdalae Trávn. et Holub ■	5	21*		Trávníček et Holub, Preslia 77: 12, 2005	
R. guttiferus Trávn. et Holub ■	4	21*		Trávníček et Holub, Preslia 77: 42, 2005	1A
R. parthenocissus Trávn. et Holub ■	4	21*		Trávníček et Holub, Preslia 77: 60, 2005	1B
R. pericrispatus Holub et Trávn. ■	5	21*		Holub et Trávníček, Preslia 77: 30, 2005	1C
R. phyllostachys P. J. Müller ▲	3	21*		P. J. Müller, Flora, Regensburg, 41: 133, 1858	1D
R. perperus H. E. Weber ■	6	21*		H. E. Weber, Ber. Bayer. Bot. Ges. 66-67: 176, 1997	1E
R. portae-moravicae Holub et Trávn. ■	3	28*		Holub et Trávníček, Preslia 77: 5, 2005	
R. praecox Bertol. ▲	4	28×	4x (2n ~ 28)×	Bertoloni Fl. Ital. 5: 220, 1842	
Ser. *Rhamnifolii* (Bab.) Focke					
R. stimulifer Plieninger ■	3	28*		Plieninger, Carolinea 53: 56, 2009	
Ser. *Micantes* Sudre					
R. chaerophylloides Spribille ▲	3		4x (2n ~ 28)×	Spribille, Deutsche Ges. Kunst Wiss. Posen. Zeitschr. Naturwiss., Abt. 9: 120, 1902	
R. silvae-bohemicae Trávn. et Žíla ■	3	28*		Holub ex Trávníček et Žíla, Preslia 83: 100, 2011	1F
Ser. *Sprengeliani* Focke					
R. capricollensis (Spribille) Spribille ▲	3	28*		Spribille, Jahresb. Schles. Ges. Vaterl. Cult. 83: 101, 1905	

Ser. *Radula* (Focke) Focke					
R. bohemo-polonicus Trávn. et Zieliński ■	3		4x (2n ~ 28)*	Trávníček et Zieliński, Acta Soc. Bot. Poloniae 73: 311, 2004	
R. muhelicus Danner ■	4		4x (2n ~ 28)*	Danner, Neilreichia 2-3: 165, 2003	
R. passaviensis Žíla ■	1		4x (2n ~ 28)*	Žíla, Ber. Bayer. Bot. Ges. 79: 111, 2009	
R. perpedatus Žíla et H. E. Weber ■	3		4x (2n ~ 28)*	Žíla et H. E. Weber, Preslia 77: 433, 2005	
R. silvae-norticae M. Lepší et P. Lepší ■	3		4x (2n ~ 28)*	M. Lepší et P. Lepší, Preslia 81: 45, 2009	
Ser. *Pallidi* W. C. R. Watson					
R. josholubii H. E. Weber ■	2	28*		H. E. Weber, Preslia 72: 234, 2000	1G
R. posnaniensis Spribille ▲	3		4x (2n ~ 28)$^{\times}$	Spribille, Deutsche Ges. Kunst. Wiss. Posen. Zeitschr. Naturwiss., Abt. 9: 123, 1902	
Ser. *Glandulosi* (W. et Gr.) Focke					
R. holzfussii Spribille ▲	3		4x (2n ~ 28)*	Spribille, Festschrift P. Ascherson's siebzigsten Geburstage 344, 1904	2B
R. lucentifolius Zieliński et Kosiński ■	1		4x (2n ~ 28)*	Zieliński et Kosiński, Polish Bot. J. 49: 5, 2004	
R. lusaticus Rostock	1		4x (2n ~ 28)	Rostock, Mitth. Voigtl. Ver. Allg. Naturk. Reichenbach 4: 22, 1884	
R. siemianicensis Spribille ▲	5		4x (2n ~ 28)$^{\times}$	Spribille, Deutsche Ges. Kunst. Wiss. Posen. Zeitschr. Naturwiss., Abt. 9: 124, 1902	
R. pedemontanus Pinkwart	1	35		Pinkwart in Baenitz Herb. Eur., no 9550, 1898	
Sect. *Corylifolii* Lindl.					
Subsect. *Subidaeus* (Focke) Hayek					
R. pruinosus Arrh. ▲	1		5x (2n ~ 35)$^{\times}$	Arrhenius Ruborum Suec. Disp. Mon. 15, 1839	
Subsect. *Sepincola* (Focke) E. H. L. Krause					
Ser. *Sepincola* (Focke) E. H. L. Krause					
R. curvaciculatus H. E. Weber ■	2		4x (2n ~ 28)*	Walsemann ex H. E. Weber, Osnabrücker Naturwiss. Mitt. 22: 111, 1996	
R. hadracanthos G. Braun	1		4x (2n ~ 28)	G. Braun Herb. Rub. Germ., Übersicht 1881 ["hadroacanthos"]	
R. kletensis M. Lepší et P. Lepší ■	3		4x (2n ~ 28)*	M. Lepší et P. Lepší, Preslia 78: 104, 2006	
Ser. *Subthyrsoidei* (Focke) Focke					
R. grossus H. E. Weber ▲	2	35*		H. E. Weber, Ber. Bayer. Bot. Ges. 60: 9, 1989	
R. wahlbergii Arrh. s.l. ▲	1		5x (2n ~ 35)$^{\times}$	Arrhenius Ruborum Suec. Disp. Mon. 45, 1839	2C
Ser. *Subcanescentes* H. E. Weber					
R. scabrosus P. J. Müller ▲	3	28*		P. J. Müller, Jahresber. Pollichia 16/17: 269, 1859	1H
Ser. *Hystricopses* H. E. Weber					
R. sendtneri Progel ▲	4	42*		Progel, Ber. Bot. Ver. Landshut. 8: 109, 1882	1I

Occurrence of newly described species as well as of those previously known from other European countries over the last 20 years was confirmed for the Czech Republic. Description of the new species published since 1996 (for an overview see Table 1) allows us to present here the chromosome numbers assigned to 10 new taxa, which were previously collected by Josef Holub and Bohumil Trávníček under provisional names. Chromosome numbers for this material and for an additional six species newly recorded in the Czech Republic, are presented in Table 1 (entry of 2n). Some of the new data on chromosomes, presented here in detail including the localities of respective plants, were previously used to assign chromosome numbers to six species (namely *R. josholubii*, *R. phyllostachys*, *R. perperus*, *R. capricollensis*, *R. grossus* and *R. sendtneri*), which were presented in the Key to the Flora of the Czech Republic (Trávníček & Havlíček 2002). Similarly, the chromosome numbers of six recently described species of the series *Discolores* (namely *R. austroslovacus*, *R. flos-amygdalae*, *R. guttiferus*, *R. parthenocissus*, *R. pericrispatus* and *R. portae-moravicae*) are cited in the descriptions of the respective species, but without any further details (Trávníček & Zázvorka 2005). The same holds for the characterization of *R. perperus* and *R. phyllostachys*, occurrence of which in the Czech Republic was recently recorded (Trávníček & Zázvorka 2005). All the karyological data mentioned above were in fact based on plant material, which is presented here with detailed specifications, including the number and description of the localities of the plants sampled.

An additional set of new karyological data presented here refers to the ploidy levels in 18 species of bramble (see entry of DNA ploidy level in the Table 1), which were determined using flow cytometry. This plant material was assembled quite recently with the aim of including all the species currently recorded in the Czech Republic for which the ploidy level/chromosome number is still unknown.

In the final part of the paper we provide an overview of the cytotypes (ploidy levels) currently recorded for European brambles. We discuss the pattern of variation in the presently known ploidy levels, especially the relationships within and between taxonomic categories below the subgeneric level (sections, subsections and series).

Materials and methods

Plant sampling

For the chromosome counts the apical part of one-year-old stems (shoot) was taken from plants of each taxon at their respective localities during late summer or in autumn. Leaves used in the flow cytometric analyses (see below) were also gathered in late summer. Individual species were collected at from one to six localities (Table 1), depending on the extent of their distribution. A detailed description of the localities is provided in the Appendix 1. Herbarium specimens of the plants analysed are deposited at PRA (the plants collected by J. Holub) and in OL (those collected by B. Trávníček).

Preparation of the chromosomes

Somatic mitoses in the apical meristem of the root tips were used for chromosome preparations. For this purpose, stems with the uppermost rooting part were cut from plants in the field and cultivated in pots filled with garden soil in a greenhouse. Fresh root tips developing

in the soil were collected and pretreated with a saturated solution of para-dichlorbenzene, rinsed in water, fixed overnight in Carnoy fixative solution and macerated in 2N HCl. The apical meristematic part was cut off and squashed in a drop of acetic carmine. A phase-contrast microscope was used for observation of the metaphases and chromosome counting. For a detailed description of the complete procedure see Krahulcová & Holub (1997).

Determination of DNA ploidy level

The DNA ploidy level (for terminology see Suda et al. 2006) was determined using Partec CyFlow ML instrument (Partec GmbH, Münster, Germany) equipped with green laser (532nm). Sample preparation followed Galbraith et al. (1983) using LB 01 buffer (Doležel et al. 1989) with modifications. Leaf tissue of sample and standard *Zea mays* CE-777, 2C = 5.43 pg (Lysák & Doležel 1998) were co-chopped with razor blade in Petri dish using 1 ml of buffer (15 mM TRIS; 2 mM EDTA; 0,5 mM spermine-4HCl; 80 mM KCl; 20 mM NaCl; 0.1 % Triton X-100; PVP 20 g/l; pH = 8,0). The suspension was filtered through nylon mesh (42 μm) and then 300 μl of buffer was added to the solution plus 50 μl of the stain propidium iodide (PI). After 10 minutes of incubation on ice the sample was measured (up to 3000 particles) using a flow cytometer. Peak position and coefficient of variance (CV) were calculated using FlowMax provided in instrument software. Due to high level of metabolites causing unspecific background, gating was used for most of the *Rubus* peaks. Only those histograms for which CV of the standard did not exceed 7.98% were accepted. Mean CV value of the standard was 5.04 %. The ploidy level of each sample was calculated from the ratio of intensity of the relative fluorescence sample/standard (FCS ratio); this ratio was compared to that obtained for the tetraploid *R. bifrons* plant (FCS ratio 0.272; for this histogram see Fig. 2D), the chromosome number of this species confirmed by chromosome counting is 2n = 4x = 28.

Results

This paper presents chromosome number/ploidy level of altogether 37 species of *Rubus* in the subgenus *Rubus* (see Table 1, where also the author citation of all the species is given). The data for 27 species are the first entries for these respective species, that for six species are new for the Czech Republic and that for the remaining four species confirm those published previously, but are based on plants from other localities. The chromosome number/ploidy level was recorded for these 37 species (Table 1, Figs 1, 2), eight of which are triploid (2n = 21, FCS ratio sample/standard = 0.206 ± 0.005, and most of these species are in the section *Rubus* series *Discolores*), 24 are tetraploid (2n = 28, FCS ratio sample/ standard = 0.271 ± 0.013, and the species are in both section *Rubus* and section *Corylifolii*, including altogether 11 series throughout the subgenus), four are pentaploid (2n = 35, FCS ratio sample/standard = 0.355 ± 0.016, namely *R. pedemontanus* in section *Rubus* series *Glandulosi* and three other species in the section *Corylifolii*) and one is hexaploid (2n = 42, namely *R. sendtneri* in section *Corylifolii*).

Relations between currently accepted names of five species (Table 1, Kurtto et al. 2010) and their respective provisional/erroneously assigned names used in the Flora of the Czech Republic (Holub 1995) and/or in the Key to the Flora of the Czech Republic (Trávníček & Havlíček 2002) are as follows:

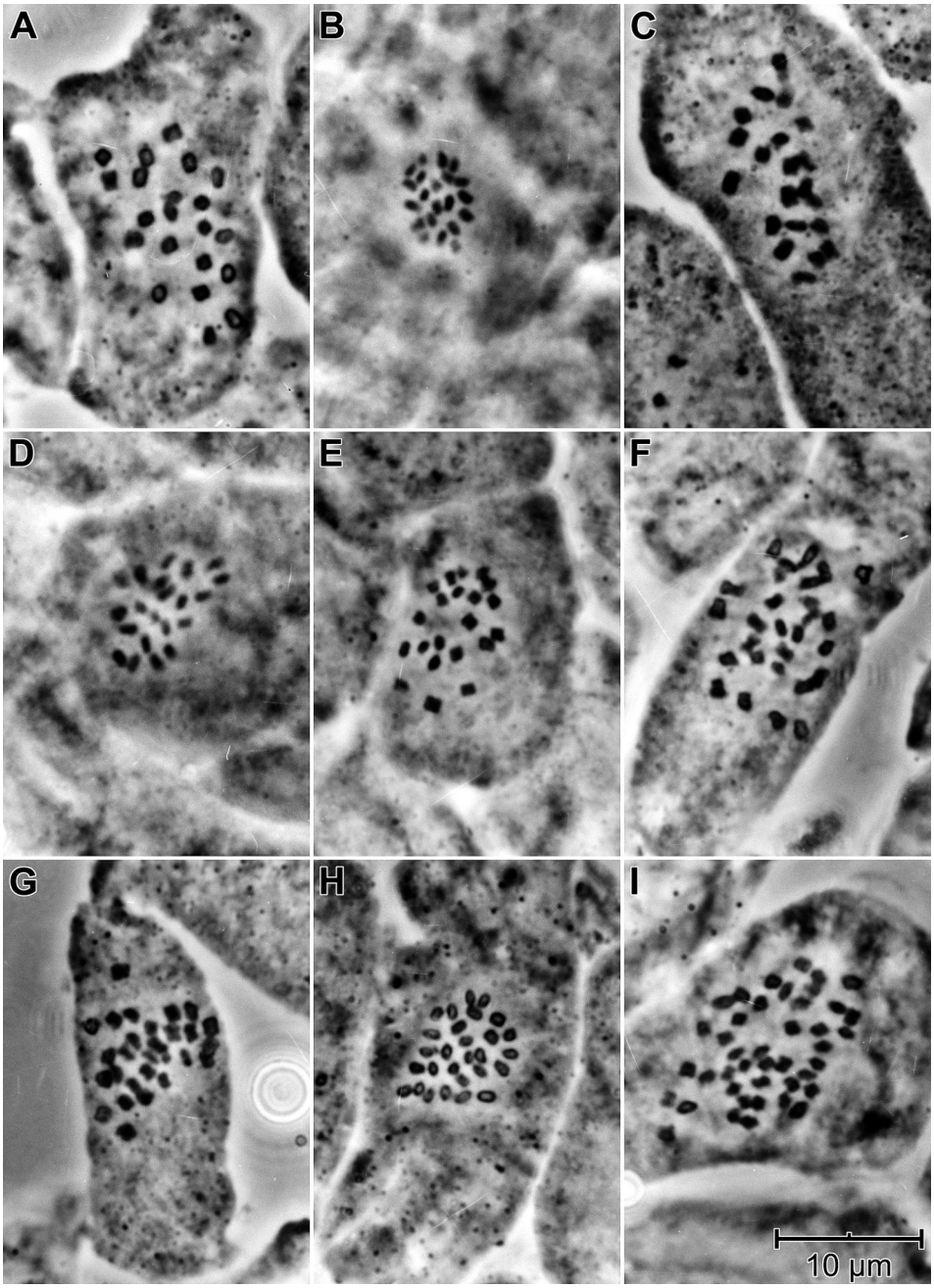

Fig. 1. – Somatic mitoses in the genus *Rubus*, subgen. *Rubus*. A – *R. guttiferus* Trávn. et Holub, 2n = 21; B – *R. parthenocissus* Trávn. et Holub, 2n = 21; C – *R. pericrispatus* Holub et Trávn., 2n = 21; D – *R. phyllostachys* P. J. Müller, 2n = 21; E – *R. perperus* H. E. Weber, 2n = 21; F – *R. silvae-bohemicae* Trávn. et Žíla, 2n = 28; G – *R. josholubii* H. E. Weber, 2n = 28; H – *R. scabrosus* P. J. Müller, 2n = 28; I – *R. sendtneri* Progel, 2n = 42.

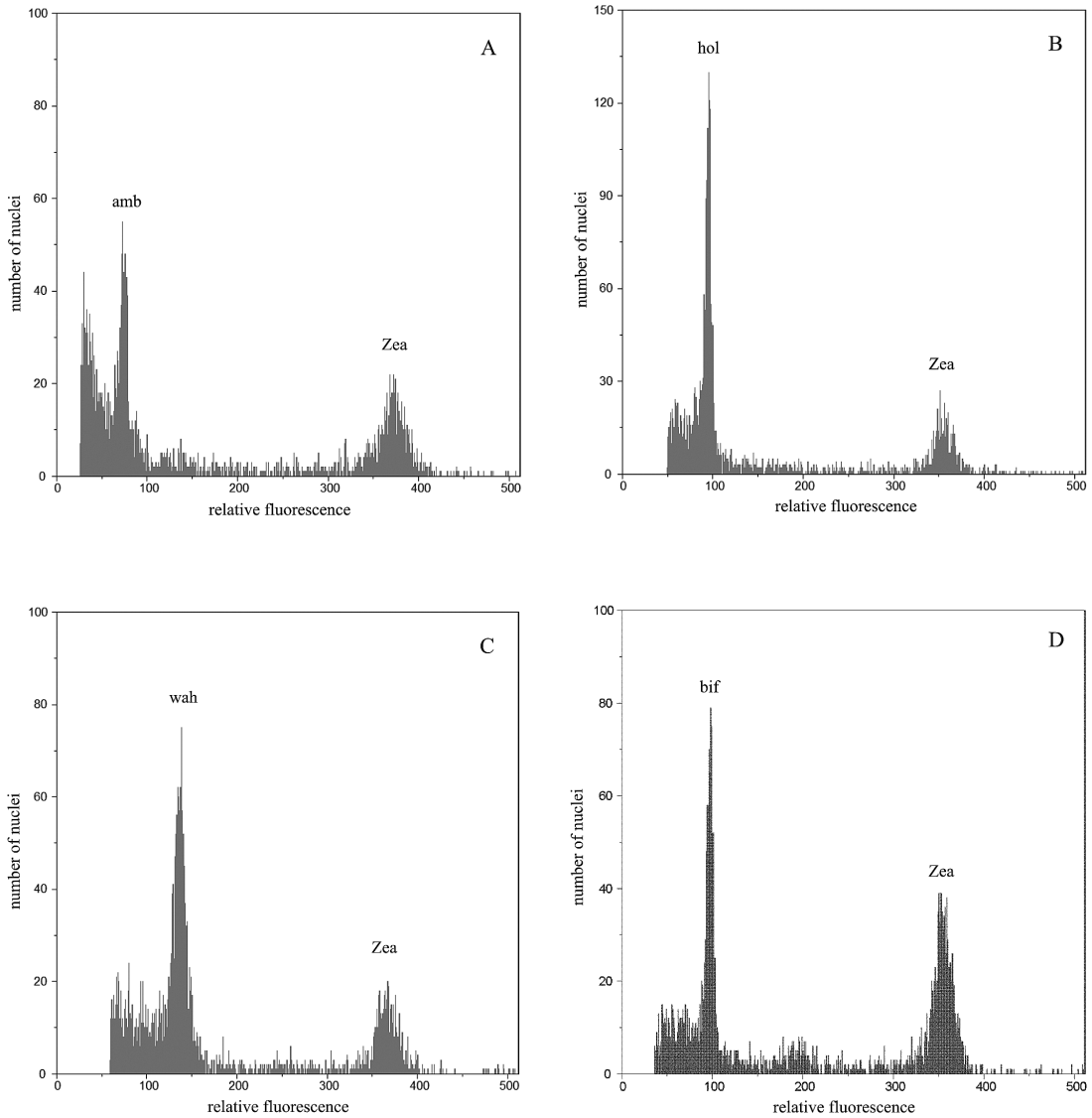

Fig. 2. – Histograms of the relative DNA content of the species of *Rubus* studied and the reference standard (*Zea mays* CE 777 = peak "Zea"), used for determining the ploidy level of all the samples: A – *R. ambrosius* (3x, peak "amb"); B – *R. holzfussii* (4x, peak "hol"); C – *R. wahlbergii* (5x, peak "wah"); D – *R. bifrons* (4x, peak "bif").

Species name (Table 1):	Earlier used provisional/erroneous name:
R. stimulifer Plieninger	*R. praecociformis* ined. (Trávníček & Havlíček 2002)
R. scabrosus P. J. Müller	*R. fasciculatiformis* H. E. Weber (Holub 1995)
	R. semmonicus H. E. Weber ined. (Trávníček & Havlíček 2002)
R. holzfussii Spribille	*R. pallidifrons* ined. (Trávníček & Havlíček 2002)
R. bohemo-polonicus Trávn. et Zieliński	*R. radellus* ined. (Trávníček & Havlíček 2002)
R. scissus W. C. R. Watson	*R. nessensis* subsp. *scissoides* H. E. Weber, nom. inval. (Trávníček & Havlíček 2002)

Discussion

New karyological data assigned to plants from the Czech Republic

Generally, the pattern in the variation in ploidy level corresponds to that recorded previously for the same area (Krahulcová & Holub 1997, 1998a, b, c, Trávníček et al. 2000). Namely, the tetraploid species are the most common in this subgenus; nevertheless, they are rarer in the section *Rubus* series *Discolores*, where triploid species prevail. The pentaploid species occur rarely in the section *Rubus* series *Glandulosi*, being rather more frequent among the representatives of the section *Corylifolii*. The hexaploid chromosome number (2n = 42, Fig. 1I) recorded here for *R. sendtneri* is the first record of a hexaploid species of *Rubus* in the Czech Republic. In fact, the hexaploid ploidy level is rare in central-European brambles (Kurtto et al. 2010).

In some cases, the different karyological characters correspond to the taxonomic diversity among morphologically similar species. For example, *R. ambrosius* is a triploid and in being so differs from the morphologically similar tetraploid *R. sulcatus* Vest (see Trávníček et al. 2005). In contrast, due to the pattern of karyological variation within this subgenus, most species have the same ploidy level. Thus closely related species, such as *R. portae-moravicae*/*R. praecox*, or *R. scissus*/*R. nessensis* W. Hall (all species tetraploid) have the same karyological characters. Similarly, the closely related *R. grossus*, *R. kuleszae* Zieliński and the Czech morphotype of *R. wahlbergii* (= *R. wahlbergii* sensu Weber 2000) are all pentaploid (Table 1, Electronic Appendix 1, Kurtto et al. 2010).

We cannot exclude that some of the previously published chromosome numbers refer to a different, but closely related species. For example, the tetraploid ploidy level in *R. praecox* was reported from Germany by Iwatsubo et al. (1995). According to the authors cited, all karyological data published before 1995 refer to a related species, *R. armeniacus* Focke, which was often confused with *R. praecox*. Karyological data for *R. grossus*, published previously from Poland (Boratyńska 1994), refer to a recently separated species, *R. kuleszae* (cf. Krahulcová & Holub 1998a). Unlike the taxonomic treatment in Atlas Florae Europaeae (Kurtto et al. 2010), we treat *Rubus flos-amygdalae* and *Rubus montanus* (both triploids in the series *Discolores*) as separate species. Although the nomenclature of the *R. montanus* group is not completely resolved (the lectotype of *R. montanus* does not provide an unambiguous interpretation of this name), we follow the taxonomic concept of Trávníček & Zázvorka (2005), which accords with many illustrations of *R. montanus* in recent literature (for example see Weber 1986: 241, Holub 1995: 111, Zieliński 2004: 71–72, Henker & Kiesewetter 2009: 103). The tetraploid ploidy level in *R. constrictus* was previously reported by A. Krahulcová and J. Holub for plants collected from six localities in the Czech Republic (Krahulcová & Holub 1998c); on the other hand, the triploids were reported earlier by other authors in south-western and central Europe, outside the Czech Republic (cited in Krahulcová & Holub 1998c). However, an additional examination of the plants collected close to the south-western border of the Czech Republic confirmed that *R. constrictus* in this country is tetraploid. The data in the literature for a triploid cytotype of *R. constrictus* may be more correctly attributed to a triploid biotype of the series *Discolores*, because in the past *R. constrictus* was occassionally confused with taxa close to the triploid *R. montanus* from this series. Likewise there are two cytotypes of *R. pedemontanus* recorded in Europe, namely pentaploids and tetraploids (Electronic Appendix 1). However, also in this case, *R. pedemontanus* may

be confused with a morphologically similar biotype of the series *Glandulosi*. Nevertheless, only the pentaploid cytotype of *R. pedemontanus* was found in the Czech Republic (five localities hitherto, Krahulcová & Holub 1998a). The additional count of 2n = 35 presented here (Table 1) confirms the occurrence of pentaploids in this country. Using flow cytometry, the tetraploid ploidy level is here confirmed in *R. lusaticus* and *R. hadracanthos* (Table 1). The previous chromosome counts for these species were published by Krahulcová & Holub (1998a) and Krahulcová & Holub (1997), respectively.

Some of the species newly investigated here, occur only in central Europe. So far, the tetraploid species *R. stimulifer* is only known from Germany, from where it was described (Kurtto et al. 2010). The present knowledge about the distribution of the tetraploid *R. josholubii* (Table 1, Fig. 1G) refers to the Czech Republic (Bohemia) (Kurtto et al. 2010). In addition four tetraploid species, namely *R. bohemiicola*, *R. vratnensis*, *R. centrobohemicus* and *R. brdensis* (all described by Josef Holub), are also endemic to the Czech Republic (Electronic Appendix 1). The following three species, the chromosome numbers of which are reported here for the first time (Table 1), also occur in central Europe (Kurtto et al. 2010): *R. perperus* (Fig. 1E), *R. capricollensis* and *R. scabrosus* (Fig. 1H).

Variation in the ploidy levels of European brambles: current state of knowledge

Extent of exploration

With respect to total number of species in individual sections, subsections and series (Table 2), this overview takes into consideration all the native European species of the subgenus *Rubus,* which are accepted in Atlas Florae Europaeae (Kurtto et al. 2010), and, in addition, *R. flos-amygdalae,* which is synonymized with *R. montanus* in the work mentioned. However, we did not include the collective heterogenous "species" *R. hirtus* (series *Glandulosi*). The following 11 new species described after the Atlas Florae Europaeae was published, are added: (i) sect. *Rubus*, subsect. *Hiemales*: *R. dufftianus* W. Jansen (ser. *Discolores*; Jansen W., Haussknechtia 12: 28, 2010); *R. silvae-bohemicae* Holub ex Trávn. et Žíla (ser. *Micantes*; Trávníček B. & Žíla V., Preslia 83: 100, 2011); *R. silvae-thuringiae* W. Jansen (ser. *Pallidi*; Jansen W., Haussknechtia 12: 36, 2010); *R. schleicheriformis* W. Jansen (ser. *Hystrix*; Jansen W., Haussknechtia 12: 32, 2010); (ii) sect. *Corylifolii*, subsect. *Sepincola*: *R. tilioides* W. Jansen et H. E. Weber (ser. *Sepincola*; Weber H. E., Mitt. Florist. Kart. Sachsen-Anhalt (Halle 2010) 15: 4, 2010); *R. anhaltianus* H. E. Weber (ser. *Sepincola*; Weber H. E., Mitt. Florist. Kart. Sachsen-Anhalt (Halle 2010) 15: 8, 2010); *R. orbifrons* H. E. Weber (ser. *Sepincola*; Weber H. E., Mitt. Florist. Kart. Sachsen-Anhalt (Halle 2010) 15: 12, 2010); *R. appropinquatus* Plieninger (ser. *Suberectigeni*; Plieninger W., Carolinea 69: 6, 2011); *R. histrionicus* Plieninger (ser. *Subthyrsoidei*; Plieninger W., Carolinea 69: 11, 2011); *R. lictorum* Plieninger (ser. *Subcanescentes*; Plieninger W., Carolinea 69: 16, 2011); *R. remotifolius* Plieninger (ser. *Subcanescentes*; Plieninger W., Carolinea 69: 20, 2011).

Data on ploidy level published for species of the subgenus *Rubus* up to 2010, are adopted according to species characterizations, as they are presented in Atlas Florae Europaeae (Kurtto et al. 2010). To illustrate the karyological variation in European species of bramble (Table 2, Electronic Appendix 1), we added the new data presented in this paper in the Table 1, and also the new karyological data cited in Ryde (2011) for three species of the sect. *Corylifolii*, namely *R. norvegicus*, *R. mortensenii* and *R. vikensis*.

Table 2. – List of series of the subgenus *Rubus* (sections *Rubus, Corylifolii* and *Caesii*), the species of which are native to Europe. Each of these series comprises at least one species with a known ploidy level. Total number of species recognized in individual series corresponds to Kurtto et al. (2010); 11 new species, described in 2010 and 2011, are included (for detailed information see Discussion, chapter Extent of exploration). Karyological data are adapted according to Kurtto et al. (2010), the new data presented in this paper and that by Ryde (2011) are included. Entries on ploidy level are inferred from both the chromosome counts and flow cytometric determinations of DNA content (the latter being represented by the new data in this paper and, in addition, that published by Ryde in 2011). For detailed information about individual species see Electronic Appendix 1: the six introduced/cultivated species presented there, namely *R. canadensis*, *R. allegheniensis*, *R. armeniacus*, *R. laciniatus*, *R. flagellaris* and *R. loganobatus*, were not included in this Table.

Section	Subsection	Series	No. of species		No. of species with the different ploidy levels					
			Total	Known ploidy	2x	3x	4x	5x	6x	Mixed within species
Rubus	Rubus	*Nessenses*	4	4			4			
Rubus	Rubus	*Rubus*	30	14		6	7			1: 3x/4x
Rubus	Hiemales	*Discolores*	76	30	2	15	13			
Rubus	Hiemales	*Rhamnifolii*	45	14			14			
Rubus	Hiemales	*Sylvatici*	76	17			17			
Rubus	Hiemales	*Sprengeliani*	15	6		1	5			
Rubus	Hiemales	*Canescentes*	1	1	1					
Rubus	Hiemales	*Vestiti*	54	6			6			
Rubus	Hiemales	*Mucronati*	17	6			6			
Rubus	Hiemales	*Micantes*	58	21			21			
Rubus	Hiemales	*Anisacanthi*	23	2			2			
Rubus	Hiemales	*Radula*	36	15			15			
Rubus	Hiemales	*Pallidi*	61	11			11			
Rubus	Hiemales	*Hystrix*	55	12			12			
Rubus	Hiemales	*Glandulosi*	34	11			9			1: 4x/5x; 1: 4x/5x/6x
Corylifolii	Subidaeus		16	4			1	3		
Corylifolii	Sepincola	*Suberectigeni*	17	7			5		1	1: 4x/6x
Corylifolii	Sepincola	*Sepincola*	24	12			12			
Corylifolii	Sepincola	*Subthyrsoidei*	38	14			9	5		
Corylifolii	Sepincola	*Subsilvatici*	12	4			3		1	
Corylifolii	Sepincola	*Subcanescentes*	16	6			6			
Corylifolii	Sepincola	*Vestitiusculi*	5	1					1	
Corylifolii	Sepincola	*Subradula*	22	3			1	2		
Corylifolii	Sepincola	*Hystricopses*	18	6			3	1	2	
Caesii			1	1			1			
		Total no.	754	228	3	22	183	11	5	4

Consequently, a total of 754 species of the subgenus *Rubus*, native to Europe, are currently recognized (Table 2). Although information on ploidy level is available for approximately 30% of them (228 species) there are karyologically examined species for each of the 25 series (Table 2). The only three groups for which there is data for all the species are the series *Nessenses*, *Canescentes* and the section *Caesii*, which are the least abundant in species. Almost half of the species (40% and more) have been karyologically examined within the series *Rubus*, *Radula*, *Sprengeliani* (all belonging to section *Rubus*), *Suberectigeni* and *Sepincola* (both belonging to section *Corylifolii*) (Table 2, Electronic Appendix 1).

Pattern of variation

Tetraploids prevail among European brambles, comprising approximately 80% of the species for which the ploidy level/chromosome number has been determined (Fig. 3). The remaining cytotypes, arranged in order of declining abundance, are triploids, pentaploids, hexaploids and diploids (Fig. 3). Occurrence of different cytotypes within species is rare and has been documented in less than 2% of the species (Figs 3, 4).

If we compare the current pattern in the variation in ploidy level in European brambles with that overviewed by Thompson (1997), certain differences are obvious. Whereas the present frequency of tetraploid species has increased slightly (from 74.0% to 80.3%) that of triploid species increased more than threefold (from 3.1% to 9.6%). On the other hand, the frequency of species with infraspecific variation in ploidy level decreased strongly (from 12.0% to 1.8%). There may be several reasons for this diference, for example, differences in the taxonomic treatment (different criteria used to separate species). In addition, several triploid biotypes were described as species relatively recently, especially in the series *Discolores*. This could have contributed to the increased frequency of triploid species. The numbers of plants that have been examined karyologically has greatly increased since Thompson's review, including those of individual species from more localities within their distribution area. Probably a substantial part of the sporadic and "unusual" chromosome counts that were previously attributed to infraspecific variation, were reevaluated for the Atlas Florae Europaeae (Kurtto et al. 2010) and excluded.

Most of the 15 series of the section *Rubus* are tetraploid (Fig. 4A). The same holds for the section *Caesii*, in which there is only the tetraploid species *R. caesius* (Fig. 4B). Rare diploid species in the section *Rubus* occur entirely in the series *Discolores* and *Canescentes*. More abundant triploid species occur commonly in the series *Rubus* and *Discolores* (in the latter series they are the most frequent cytotype), whereas there is an isolated occurrence of a triploid species (*R. brevistaminosus*) in the series *Sprengeliani*. Cytotypes above the tetraploid level are rare in the section *Rubus*, being always accompanied by tetraploids, which co-occur within the respective species (Fig. 4A). Species with a variable ploidy level are reported in the series *Rubus* (*R. constrictus*) and *Glandulosi*

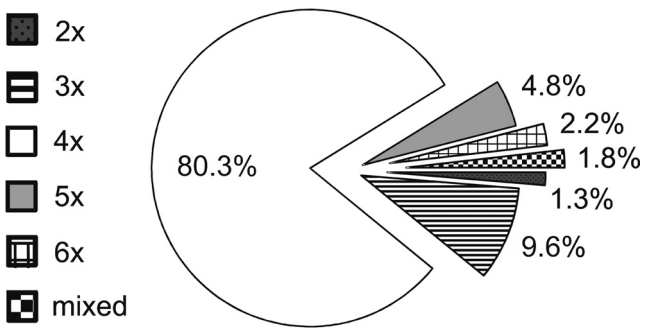

Fig. 3. – Frequency of cytotypes (ploidy levels) among 228 species of the subgenus *Rubus* native in Europe. For the source of this data see Table 2.

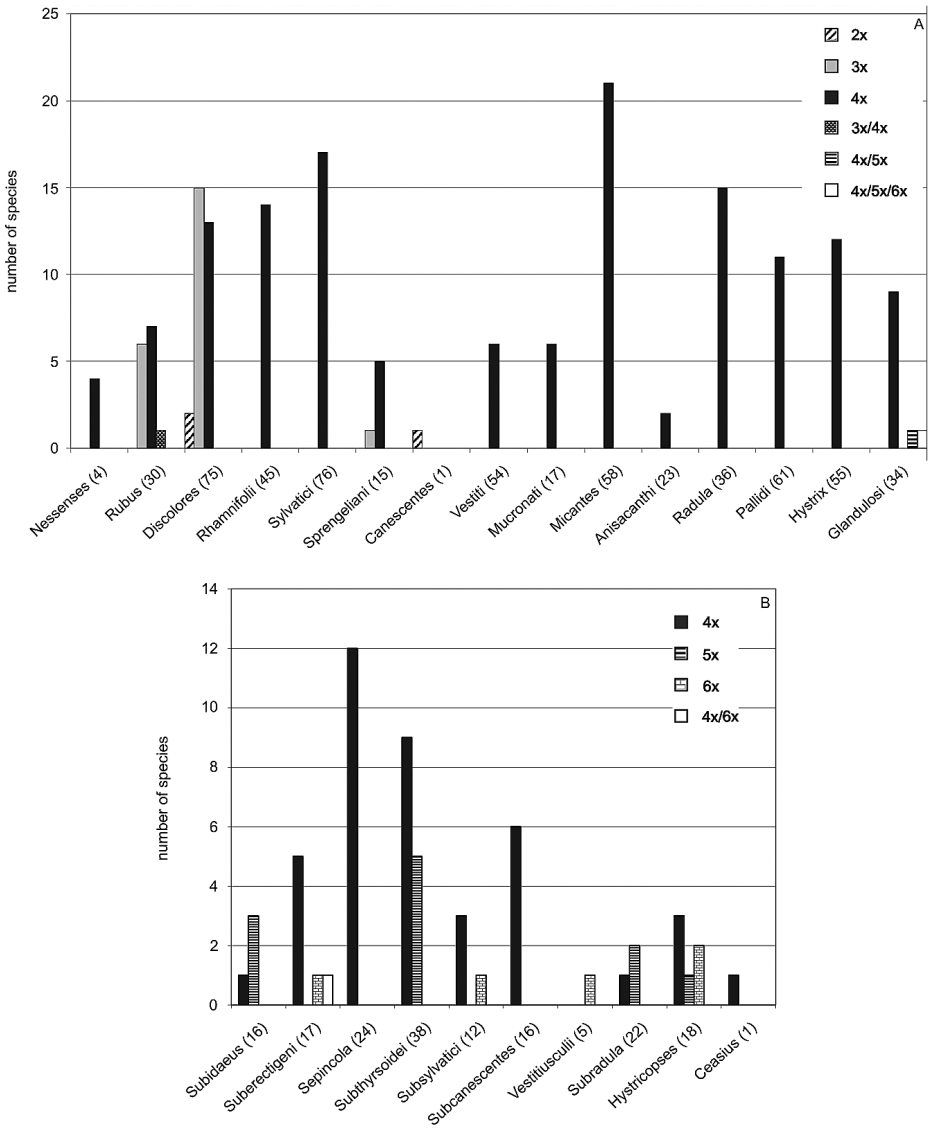

Fig. 4. – Frequency of species in the subgenus *Rubus* native in Europe based on the ploidy level within individual intrasub-generic taxa: A – section *Rubus* (fifteen series comprising the subsections *Rubus* and *Hiemales*); B – section *Corylifolii* (subsection *Subidaeus* and eight series comprising the subsection *Sepincola*) and the section *Caesii*. Total number of species recognized in individual taxonomic entities is presented in parentheses behind their name. For the source of this data see Table 2 and Electronic Appendix 1.

(*R. pedemontanus*, *R. guentheri*) (Electronic Appendix 1). However, it is possible that in these cases there are incorrect taxonomic determinations. Taxonomic revision of herbarium specimens of those plants that have atypical chromosome numbers is necessary: otherwise, it is difficult to prove that the determinations were incorrect.

The pattern of karyological variation in the section *Corylifolii*, comprising nine series, is different (Fig. 4B). Unlike in section *Rubus* there are no cytotypes below the tetraploid level in section *Corylifolii*. Tetraploid species prevail and they are represented in each of the nine series of this section (Fig. 4B). Out of 57 species in the section *Corylifolii* examined karyologically, 40 (70%) are tetraploid (Table 2). However, there are only tetraploid species in two of the series in this section, namely the series *Sepincola* and *Subcanescentes*, whereas in the remaining six series and the subsection *Subidaeus* there are pentaploid and/or hexaploid species in addititon to tetraploids (Table 2, Fig. 4B). Future karyological studies may also reveal some variation in ploidy level in the currently purely tetraploid series *Sepincola* and *Subcanescentes*, because less than half of the species in these series have been examined (Table 2, Electronic Appendix 1). The highest variation in ploidy level seems to occur in the series *Hystricopses*, in which three cytotypes (namely tetraploids, pentaploids and hexaploids) are reported (Fig. 4B). There appears to be little intraspecific variation in ploidy level in the section *Corylifolii*: the only species recorded with more than one cytotype, is *R. dissimulans* of the series *Suberectigeni*, in which tetraploids and hexaploids are recorded (Fig. 4B, Electronic Appendix 1).

Taxonomic and evolutionary implications

Modern taxonomic classification of the subgenus *Rubus* includes as species only those biotypes, which although they may be of hybrid origin, are stabilized by apomixis (Weber 1996, Kurtto et al. 2010). Due to high expressivity of apomixis, such stabilization is usually associated with little morphological variation in the respective biotype and in most cases its occupation of a rather broad territory (e.g. Kurtto et al. 2010). This approach properly excludes early generations of newly formed hybrids from taxonomical classification. In fact, the morphological diversity of such progeny may be so high, that the individual progeny from a single F_1 hybrid individual may be classified into several different series (Maurer & Weber 2000). This variation seems to be consistent with sexual reproduction occurring in some products of interspecific hybridization of two facultatively apomictic species (Nybom 1988, Asker & Jerling 1992).

For the reasons mentioned above, identification of parental species of stabilized interspecific hybrids is impossible in many cases. The situation is rather easier in recent hybrids that are intermediate between their putative parents in morphology and coexist with them at the same locality (Holub 1997). Nevertheless, hypotheses on origin of some intrasubgeneric taxa are still accepted. Representatives of the section *Corylifolii* belong to evolutionary younger biotypes within the subgenus *Rubus* (Holub 1997): they are assumed to be hybrids and segregation products of crosses between biotypes of the section *Rubus* (formerly classified as "Eubati veri") and tetraploid *R. caesius* of the section *Caesii* (Gustafsson 1939). Certain of the polyploid cytotypes in the subgenus *Rubus* could have evolved via hybridization of specific parental cytotypes, some of which might have belonged to other subgenera: for example, the maternal parent of the pentaploids that prevail in subsection *Subidaeus* of section *Corylifolii*, could have originated from a cross between the tetraploid *R. caesius* (subgenus *Rubus*) and diploid *R. idaeus* L. (subgenus *Idaeobatus* Focke). Consequently, an unreduced female gamete of such a triploid hybrid, fertilized by a reduced pollen from some tetraploid biotype, could give rise to pentaploid progeny (Weber 1995). Alternatively, the unreduced tetraploid *Corylifolii* gametes,

fertilized by a reduced pollen of *R. idaeus*, could result in the pentaploid representatives in the section *Corylifolii* subsection *Subidaeus*, such as *R. pruinosus* (Gustafsson 1939).

Taxonomy of brambles is almost exclusively based on evaluation of morphological characters. Classification of taxa into intrasubgeneric groups, namely into series, reflects broad morphological distinctness among these groups within the subgenus (Holub 1995, 1997, Weber 1995). Nevertheless, many of the series are taxonomically not entirely homogenous and represent rather categories that resulted from a utilitarian classification below the subgenus level (Holub 1995). In spite of this, some of the series may reflect the common biological character of the taxa comprising them: for example in the series *Glandulosi* there are many species that have a prevalent sexuality and a great diversity of morphotypes (Holub 1997, Šarhanová et al. 2012). The supposed polyphyletic origin of this group (Weber 1995) needs to be confirmed. Recently, molecular tools, such as allozymes, AFLPs and minisatellites have been used to investigate the genetic diversity of either plants of different/similar morphotypes within/between species (e.g. Kraft & Nybom 1995), or the seedling progenies derived from a facultatively apomictic parent (Kollmann et al. 2000). This approach implies that the populations that share identical genotypes should be regarded as conspecific, while the genetically different populations may, but may not necessarily belong to one species (Kraft & Nybom 1995). However, this approach to studying *Rubus* taxonomy can be very expensive, laborious and time consuming. On the other hand, studies of the genetic structure of populations, which focus on the relationships between the putative parents and their progeny, could elucidate the origin of recently formed hybridogenous taxa (Kraft et al. 1995). Determination of the ploidy level of the putative parents and their hybrids may provide support for the interpretation of the origin of the derived biotypes. In this respect, the karyological survey presented here contributes to the basic knowledge of the taxonomically intricate subgenus *Rubus*.

See www.preslia.cz for Electronic Appendix 1

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Souhrn

Polyploidní agamický komplex podrodu *Rubus* (ostružiník) patří k nejsložitějším skupinám evropské flóry. Taxonomická složitost a obtížnost je způsobena polyploidií, neúplnou (fakultativní) apomixí, projevy minulé i recentní hybridizace s následnou segregací morfotypů a modifikací prostředím. Práce podává aktualizovaný přehled ploidní úrovně/počtu chromozomů u 228 druhů podrodu původních v Evropě, pro něž do současnosti existují karyologické údaje. První část článku se týká nových karyologických dat získaných z území České republiky v posledních 14 letech pomocí počítání chromozomomů a metody průtokové cytometrie. Tyto údaje zahrnují hlavně 19 druhů recentně popsaných z Evropy a 14 dříve popsaných druhů, jejichž přítomnost byla v České republice nově objevena. Vůbec první karyologická data jsou uvedena pro 27 druhů, tvořených triploidy (2n = 21 – 8 druhů), tetraploidy (2n = 28 – 17 druhů), pentaploidem (2n = 35 – 1 druh) a hexaploidem (2n = 42 – 1 druh). *Rubus sendtneri*

Progel je prvním hexaploidním druhem zjištěným v České republice. Ve druhé části publikace jsou prezentovány a diskutovány dosud známé karyologické charakteristiky druhů původních v Evropě. Tento přehled obsahuje data převzatá z díla Atlas Florae Europaeae (Kurtto et al. 2010) a nová data prezentovaná v první části článku. Stupeň ploidie je dosud znám u 30 % druhů z celkového množství více než 750, které jsou dnes v Evropě uznávány. Tetraploidní druhy (2n = 28) přitom tvoří 80 % z karyologicky prozkoumaných druhů. Jsou známy pouze tři diploidní sexuální druhy, které jsou v Evropě původní. Triploidi jsou soustředěni hlavně do série *Discolores* a *Rubus* v sekci *Rubus*, zatímco se vůbec nevyskytují v evolučně odvozenější sekci *Corylifolii*. Pentaploidní druhy jsou v podrodu *Rubus* méně časté; přece se však vyskytují častěji v sekci *Corylifolii* než v sekci *Rubus*. Vzácní hexaploidi jsou známi pouze v sekci *Corylifolii*. Diferenciace jednoho druhu do více cytotypů je u evropských ostružiníků velmi vzácná. Zástupci sekce *Corylifolii* jsou pokládáni za produkty hybridizace a segregace vzniklé z křížení mezi zástupci sekce *Rubus* a tetraploidního druhu *R. caesius* (sekce *Caesii*). Obraz variability v ploidii předpokládá v tomto případě účast neredukovaných gamet při vytváření zástupců sekce *Corylifolii*. Taxonomie ostružiníků je téměř výhradně založena na morfologických znacích příslušných taxonů. Zde prezentovaný karyologický přehled může přispět k pochopení procesů, které formovaly současnou strukturu polyploidního komplexu, zajména pak pochopení původu recentních hybridů, ale i některých hybridogenních apomiktických mikrospecií.

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Appendix 1. **–** List of localities where the plants were collected (the species are arranged alphabetically).

Rubus ambrosius. **1.** S Bohemia; distr. Strakonice; woodland edge of the forest road 0.7 km SE of Kočín village, ca. 5.5 km WNW of Bavorov town, 550 m a.s.l., 49°07'57"N, 14°00'03"E. Coll. B. Trávníček 1. 9. 2011 (OL, R222/11). **2**. N Bohemia; distr. Litoměřice; in the wood near the road 0.9 km NNE of Hrdly village, ca. 2.5 km SE of Terezín, 160 m a.s.l., 50°29'36"N, 14°10'42"E. Coll. B. Trávníček 4. 9. 2011 (OL, R262/11). **3.** N Moravia; distr. Opava; in the wood near the road 1.5 km WSW of the village Šilheřovice, ca. 5 km NE of Hlučín, 260 m a.s.l., 49°55'21"N, 18°15'02"E (locus classicus of the species). Coll. B. Trávníček 6. 9. 2011 (OL, R272/11).

Rubus austroslovacus. **1**. E Moravia; distr. Zlín; Zlínské vrchy Hills, beside the road 1 km SSE of the SE periphery of the village Kudlov (now a part of Zlín), 420 m a.s.l., 49°11'30"N, 17°42'00"E. Coll. B. Trávníček 23. 10. 1997. **2**. E Moravia; distr. Zlín; Hostýnské vrchy Hills, at the edge of the wood on the NE periphery of the village Veliková, 390 m a.s.l., 49°17'00"N, 17°45'50"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996. **3**. S Moravia; distr. Uherské Hradiště; edge of woodland beside the road between the village Březolupy and Šarovy settlement, ca. 1.8 km NNE of the village Březolupy, 230 m a.s.l., 49°08'00"N, 17°35'50"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996.

Rubus bohemo-polonicus. **1**. C Bohemia; distr. Rakovník; woodland edge 0.8 km NW of the village Novosedly, ca 10 km S of Rakovník, 430 m a.s.l., 50°00'52"N, 13°44'48"E. Coll. B. Trávníček 4. 9. 2011 (OL, R256/11). **2**. E Bohemia; distr. Svitavy; in the wood along forest road 1.6 km SSE of the village Pohodlí, ca. 7 km S of Litomyšl, 440 m a.s.l., 49°48'22"N, 16°18'07"E. Coll. B. Trávníček 5. 9. 2011 (OL, R268/11). **3**. N Moravia; distr. Opava; in wood near the road between the villages of Brumovice and Sosnová, 2.1 km SSE of the village Úblo, 360 m a.s.l., 50°00'14"N, 17°42'25"E. Coll. B. Trávníček 6. 9. 2011 (OL, R278/11).

Rubus capricollensis. **1**. S Moravia; distr. Brno-venkov [Brno-country district]; along woodland path 1.7 km ENE of the village Újezd (ca. 11 km WNW of Rosice town), 510 m a.s.l., 49°13'50"N, 16°16'40"E. Coll. B. Trávníček and A. Krahulcová 18. 10. 1996. **2**. C Moravia; distr. Prostějov; along woodland path at the clearing 1 km NW of the village Prostějovičky, 410 m a.s.l., 49°26'00"N, 16°59'00"E. Coll. B. Trávníček and A. Krahulcová 16.10. 1996. **3**. C Moravia; distr. Prostějov; at edge of woodland at the crossing 0.3 km SE of the place "Zámeček" SW of the village Hluchov, 350 m a.s.l., 49°31'50"N, 16°58'30"E. Coll. B. Trávníček and A. Krahulcová 16. 10. 1996.

Rubus chaerophylloides. **1**. S Bohemia; distr. České Budějovice; in wood 1.2 km NNW of the village Hosín ca. 2.5 km ESE of Hluboká nad Vltavou, 460 m a.s.l., 49°02'52"N, 14°28'15"E. Coll. B. Trávníček 1. 9. 2011 (OL, R227/11). **2**. S Bohemia; distr. České Budějovice; in wood 0.3 km S of Kaliště settlement, ca. 4 km SE of Rudolfov, 510 m a.s.l., 48°57'46"N, 14°34'26"E. Coll. B. Trávníček 2. 9. 2011 (OL, R253/11). **3**. S Moravia; distr. Blansko; at edge of woodland along the road between the villages of Senetářov and Podomí, 1.1 km SE of the village Senetářov, 560 m a.s.l., 49°20'49"N, 16°49'10"E. Coll. B. Trávníček 8. 9. 2011 (OL, R287/11).

Rubus constrictus. **1**. SW Bohemia; distr. Domažlice; in a ditch along the road between the villages of Česká Kubice and Spálenec, 1.3 km E of the village Česká Kubice, 530 m a.s.l., 49°22'00"N, 12°52'40"E. Coll. J. Holub and A. Krahulcová 12. 10. 1998.

Rubus curvaciculatus. **1**. C Bohemia; distr. Rakovník; scrub between the road and railway 0.7 km NE of the village Lužná II, ca. 5.5 km NE of Rakovník, 380 m a.s.l., 50°08'42"N, 13°46'35"E. Coll. B. Trávníček 4. 9. 2011 (OL, R258/11). **2**. C Bohemia; distr. Rakovník; at the edge of the woodland along the forest road 1.5 km E of the village Srbeč, ca. 8 km N od Nové Strašecí, 310 m a.s.l., 50°13'36"N, 13°54'15"E. Coll. B. Trávníček 4. 9. 2011 (OL, R260/11).

Rubus flos-amygdalae. **1**. C Bohemia; distr. Mladá Boleslav; the protected landscape area Český ráj; in the wood beside the road 1.2 km SE of the village Žehrov, 290 m a.s.l., 50°31'30"N, 15°06'30"E. Coll. J. Holub and A. Krahulcová 7. 8. 1996. **2**. C Bohemia; distr. Kolín; clearing in woods in the valley NW of the village Kostelec nad Černými Lesy, 345 m a.s.l., 50°00'10"N, 14°51'20"E. Coll. J. Holub 14. 10. 1995. **3**. C Bohemia; distr. Příbram; at the edge of woodland situated in the SE foothills of Rohatec hill (517m) near the village Borotice, 400 m a.s.l., 49°44'10"N, 14°17'50"E. Coll. J. Holub and A. Krahulcová 2. 10. 1996. **4**. N Moravia (Moravian Silesia); distr. Karviná; the village Albrechtice, at the edge of woodland Loucký les close to the railway, ca. 2.2 km E of the railway station, 280 m a.s.l., 49°47'40"N, 18°33'30"E. Coll. B. Trávníček 10. 10. 1996. **5**. C Moravia; distr. Kroměříž; Chropyně town, in clearing in the wood Rasina ca. 2 km NE of the railway station, 190 m a.s.l., 49°23'00"N, 17°23'00"E. Coll. B. Trávníček 8. 10. 1996.

Rubus grossus. **1**. S Bohemia; distr. Strakonice; at the edge of woodland along the road 0.5 km NW of the village Třebohostice , 500 m a.s.l., 49°20'20"N, 13°51'20"E. Coll. J. Holub, V. Žíla and A. Krahulcová 1. 10. 1998. **2**. SW Bohemia; distr. Klatovy; at the edge of woodland below the road in the western part of Karlovecký hřbet ridge, 1.75 km NNW of the village Kejnice S of Horaždovice, 560 m a.s.l., 49°17'00"N, 13°41'30"E. Coll. J. Holub, V. Žíla and A. Krahulcová 1. 10. 1998.

Rubus guttiferus. **1**. S Bohemia; distr. Strakonice; at the edge of woodland on the E slope of Zámek hill (520 m) 1 km NW of the village Nemětice, ca. 4.5 km NNW of Volyně, 500 m a.s.l., 49°12'10"N, 13°52'20"E. Coll. J. Holub and A. Krahulcová 3. 10. 1996. **2**. SW Bohemia; distr. Domažlice; beside the road 0.5 km SW of the village Pocinovice, ca. 8 km SE of Kdyně, 450 m a.s.l., 49°20'20"N, 13°07'30"E. Coll. J. Holub and A. Krahulcová 4. 10. 1996. **3**. SW Moravia; distr. Třebíč; at the edge of woodland along the road between the villages of Slavětice and Kramolín, 1.5 km NNE of the village Slavětice, 410 m a.s.l., 49°06'50"N, 16°07'10"E. Coll. B. Trávníček and A. Krahulcová 18. 10. 1996. **4**. C Moravia; distr. Olomouc; at the edge of woodland on the north-eastern periphery of the village Loučka, 370 m a.s.l., 49°39'40"N, 17°01'20"E. Coll. B. Trávníček and A. Krahulcová 16. 10. 1996.

Rubus hadracanthos. **1**. C Bohemia; distr. Příbram; scrub along the road 0.7 km SSW of Cholín settlement, ca. 9.5 km NW of Sedlčany, 310 m a.s.l., 49°42'40"N, 14°19'46"E. Coll. B. Trávníček 4. 9. 2011 (OL, R254/11).

Rubus holzfussii. **1**. S Moravia; distr. Blansko; at the edge of woodland at the road between the villages of Češkovice and Obůrka, 0.8 km NE of the village Češkovice, ca. 3 km NE of Blansko, 500 m a.s.l., 49°22'45"N, 16°41'06"E. Coll. B. Trávníček 5. 9. 2011 (OL, R270/11). **2**. S Moravia; distr. Brno – město [Brno – City]; at the edge of woodland near the NW margin of the village Útěchov, ca. 2.5 km W(-WSW) of Adamov, 490 m a.s.l., 49°17'27"N, 16°37'42"E. . Coll. B. Trávníček 8. 9. 2011 (OL, R289/11). **3**. N Moravia; distr. Opava; in the wood near the road between the villages of Brumovice and Sosnová, 2.1 km SSE of the village Úblo, 360 m a.s.l., 50°00'14"N, 17°42'25"E. Coll. B. Trávníček 6. 9. 2011 (OL, R277/11).

Rubus josholubii. **1**. C Bohemia; distr. Praha – východ [Prague – East]; along the road in the wood 0.5 km W of the village Vojkov near Říčany, 430 m a.s.l., 49°59'10"N, 14°41'40"E. Coll. J. Holub and A. Krahulcová 7. 8. 1996. **2**. NE Bohemia; distr. Trutnov; in the wood along the road near the crossing, ca. 1.5 km E of the village Horní Dehtov, 400 m a.s.l., 50°25'30"N, 15°45'30"E. Coll. J. Holub and A. Krahulcová 16. 9. 1997.

Rubus kletensis. **1**. S Bohemia; distr. Strakonice; at the edge of woodland along the road 1.3 km ESE of the village Hlupín, ca. 9 km E of Horažďovice, 490 m a.s.l., 49°19'53"N, 13°49'31"E. Coll. B. Trávníček 1. 9. 2011 (OL, R220/11). **2**. S Bohemia; distr. Český Krumlov; ruderal place 1.1 km E(-ENE) from the railway station of the village Zlatá Koruna, NE of Český Krumlov, 450 m a.s.l., 48°51'07"N, 14°21'59"E. Coll. B. Trávníček 1. 9. 2011 (OL, R231/11). **3**. S Bohemia; distr. Český Krumlov; scrub along the road 0.9 km NE of the village Mříč, SW of České Budějovice, 540 m a.s.l., 48°54'45"N, 14°20'21"E. Coll. B. Trávníček 2. 9. 2011 (OL, R235/11).

Rubus lucentifolius. **1**. N Moravia; distr. Bruntál; at the edge of woodland 0.8 km NW of the church in the village Janov, ca. 5.5 km E of Zlaté Hory, 480 m a.s.l., 50°15'10"N, 17°28'29"E. Coll. B. Trávníček 6. 9. 2011 (OL, R282/11). In the Czech Republic, this species was discovered for the first time by J. Hadinec, R. Hlaváček and B. Trávníček in 2011.

Rubus lusaticus. **1**. N Bohemia; distr. Děčín; in the wood on the SE slope of Růžovský vrch hill (619 m) 1.2 km SW of the church in the village Srbská Kamenice, ca. 6.5 km NW of Česká Kamenice, 340 m a.s.l., 50°49'33"N, 14°20'18"E. Coll. B. Trávníček 4. 9. 2011 (OL, R264/11).

Rubus muhelicus. **1**. S Bohemia; distr. České Budějovice; in S part of Rudolfův Kout wood 1.3 km ENE of the village Munice, ca. 1.5 km NNW of Hluboká nad Vltavou, 420 m a.s.l., 49°03'52"N, 14°25'27"E. Coll. B. Trávníček 1. 9. 2011 (OL, R228/11). **2**. S Bohemia; distr. Český Krumlov; in wood near Konopáč settlement 1.0 km W of Plešovice settlement, ca. 5.5 km NNE of Český Krumlov, 640 m a.s.l., 48°51'45"N, 14°20'23"E. Coll. B. Trávníček 2. 9. 2011 (OL, R233/11). **3**. S Bohemia; distr. České Budějovice; scrub along the road 0.5 km SW of the village Straňany, S of České Budějovice, 450 m a.s.l., 48°53'09"N, 14°29'55"E. Coll. B. Trávníček 2. 9. 2011 (OL, R239/11). **4**. S Bohemia; distr. České Budějovice; at the edge of woodland along the road 1.8 km E of the village Čakov, W of České Budějovice, 450 m a.s.l., 48°58'47"N, 14°20'02"E. Coll. B. Trávníček 14. 8. 2008 (OL, R51/08).

Rubus parthenocissus. **1**. C Bohemia; distr. Praha – západ [Prague – West]; along the road at the edge of woodland ca. 0.75 km N of the village Slapy, SE of Vyhlídka hill (437 m), 410 m a.s.l., 49°49'20"N, 14°23'50"E. Coll. J. Holub and A. Krahulcová 2. 10. 1996. **2**. C Bohemia; distr. Benešov; Živohošť, in wood W of the village. Coll. J. Holub 28. 10. 1995. **3**. E Bohemia; distr. Rychnov nad Kněžnou; in the wood Zádušní les ca. 0.5 km NNE of the village Voděrady S of Dobruška, 350 m a.s.l., 50°12'50"N, 16°09'30"E. Coll. J. Holub and A. Krahulcová 27. 9. 1996. **4**. S Moravia; distr. Kroměříž; Chřiby Hills; along path in the wood close to Bunč 4 km SEE of the village Roštín, 490 m a.s.l., 49°10'50"N, 17°20'50"E. Coll. B. Trávníček and A. Krahulcová 16. 10. 1996.

Rubus passaviensis. **1**. S Bohemia; distr. Český Krumlov; in wood 0.4 km NE of the village Malčice, ca. 6 km ESE of Český Krumlov, 580 m a.s.l., 48°47'54"N, 14°23'37"E. Coll. B. Trávníček 2. 9. 2011 (OL, R250/11).

Rubus pedemontanus. **1**. C Bohemia; distr. Kladno; Kožova hora hill, in wood ca. 1 km NNE of the excursion restaurant, 420 m a.s.l., 50°07'20"N, 14°06'50"E. Coll. J. Holub and A. Krahulcová 29. 10. 1996.

Rubus pericrispatus. **1**. C Bohemia; distr. Benešov; in wood along the road between the villages of Ostředek and Divišov, ca. 2.5 km NNW of the village Divišov, 420 m a.s.l., 49°48'40"N, 14°51'40"E. Coll. J. Holub and A. Krahulcová 13. 10. 1995. **2**. E Bohemia; distr. Chrudim; along road between the towns of Čáslav and Chrudim, at

the sharp bend N of the village Podhořany u Ronova, 350 m a.s.l., 49°56'40"N, 15°32'20"E. Coll. J. Holub and A. Krahulcová 27. 9. 1996. **3**. C Moravia; distr. Zlín; at the edge of a clearing in the wood at Paseky, ca. 1.5 km SSE of Paseky settlement near the village Bohuslavice u Zlína, 370 m a.s.l., 49°49'10"N, 17°38'50"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996. **4.** C Moravia; distr. Olomouc; at the edge of woodland 0.8 km SEE of the village Tovéř, 300 m a.s.l., 49°38'20"N, 17°20'00"E. Coll. B. Trávníček 3. 10. 1996.

Rubus perpedatus. **1**. SW Bohemia; distr. Domažlice; along the road at the edge of woodland 0.3 km NE of Spáleneček settlement, ca. 9 km SSW of Domažlice, 560 m a.s.l., 49°22'04"N, 12°53'07"E. Coll. B. Trávníček 1. 9. 2011 (OL, R218/11). **2**. SW Bohemia; distr. Domažlice; at thew edge of woodland along the road between the villages of Všeruby and Pomezí, 0.9 km NW of the village Pomezí , 470 m a.s.l., 49°19'58"N, 13°00'11"E. Coll. B. Trávníček 1. 9. 2011 (OL, R219/11). **3**. SW Bohemia; distr. Klatovy; the woodland at the way of the Cross near the N margin of the village Hory Matky Boží, ca. 7 km NW of Sušice, 695 m a.s.l., 49°16'13"N, 13°26'06"E. Coll. B. Trávníček 13. 9. 2008 (OL, R64/08).

Rubus perperus. **1**. C Bohemia; distr. Příbram; between the settlements of Evženov and Křešín, NW of the village Jince, 49°47'50"N, 13°57'20". Coll. J. Holub 19. 11. 1994. **2**. C Bohemia; distr. Příbram; in wood W of the village Felbabka close to the entrance to the military area, ca. 4 km SE of Hořovice, 450 m a.s.l., 49°48'50"N, 13°56'20"E. Coll. J. Holub and A. Krahulcová 27. 10. 1995. **3**. C Bohemia; distr. Praha – západ [Prague – West]; along the road at the edge of woodland ca. 0.75 km N of the village Slapy, SE of Vyhlídka hill (437 m), 410 m a.s.l., 49°49'20"N, 14°23'50"E. Coll. J. Holub and A. Krahulcová 2. 10. 1996. **4**. S Bohemia; distr. Prachatice; at the edge of Čarouše wood ca. 1.5 km NNE of Budkov settlement NE of the village Husinec, 500 m a.s.l., 49°05'10"N, 14°01'10"E. Coll. J. Holub and A. Krahulcová 3. 10. 1996. **5**. N Bohemia; distr. Děčín; town of Děčín, beside the road above the railway in the suburb of Prostřední Žleb, ca. 0.5 km S of the mouth of Čertova Voda brook, 140 m a.s.l., 50°48'50"N, 14°13'20"E. Coll. J. Holub and A. Krahulcová 10. 10. 1996. **6**. S Moravia; distr. Uherské Hradiště; Chřiby hills, at the edge of woodland in the area of Paseky settlement, ca. 1.7 km NNW of the village Stříbrnice, 360 m a.s.l., 49°04'20"N, 17°18'10"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996.

Rubus phyllostachys. **1**. C Moravia; distr. Olomouc;at the edge of woodland 0.8 km SEE of the village Tovéř , 300 m a.s.l., 49°38'20"N, 17°20'00"E. Coll. B. Trávníček 3. 10. 1996. **2**. E Moravia; distr. Zlín; Zlínské vrchy Hills, beside the road 0.8 km SSE of SE periphery of the village Kudlov (now a part of Zlín), 440 m a.s.l., 49°11'40"N, 17°42'00"E. Coll. B. Trávníček 23. 10. 1997. **3**. S Moravia; distr. Hodonín; the village Bohuslavice N of Kyjov, in scrubby vegetation on a slope situated 0.4 km SW of the railway station, 240 m a.s.l., 49°03'10"N, 17°07'10"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996.

Rubus portae-moravicae. **1**. C Moravia; distr. Přerov; at the edge of woodland along the road 1 km WNW of Zámecký kopec hill (359 m) situated W of the village Veselíčko, 330 m a.s.l., 49°32'20"N, 17°29'30"E. Coll. B. Trávníček 10. 10. 1996. **2**. C Moravia; distr. Přerov; in scrubby vegetation along the road 0.7 km W of the village Bohuslávky, 330 m a.s.l., 49°33'50"N, 17°33'30"E. Coll. B. Trávníček 10. 10. 1996. **3**. C Moravia; distr. Přerov; in scrubby vegetation along the road 1 km NNW of the village Loučka, 340 m a.s.l., 49°34'00"N, 17°35'10"E. Coll. B. Trávníček 10. 10. 1996.

Rubus posnaniensis. **1**. N Moravia; distr. Bruntál; at the edge of woodland along the forest road 0.8 km NNW of the village Biskupice, ca. 3.5 km NE of Město Albrechtice, 490 m a.s.l., 50°10'59"N, 17°36'23"E Coll. B. Trávníček 6. 9. 2011 (OL, R280/11). **2**. C Moravia; distr. Olomouc; at the edge of woodland along the road between the villages of Bouzov and Podolí, 1.1 km W of the village Podolí, ca. 4.5 km SSW of Loštice, 360 m a.s.l., 49°42'26"N, 16°54'18"E. Coll. B. Trávníček 6. 9. 2011 (OL, R286/11). **3**. S Moravia; distr. Blansko; in a forest glade 0.7 km ESE of the village Bukovinka, ca. 11 km NNW of Rousínov, 530 m a.s.l., 49°17'27"N, 16°48'37"E. Coll. B. Trávníček 8. 9. 2011 (OL, R288/11).

Rubus praecox. **1**. S Bohemia; distr. Strakonice; at the edge of woodland along the forest road 1.4 km SE of the village Koječín, ca. 5 km WNW of Bavorov, 550 m a.s.l., 49°07'49"N, 14°00'33"E. Coll. B. Trávníček 1. 9. 2011 (OL, R223/11). **2**. SE Moravia; distr. Uherské Hradiště; Chřiby Hills; at the edge of woodland beside the road 1.3 km SW of Chrastě hill (394 m), situated NW of the village Buchlovice, 350 m a.s.l., 49°05'50"N, 17°19'00"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996. **3**. SE Moravia; distr. Uherské Hradiště; Chřiby Hills; at the edge of a grove in Paseky settlement, 1.4 km NW of the village Stříbrnice, 400 m a.s.l., 49°04'00"N, 17°17'50"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996. **4**. E Moravia; distr. Zlín; Zlínské vrchy Hills; along the dirt track 0.5 km SE of the northern periphery of the village Kudlov (now a part of Zlín), 400 m a.s.l., 49°12'40"N, 17°41'20"E. Coll. B. Trávníček 23. 10. 1997.

Rubus pruinosus. **1**. C Bohemia; distr. Příbram; scrub along the road 0.7 km SSW of Cholín settlement, ca. 9.5 km NW of Sedlčany, 310 m a.s.l., 49°42'40"N, 14°19'46"E. Coll. B. Trávníček 4. 9. 2011 (OL, R255/11).

Rubus scabrosus. **1**. SW Bohemia; distr. Domažlice; Český les Mts, in the wood Drahotínský les along the road between the villages of Poběžovice and Drahotín, 480 m a.s.l., 49°31'00"N, 12°47'10"E. Coll. J. Holub 17. 9. 1994. **2**. SW Bohemia; distr. Domažlice; Český les Mts, along the road 0.4 km W of the village of Hora Sv.

Václava, 610 m a.s.l., 49°31'14"N, 12°44'04"E. Coll. J. Holub and P. Havlíček 18. 10. 1997. **3**. SW Bohemia; distr. Domažlice; Český les Mts close to small ponds on the E periphery of the village Pivoň, 570 m a.s.l., 49°29'10"N, 12°44'40"E. Coll. J. Holub 18. 10. 1997.

Rubus scissus. **1**. S Bohemia; distr. České Budějovice; in wood 0.8 km NE of the village Borovnice, SE of České Budějovice, 450 m a.s.l., 48°54'59"N, 14°31'50"E. Coll. B. Trávníček 2. 9. 2011 (OL, R241/11). **2**. S Bohemia; distr. České Budějovice; scrub along the road 1.0 km NW of Šejby settlement, ca. 6.5 km SSW(-S) of Nové Hrady, 600 m a.s.l., 48°44'00"N, 14°45'56"E. Coll. B. Trávníček 2. 9. 2011 (OL, R243/11). **3**. N Moravia; distr. Opava; in the wood along the road 0.5 km NE of Svoboda settlement, ca. 4.5 km N of Kravaře, 270 m a.s.l., 49°58'25"N, 18°01'00"E. Coll. B. Trávníček 6. 9. 2011 (OL, R275/11).

Rubus sendtneri. **1**. SW Bohemia; distr. Domažlice; at the edge of woodland along the road between the villages of Poběžovice and Hostouň, 2.5 km NNW of the village Poběžovice, 450 m a.s.l., 49°32'00"N, 12°47'50"E. Coll. J. Holub and A. Krahulcová 12. 10. 1998. **2**. SW Bohemia; distr. Domažlice; Český les Mts; at the edge of woodland close to parking area at the excursion resort Výhledy, situated 2.5 km SSW of Klenčí p. Čerchovem village, 700 m a.s.l., 49°24'50"N, 12°48'20"E. Coll. J. Holub and A. Krahulcová 12. 10. 1998. **3**. SW Bohemia; distr. Domažlice; in the wood along the road on the SSW foothill of Jezvinec hill (739 m), close to gamekeeper's lodge, 2.5 km SW of the village Orlovice, 570 m a.s.l., 49°19'00"N, 13°04'10"E. Coll. J. Holub and A. Krahulcová 12. 10. 1998. **4**. SW Bohemia; distr. Domažlice; along the road in the NEE periphery of Pláně settlement situated 8 km NWW of the village Nýrsko, 480 m a.s.l., 49°19'30"N, 13°02'20"E. Coll. J. Holub and A. Krahulcová 12. 10. 1998.

Rubus siemianicensis. **1**. N Moravia; distr. Opava; in wood near the SW margin of the village Bobrovníky, ca. 3 km SSE of Hlučín, 290 m a.s.l., 49°52'09"N, 18°12'10"E. Coll. B. Trávníček 6. 9. 2011 (OL, R271/11). **2**. N Moravia; distr. Opava; in wood near the road 1.5 km WSW of the village Šilheřovice, ca. 5 km NE of Hlučín, 260 m a.s.l., 49°55'21"N, 18°15'02"E. Coll. B. Trávníček 6. 9. 2011 (OL, R273/11). **3**. N Moravia; distr. Opava; in wood near the road between the villages of Brumovice and Sosnová, 2.1 km SSE of the village Úblo, 360 m a.s.l., 50°00'14"N, 17°42'25"E. Coll. B. Trávníček 6. 9. 2011 (OL, R276/11). **4**. N Moravia; distr. Bruntál; at the edge of woodland along the forest road 0.8 km NNW of the village Biskupice, ca. 3.5 km NE of Město Albrechtice, 490 m a.s.l., 50°10'59"N, 17°36'23"E Coll. B. Trávníček 6. 9. 2011 (OL, R281/11). **5**. W Moravia; distr. Žďár nad Sázavou; at the edge of woodland along the forest road 0.9 km S of Ondrušky settlement, ca. 6 km NNW of Velká Bíteš, 530 m a.s.l., 49°20'21"N, 16°11'37"E. Coll. B. Trávníček 8. 9. 2011 (OL, R295/11).

Rubus silvae-bohemicae. 1. SW Bohemia; distr. Domažlice; Český les Mts, along the road in the wood Drahotínský les 1.5 km WNW of the village Poběžovice, ca. 12 km NW of Domažlice, 480 m a.s.l., 49°31'00"N, 12°47'00"E. Coll. J. Holub and A. Krahulcová 4. 10. 1996. 2. SW Bohemia; distr. Domažlice; Český les Mts, beside the road in the wood Závistský les 0.75 km E of the village Závist, 630 m a.s.l., 49°29'30"N, 12°41'50"E. Coll. J. Holub and A. Krahulcová 4. 10. 1996. 3. SW Bohemia; distr. Domažlice; Český les Mts, beside the road 0.5 km NE of Pivoň settlement, close to cemetery, ca. 5.5 km SW of the village Poběžovice, 620 m a.s.l., 49°29'20"N, 12°44'50"E. Coll. J. Holub and P. Havlíček 18. 10. 1997.

Rubus silvae-norticae. **1**. S Bohemia; distr. České Budějovice; at the edge of woodland along the road 0.5 km S of road bridge in the village Boršov nad Vltavou, SSW of České Budějovice, 440 m a.s.l., 48°55'06"N, 14°26'08"E. Coll. B. Trávníček 1. 9. 2011 (OL, R230/11). **2**. S Bohemia; distr. Český Krumlov; ruderal place near the crossroad in N part of Veselka settlement, ca. 2 km NNW of Veleš́n, 530 m a.s.l., 48°50'49"N, 14°27'18"E. Coll. B. Trávníček 2. 9. 2011 (OL, R238/11). **3**. S Bohemia; distr. Český Krumlov; in the wood on the N slope of Kuřský vrch hill (806 m) 0.3 km S of Pila Gabriela settlement, near the S margin of the village Benešov nad Černou, 680 m a.s.l., 48°43'14"N, 14°37'45"E. Coll. B. Trávníček 2. 9. 2011 (OL, R247/11).

Rubus sorbicus. **1**. N Bohemia; distr. Děčín; in wood on the SE slope of Růžovský vrch hill (619 m) 1.2 km SW of the church in the village Srbská Kamenice, ca. 6.5 km NW of Česká Kamenice, 340 m a.s.l., 50°49'33"N, 14°20'18"E. Coll. B. Trávníček 4. 9. 2011 (OL, R265/11). The first discovery of *R. sorbicus* in the Czech Republic was recorded by J. Hadinec in 2008.

Rubus stimulifer. **1**. S Moravia; distr. Brno-venkov [Brno-country district]; at the edge of woodland beside the road between the villages of Hlína and Prštice near Ivančice, ca. 1.5 km NE of Hlína village, 420 m a.s.l., 49°07'20"N, 16°26'40"E. Coll. B. Trávníček and A. Krahulcová 18. 10. 1996. **2**. S Moravia; distr. Kroměříž; Chřiby Hills; beside the road along the reservoir of the Stupava river, ca. 2.5 km E of the village Koryčany, 1.2 km NNE of Vršava hill (500 m), 310 m a.s.l., 49°06'50"N, 17°11'40"E. Coll. B. Trávníček and A. Krahulcová 17. 10. 1996. **3.** S Moravia; distr.Kroměříž; at the edge of a clearing in the wood beside the road, 1.5 km E of the village Kostelany, 380 m a.s.l., 49°12'10"N, 17°24'30"E. Coll. B. Trávníček and A. Krahulcová 16. 10. 1996.

Rubus wahlbergii s.l. **1**. N Moravia; distr. Bruntál; at the edge of woodland along the road 0.9 km ESE of Guntramovice settlement, SSW of Krnov, 430 m a.s.l., 50°03'55"N, 17°40'34"E. Coll. B. Trávníček 6. 9. 2011 (OL, R279/11).