Sorbus milensis, a new hybridogenous species from northwestern Bohemia

***Sorbus milensis*, nový hybridogenní druh jeřábu ze severozápadních Čech**

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A new apomictic triploid (2n = 3x = 51) species belonging to the *Sorbus latifolia* group, *S. milensis* M. Lepší, K. Boublík, P. Lepší et P. Vít, putatively of hybridogenous origin between sexual *Sorbus aria* s.l. and *S. torminalis*, is described from the České středohoří Mts (northwestern Bohemia, Czech Republic). Several biosystematic techniques, including molecular (nuclear microsatellite markers), karyological (chromosome counts, genome size) and multivariate morphometrics were used to assess the variation in this species and justify its independent taxonomic status. The only known population of *S. milensis* consists of 38 adult and 19 juvenile individuals, is phenotypically homogenous and distinct from other Bohemian hybridogenous *Sorbus* species. All sampled individuals were karyologically uniform and showed little genetic variation. *Sorbus milensis* is a stenoendemic occurring on Milá hill (situated ca 9.5 km NNW of the town of Louny) where it grows on basaltic rocks, in ravines and on screes. The majority of the individuals grow in scree forests of the *Tilio-Acerion* alliance; other vegetation types include xeric scrub of the *Prunion spinosae* alliance and xerothermophilous grassland communities of the *Festucion valesiacae* alliance. A detailed distribution map for this species is provided as well as photographs of the type specimen.

K e y w o r d s: apomixis, Czech Republic, endemic, hybridization, karyology, multivariate morphometrics, phytosociology, *Rosaceae*, SSR markers, *Sorbus latifolia* agg., taxonomy

Introduction

The genus *Sorbus* L. (rowans; *Rosaceae*, *Maloideae*) is one of the most taxonomically challenging groups of vascular plants in Central Europe. Its taxonomic complexity is primarily a consequence of frequent interspecific hybridization accompanied by genome duplication and subsequent stabilization of the crosses by agamospermous reproduction. Primary hybrids notwithstanding, two groups of species are generally recognized: (i) sexual species, which are usually well delineated phenotypically (exceptions being microspecies of *S. aria* s.l.), often have a diploid number of chromosomes (2n = 2x = 34) and are widely distributed (five basic species, often recognized as separate subgenera, belong to this group; see Challice & Kovanda 1978, Jankun 1993, Kutzelnigg 1995); and (ii) hybridogenous, polyploid species originating from interspecific crosses between *Sorbus aria* s.l. and *S. aucuparia*, *S. torminalis* or *S. chamaemespilus.* They reproduce, at least

partly, via agamospermy, usually have restricted distributions (i.e., local endemics; Kárpáti 1960), and are difficult to identify due to their phenotypic similarity (Liljefors 1953, Challice & Kovanda 1978).

The complex evolutionary history of many *Sorbus* biotypes has triggered a long-term dispute concerning the species concept and the boundaries between taxa (especially in hybridogenous apomictic groups). The criteria appropriate to panmictic groups (i.e., the biological species concept; Mishler & Donoghue 1982) can hardly be applied to apomictic species of *Sorbus* with uniparental reproduction. Frequent interspecific hybridization involving the same parental species raises the problem of polyphyly. To avoid this issue, some authors treat the products of separate hybridization events as independent taxa. However, recent studies revealed that the use of monophyly in species definition raises problems, as monophyletic units do not necessarily correspond to phenotypic units and morphologically homogenous populations may arise recurrently (Robertson et al. 2004). In order to cope with the evolutionary complexity of apomictic hybridogenous rowans, the morphospecies concept coupled with distributional data is, for practical reasons, the primary basis for species recognition (Campbell & Dickinson 1990, Bernátová & Májovský 2003). These key characters should be accompanied by surveys of karyological, genotypic and reproductive variation. Generally, hybridogenous *Sorbus* biotypes, which form morphologically homogenous and distinguishable units, have distinct areas of distribution, are reproductively isolated and produce genetically identical offspring, are given different names. The extent of the distribution of *Sorbus* species is not a crucial requirement for the evaluation of taxa unlike in the genus *Rubus* (e.g., Trávníček & Zázvorka 2005, Žíla & Weber 2005, Lepší & Lepší 2006, Bijlsma & Haveman 2007).

While a solely morphological approach dominated early taxonomic research on rowans (Kárpáti 1960), recent years have seen an increase in the number of traits on which taxonomic decisions are made, in particular karyological (Kovanda 1996a, Mikoláš 2003) and/or genetic markers (Robertson & Sydes 2006). With the advent of molecular techniques, it was possible to obtain novel insights into the evolutionary history of several hybridogenous species, documenting, for example, modes of genesis of taxa (i.e., simple versus multiple hybridization) or numbers of independent origins (e.g., Nelson-Jones et al. 2002, Robertson et al. 2004, Vít 2006). Nevertheless, a holistic taxonomic approach, based on overall appraisal of various kinds of data, is still rare.

Despite improvement in the systematic treatment of rowans in the Czech Republic during the last two decades (Kovanda 1996a, 1996b), some phenotypically distinct populations, which may deserve independent taxonomic status, remained undetected. Therefore, the aim of this study was to perform a comprehensive taxonomic evaluation of a distinct population from the České středohoří Mts (NW Bohemia), employing morphological, karyological and genetic markers.

Material and methods

Plant material and field work

The unique *Sorbus* population on Milá hill was first recorded in 1999 and monitored regularly since then. Fifteen mature and well developed individuals (= ca 38.5% of the total

population size, excluding juvenile plants) were selected for the study of phenotypic and genetic variation.

Four other hybridogenous *Sorbus* taxa of the same putative parental combination occurring in Bohemia were included in the morphometric analyses in order to assess the phenotypic variation in a group of taxa closely related to the *Sorbus* population on Milá hill and to determine species-specific characters (see Table 1 for locality details). They were *S. bohemica* (111 individuals), *S. eximia* (45 individuals), *S. gemella* (10 individuals) and *S. rhodanthera* (12 individuals). Samples from type localities were available for all taxa. The number of individuals analysed reflects the population sizes of particular species, the most representative sampling was for *S. bohemica*, which co-occurs with the newly described taxon in the same geographic area. Specimens were collected during 2004–2005 observing the recommendations of Kutzelnigg (1995) and Meyer et al. (2005) and attention paid to sampling the same stage (generally, flowering and fruiting trees were collected in early May – middle June and September, respectively). Colours of generative parts were recorded, and flower parts were stored in 70% ethanol. Unless indicated otherwise, herbarium vouchers are deposited in CB and PRC.

Species nomenclature is unified according to Kubát et al. (2002) except for *Sorbus chamaemespilus* and *S. latifolia*, which follow Kutzelnigg (1995). Phytosociological nomenclature follows Moravec et al. (1995). For abbreviations of public herbaria, see Holmgren et al. (1990).

Karyology

Short, two-year old branches with well-developed leaf buds were collected from specimens of the population on Milá hill in January 2007 and kept at room temperature for 1–3 days (with basal parts submerged in water). Actively growing vegetative tissue was then cut off, pre-treated with a saturated water solution of p-dichlorbenzen (2–3 hours at room temperature) and fixed in ice-cold 3:1 ethanol : acetic acid overnight. The maceration lasted for 30–60 s in 1:1 ethanol : HCl at 22 °C, after which meristematic tissues were squashed in a drop of lacto-propionic orceine. Chromosomes were counted under a light microscope (Carl Zeiss NU, Jena, Germany) at a magnification of 1000 times. Three *Sorbus* individuals (incl. the type specimen) were karyologically examined and at least three well-spread mitoses per plant counted.

Flow cytometry

Genome size (C- and Cx-values) of three specimens from the *Sorbus* population on Milá hill (the same as investigated karyologically) was estimated using propidium iodide flow cytometry. *Bellis perennis* (2C = 3.38 pg; J. Suda et al., unpubl.) was selected as a suitable internal reference standard with a close, but not overlapping, genome size to the new taxon. An intact leaf petiole of the analyzed rowan was chopped together with an appropriate volume of fresh leaf tissue of the internal standard in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (Otto 1990). The crude suspension was filtered through a 42 µm nylon mesh and incubated at room temperature for 30 min, after which a staining solution consisting of 1 ml of Otto II buffer supplemented with propidium iodide (final concentration 50 µl/ml; Sigma, Steinheim, Germany), RNase IIA (50 ěl/ml; Sigma, Steinheim, Germany) and 2-mercaptoethanol (2 µl/ml; Fluka, Buchs, Germany) was added. Samples

Table 1. – Locality details of *Sorbus* species included in the morphometric analyses. Classification of phytogeographical regions follows Skalický (1988).

Species	Phytogeographic region	Locality	Geographical coordinates	Altitude (m a.s.l.)	Number of individuals analysed
S. milensis	Lounské středohoří Mts	Milá hill near the village of Milá (locus classicus)	50°26'03" N 13°45'30" E	450	15
S. bohemica	Labské středohoří Mts	Lovoš hill near the town of Lovosice (locus classicus)	50°31'43" N 14°01'05" E	570	27
	Labské středohoří Mts	Boreč hill near the village of Boreč	50°30'50" N 13°59'14" E	420	31
	Labské středohoří Mts	Opárenské údolí valley near the village of Opárno	50°32'27" N 14°01'18" E	240	6
	Labské středohoří Mts	Debus hill near the village of Radejčín	50°34'46" N 14°00'55" E	370	6
	Labské středohoří Mts	Výsluní hill near the village of Dubice	50°35'12" N 14°01'28" E	300	4
	Labské středohoří Mts	Deblík hill near the village of Sebuzín	50°35'08" N 14°03'11" E	400	10
	Labské středohoří Mts	Krkavčí skála hill near the village of Sebuzín	50°35'07" N 14°05'34" E	350	24
	Labské středohoří Mts	Hradiště hill near the village of Hlinná	50°34'07" N 14°07'01" E	520	3
S. eximia	Bohemian Karst	Koda hill near the village of Srbsko	49°56'08" N 14°07'29" E	390	45
S. gemella	Džbán region	hill ca 0.5 km east of the village of Konětopy (locus classicus)	50°16'01" N 13°44'38" E	460	10
S. rhodanthera	Žlutická pahorkatina hills	Chlumská hora hill near the town of Manětín (locus classicus)	50°00'43" N 13°11'50" E	610	12

were kept for 5 min at room temperature and then analysed on a Partec CyFlow cytometer (Partec GmbH, Münster, Germany) equipped with a green solid state laser (Cobolt Samba 532 nm, 100 mW; Cobolt, Stockholm, Sweden) as an excitation source. Fluorescence intensity of 5000 particles was recorded, and only histograms with both peaks approximately of the same height were considered. To eliminate potential instrument fluctuation, each plant was measured three times on different days.

In order to check the ploidy homogeneity of the population, DAPI flow cytometry was employed (instrument Partec PA II equipped with a mercury arc lamp, Otto methodology as described above except for using fluorochrome DAPI at final concentration 4 µl/ml; Sigma, Steinheim, Germany).

Morphometric data and analyses

Sixteen quantitative characters (Table 2) were measured and scored on 193 individuals of five hybridogenous apomictic *Sorbus* species (incl. 15 individuals from Milá hill). The character set was selected on the basis of published determination keys, floras and our own observations. Data were analysed using CANDISC and DISCRIM procedures available in the SAS package (version 9.1; SAS Institute, Cary, NC, USA) following the methodology provided by Klecka (1980). Individual plants as operational taxonomic units (OTUs) and species as groups were used. Because the data distributions within the groups were not multivariate normal, a non-parametric *k*-nearest-neighbour discriminant function was employed. The discriminant power was determined by cross-validation (Klecka 1980). Various modifications of discriminant analyses were performed, including all five groups analysed together and analyses of pairs of groups (i.e., the new taxon from Milá hill and each of the four related taxa).

Nuclear microsatellite markers (SSR)

Total genomic DNA was extracted from silica-dried leaves following the CTAB-protocol (Doyle & Doyle 1987) with minor modifications as described by Pfosser et al. (2005). The powdered leaf material, together with 5 mg PVP K-30 (Roth, Karlsruhe, Germany), was extracted in 700 µl CTAB buffer (2% CTAB, 100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 0.2% 2-mercaptoethanol, pH 8.0) for 30 min at 60 °C; 500 µl 24:1 chloroform : isoamylalcohol was then added and the extract incubated for 15 min at 4 °C. After centrifugation, the DNA was precipitated with 500 µl isopropanol. The pellet was washed with 96% and 70% ethanol and dissolved in 200 µl TE buffer. Microsatellite primers developed for genera *Sorbus* (Mss1, Mss5, Mss6, Ms6g, and Ms14; Oddou-Muratorio et al. 2001, Nelson-Jones et al. 2002) and *Malus* (CH02D11 and CH01H10; Gianfrancesci et al. 1998) were used for the determination of intraspecific genetic variation, following the methodology provided by the original authors. PCR mixtures (20 µL total volume) contained the following components/concentrations: 1 unit of Red Taq Jumpstart DNA polymerase (Sigma, Steinheim, Germany), 2 µL of 10× PCR buffer Jumpstart (Sigma, Steinheim, Germany), 0.2 mM of each dNTP (Sigma, Steinheim, Germany), with 0.5 µM of each forward and reverse primer and 40–50 ng of genomic DNA. PCRs were done in a XP thermal cycler (Bioer, Tokyo, Japan). The following programme was used: an initial denaturation at 94 °C for 2 min followed by 40–50 cycles at 94 °C for 30 sec., 48 °C (for primer Mss5, Ms6g, and Ms14) or 55 °C (Mss1, CH02D11, CH01H10, and Mss6) for 45

Table 2. – Summary of quantitative and qualitative characters with corresponding values and character states, respectively, for five Bohemian members of the *Sorbus latifolia* agg. Values and character states in bold indicate features useful for separation of *S. milensis* from other species. Only quantitative characters were used in the morphometric analyses.

Character (scale)	*S. milensis*	*S. rhodanthera*	*S. gemella*	*S. eximia*	*S. bohemica*
Leaf lamina length (cm)	(8.2–) 9.0–10.3 (–11.3)	(8.3–) 9.6–10.7 (–11.6)	(6.5–) 8.2–9.5 (–11.0)	(7.5–) 8.6–9.3 (–11.2)	**(5.1–) 7.0–8.4 (–10.6)**
Leaf lamina width (cm)	(6.0–) 6.8–7.8 (–9.6)	(6.4–) 7.8–9.0 (–9.4)	(4.6–) 6.8–8.0 (–8.6)	(5.4–) 6.6–7.4 (–9.4)	**(3.4–) 5.0–6.0 (–8.2)**
Petiole length (cm)	(1.4–) 1.9–2.3 (–2.5)	(1.8–) 2.2–2.4 (–2.7)	(1.6–) 1.8–2.1 (–2.8)	(1.5–) 1.9–2.1 (–3.0)	**(1.2–) 1.6–1.9 (–2.5)**
Width of the 3rd lamina lobe from the leaf base (cm)	(0.7–) 1.0–1.2 (–1.5)	(0.7–) 1.2–1.4 (–1.7)	(0.8–) 1.1–1.4 (–1.6)	(1.0–) 1.1–1.3 (–1.7)	(0.6–) 0.8–1.1 (–1.4)
Incission between the 2nd and 3rd lobe of the leaf lamina (cm)	(0.3–) 0.6–0.7 (–0.9)	**(0.4–) 0.8–1.0 (–1.2)**	(0.5–) 0.8–0.9 (–1.0)	**(0.3–) 0.4 (–0.5)**	(0.2–) 0.4–0.6 (–0.9)
Number of lateral leaf veins	(9–) 10–11 (–13)	(8–) 9–10 (–11)	(8–) 9–10 (–11)	**(6–) 7–8 (–10)**	(7–) 8–9 (–11)
Number of lamina lobes wider than 1 cm	(1–) 2 (–3)	(2–) 3–4 (–5)	(2–) 3–4	(2–) 3–4	(1–) 2–3 (–4)
Distance between the insertion of the petiole and the tip of the 1st lamina lobe (cm)	(3.4–) 5.0–6.0 (–7.2)	(4.2–) 5.4–6.1 (–6.5)	(3.4–) 4.7–5.5 (–6.6)	(3.6–) 4.6–5.3 (–6.5)	(2.7–) 3.7–4.5 (–5.8)
Fruit width (mm)	(13–) 14–15 (–16)	(12–) 13 (–14)	(11–) 12 (–13)	**(11–) 12 (–13)**	**(9–) 11–12 (–14)**
Fruit length (mm)	(12–) 13–14 (–16)	(11–) 12 (–13)	(11–) 12 (–14)	(11–) 12 (–13)	**(9–) 10 (–14)**
Corolla length (mm)	(5.7–) 6.7–7.3 (–7.8)	(6.0–) 6.7–7.0 (–8.3)	**(5.2–) 5.8–6.1 (–7.3)**	**(5.1–) 5.7–6.1 (–6.7)**	(5.6–) 6.2–6.5 (–8.2)
Corolla width (mm)	(4.8–) 5.1–5.5 (–6.2)	(4.3–) 5.2–5.5 (–6.1)	**(4.0–) 4.3–4.6 (–4.8)**	(3.8–) 4.6–4.9 (–5.2)	(3.7–) 4.7–4.8 (–5.4)
Calyx length (mm)	(2.7–) 3.1–3.6 (–4.5)	**(1.9–) 2.8–3.1 (–4.0)**	(2.4–) 3.1–3.4 (–3.8)	(1.7–) 2.1–2.5 (–2.9)	(1.8–) 2.4–2.6 (–4.2)
Calyx width (mm)	(2.0–) 2.3–2.7	**(2.6–) 2.8 (–3.1)**	(2.1–) 2.7 (–3.0)	(1.8–) 2.6–2.9 (–3.3)	(2.0–) 2.6–2.7 (–3.2)
Anther length (mm)	(1.2–) 1.4–1.6 (–1.7)	**(1.2–) 1.3–1.4 (–1.6)**	**(1.2–) 1.3 (–1.4)**	(1.0–) 1.2–1.4 (–1.6)	**(1.2–) 1.3 (–1.6)**
Stigma width (mm)	0.5–0.6 (–0.7)	(0.4–) 0.5–0.6	**0.6–0.7**	(0.6–) 0.6–0.7 (–0.8)	**(0.5–) 0.6–0.7 (–0.8)**
Colour of anthers	pale rose	**rose**	**yellow**	**yellow**	**yellow**
Shape of lamina	rhomboidal to broadly ovate	rhomboidal to broadly ovate	rhomboidal to broadly ovate	**broadly ovate to broadly elliptic**	**elliptic to ovate elliptic**
Colour of fruits	orange-red	orange-red	**orange**	**orange**	orange-red

sec. and 72 °C for 45 sec. A final 15-min extension step at 72 °C was included. Final visualization of fluorescently-labelled fragments (NED, 6-FAM, HEX; Applied Biosystems, Foster City, CA, USA) was carried out using an automatic sequencer Avant Genetic Analyser 3100 (Applied Biosystems, Foster City, CA, USA).

Results and discussion

Sorbus milensis M. Lepší, K. Boublík, P. Lepší et P. Vít, **spec. nova**

D e s c r i p t i o: Arbores (vel frutices) usque 10 m alti; foliis (in brachyblastis fertilibus) simplicibus, laminis ambitu fere rhombeis usque late ovatis, pinnato-lobatis (lobis acutis vel acuminatis, serratis), in parte superiore tantum duplicato-serratis, (8.2–) 9.0–10.3 (–11.3) cm longis et (6.0–) 6.8–7.8 (–9.6) cm latis, ad basin late cuneatis usque rotundatis, subintegris vel remote serratis, nitidis, obscure viridibus, subtus ochro-griseo-viride tomentosis, nervis ab utroque latere (9–) 10–11 (–13) in numero; petiolis (1.4–) 1.9–2.3 (–2.5) cm longis; corymbothyrsis multifloris, compactis, convexis, ramis plus minusve tomentosis. Dentibus calycinis triangularibus, acuminatis usque acutis, (2.7–) 3.1–3.6 (–4.5) mm longis et (2.0–) 2.3–2.7 mm latis, patentibus, post anthesin reclinatis, tomentosis, tempore fructificationis siccis, persistentibus; petalis late ovatis usque subrotundatis, breviter unguiculatis, (5.7–) 6.7–7.3 (–7.8) mm longis et (4.8–) 5.1–5.5 (–6.2) mm latis, albiusculis, superne ad basin sparse villosis, patentibus; staminibus ca 20, antheris pallide rosaceis, (1.2–) 1.4–1.6 (–1.7) mm longis; ovario semi-infero; stylis 2 (3) ad (1/5–) 2/5 usque 3/5 (–5/5) coalescentibus, ad basin villosis, luteo-viridis, (3.5–) 4.3–4.7 (–5.2) mm longis, stigmatibus plus minusve planis; fructibus subglobosis, (12–) 13–14 (–16) mm longis et (13–) 14–15 (–16) mm latis, maturitate rubris, glabris vel fere glabris, nitidis, cum (7–) 9–14 (–26) lenticellis parvis, pallido-fuscis ad 0.25 cm^2; mesocarpio heterogeneo; endocarpio cartillagineo, seminibus atro-fuscis. Numerus chromosomatum triploideus 2n = 51. Planta apomicta. Floret V.

H o l o t y p u s: Bohemia septentrionalis, distr. Louny, pagus Milá (5548d): ca. 0.6 km situ sept.-orient. a pago, in rupibus praeruptis in declivibus meridionalibus collis Milá; solo basaltico; 450 m s. m., 50°26'02.88" N, 13°45'30.30" E; numerus arboris 13; leg. 16. 5. 2002 M. Lepší; CB, no. 33262 (Fig. 1). – I s o t y p i: CB, nos. 33261, 33263, 33264, 33275–33279; PRC, no. 33262/a; PR, no. 33279/a. – P a r a t y p i: leg. October 1999 M. Lepší et K. Boublík, CB, no. 39785; leg. 29. 5. 2000 P. Lepší et K. Boublík, PRA, no. 320; leg. 1. 9. 2000 M. Lepší, P. Lepší et K. Boublík, CB, no. 39787; leg. 1. 9. 2000 P. Lepší et K. Boublík, LIT, no. 299; leg. 4. 8. 2002 M. Lepší, CB, nos. 39166–39182; leg. 2003 M. Lepší et K. Boublík, CB, no. 38992; leg. 15. 9. 2004 M. Lepší, P. Lepší et K. Boublík, CB, nos. 39187–39197.

D e s c r i p t i o n: Trees (or shrubs) up to 10 m tall with an ovate to broadly ovate crown, often with several trunks. Trunk up to 1 m in circumference. Bark grey to dark grey, smooth when young, with vertical fissures (particularly at the trunk base) at maturity, with (5–) 7–9 (–13) mm long and (3–) 5–8 (–13) mm wide lenticels. Branches held at an angle of 50–80° to the trunk; twigs greyish-brown, thick; young shoots pale brown and tomentose, but almost glabrous at maturity, with numerous subrotund and elliptical lenticels. Buds 8–14 mm long and 4–7 mm wide, ovoid; scales green, with narrow brown sparsely tomentose margins. Leaves (of short fertile shoots) simple; laminas more or less rhomboidal or broadly ovate, occasionally oval, usually concave, glossy, dark green above, yellowish-greyish-green beneath, undulated at margins, more or less acute at apex, usually broadly cuneate or rounded and partly serrate at base, almost glabrous on upper surface, evenly tomentose on lower surface, (8.2–) 9.0–10.3 (–11.3) cm long and (6.0–) 6.8–7.8 (–9.6) cm wide, widest at (34–) 46–56 (–64)% of the lamina length (from the tip), shallowly lobed (doubly serrate apically); lobes acute or acuminate, serrate or doubly serrate with acuminate teeth terminating the main veins, other teeth smaller, acute or acuminate; sides of lobes convex to straight; the third lobe (from the base) (0.7–) 1.0–1.2 (–1.5) cm broad; incision between the second and the third lobe (0.3–) 0.6–0.7 (–0.9) cm; lobes broader than 1 cm (1–) 2 (–3) on each side; veins (9–) 10–11 (–13) on each side; petioles (1.4–)

Fig. 1. – Holotypus of *Sorbus milensis* M. Lepší, K. Boublík, P. Lepší et P. Vít (photo H. Jakešová 2006).

1.9–2.3 (–2.5) cm long, more or less tomentose. Inflorescences with 35–65 flowers, 4.5–7.5 cm in diameter, compact, convex; branchlets more or less tomentose. Hypanthium turbinate, tomentose. Sepals (2.7–) 3.1–3.6 (–4.5) mm long and (2.0–) 2.3–2.7 mm wide, triangular, acuminate or acute, tomentose on both surfaces, patent, reclinate after anthesis, persistent, dry, erect. Petals (5.7–) 6.7–7.3 (–7.8) mm long and (4.8–) 5.1–5.5 (–6.2) mm wide, broadly ovate to subrotund, concave, whitish, patent, sparsely hirsute at the base of upper surface, with a short claw. Stamens ca 20; filaments whitish; anthers pale rose, (1.2–) 1.4–1.6 (–1.7) mm long. Ovary semi-inferior. Styles 2 (3), greenish-cream, (3.5–) 4.3–4.7 (–5.2) mm long, hairy at the base, connate up to (1/5–) 2/5–3/5 (–5/5). Stigma greenish-cream, more or less flat, 0.5–0.6 (–0.7) mm wide. Fruit (12–) 13–14 (–16) mm long and (13–) 14–15 (–16) mm wide, subglobose, orange-red at maturity, glabrous or almost glabrous, glossy, with (7–) 9–14 (–26) pale brown lenticels per 0.25 cm^2; mesocarp heterogeneous; endocarp cartilagineous. Seeds fuscous. Somatic chromosome number 2n = 51 (triploid). Reproduction probably apomictic. Flowering V.

Etymology

The name "*milensis*" refers to Milá hill (510.1 m a.s.l.) in the České středohoří Mts where the species occurs. The authors propose the epithet "milský" for the Czech name.

Morphometric analyses

Canonical discriminant analyses showed that *Sorbus milensis* is well defined morphologically and is clearly separated from the four closely related taxa in the *S. latifolia* agg. occurring in Bohemia. An ordination diagram displaying the distribution of all OTUs along the first three canonical axes is presented in Fig. 2. The first canonical axis separated *S. milensis* from *S. bohemica* and *S. eximia,* while the third canonical axis was responsible for the separation of the newly described species from *S. rhodanthera* and *S. gemella*. The second discriminant axis separated the two well defined species *S. bohemica* and *S. eximia*. Calyx length, number of lateral veins and fruit diameter were most closely correlated with the first canonical axis; calyx width, calyx length and anther length were most tightly correlated with the third canonical axis. Classificatory discriminant analysis based on 16 quantitative characters resulted in the correct classification of all individuals. Separate discriminant analyses revealed characters distinguishing *S. milensis* from each of the four related taxa; Table 3 gives four characters with the highest canonical coefficients for each taxon.

Diagnostic characters

Leaf laminas are more or less rhomboidal or broadly ovate, (8.2–) 9.0–10.3 (–11.3) cm long and (6.0–) 6.8–7.8 (–9.6) cm wide, shallowly lobed – incisions between the second and the third lobe are only (0.3–) 0.6–0.7 (–0.9) cm long. Sepals (2.7–) 3.1–3.6 (–4.5) mm long and (2.0–) 2.3–2.7 mm wide, petals (5.7–) 6.7–7.3 (–7.8) mm long and (4.8–) 5.1–5.5 (–6.2) mm wide. Anthers are pale rose and (1.2–) 1.4–1.6 (–1.7) mm long. Stigma 0.5–0.6 (–0.7) mm wide. Fruit are (12–) 13–14 (–16) mm long and (13–) 14–15 (–16) mm wide, subglobose, orange-red at maturity (Fig. 4).

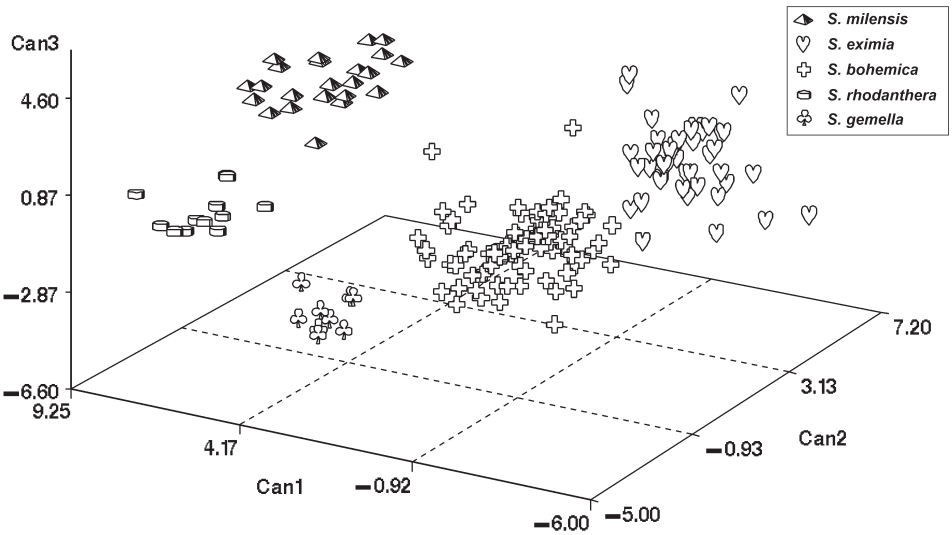

Fig. 2. – Canonical discriminant analysis of five Bohemian members of the *Sorbus latifolia* agg. All quantitative characters (see Table 2) were used. The first three axes explain 56.1%, 31.7% and 9.5% of the variation, respectively.

Chromosome and genome size variation

The species under investigation possessed 51 chromosomes in somatic cells, which corresponds to the triploid level (2n = 3x = 51; Fig. 3). Screening of the DNA ploidy levels of 35 individuals of *S. milensis* using DAPI flow cytometry did not reveal any intraspecific variation. Holoploid genome size (mean 2C-value ± SE) of *S. milensis* was estimated as 2.01 ± 0.01 pg and mean monoploid genome size (Cx-value) as 0.67 pg.

Genetic variation

The specimens of *Sorbus milensis* examined showed no variation at seven nuclear microsatellite loci, which indicates a monotopic origin (i.e., a single evolutionary lineage). This observation also applies to several other agamospermous *Sorbus* taxa occurring in the Czech Republic (e.g., *S. bohemica*, *S. rhodanthera*; P. Vít et al., unpublished results). However, distinct differences in microsatellite patterns were detected between species, supporting the independent status of *S. milensis* as a unique evolutionary unit (P. Vít et al., unpublished results). The low level of genetic (as well as phenotypic) variation indicates that apomixis is the predominant, if not the sole, mode of reproduction in *S. milensis*.

Table 3. – Results of separate canonical discriminant analyses of *S. milensis* and four other Bohemian members from the *Sorbus latifolia* agg. Four characters with the highest canonical correlation coefficients are shown for each taxon (absolute values of canonical coefficients are given in parentheses).

Species	*Sorbus milensis*			
S. bohemica	fruit length (0.94)	fruit width (0.83)	anther length (0.70)	stigma width (0.68)
S. eximia	calyx length (0.91)	incision between the 2nd and 3rd lobe of the leaf lamina (0.87)	fruit width (0.86)	number of lateral leaf veins (0.85)
S. gemella	corolla width (0.95)	anther length (0.95)	stigma width (0.93)	corolla length (0.89)
S. rhodanthera	calyx width (0.95)	anther length (0.88)	calyx length (0.85)	incision between the 2nd and 3rd lobe of the leaf lamina (0.81)

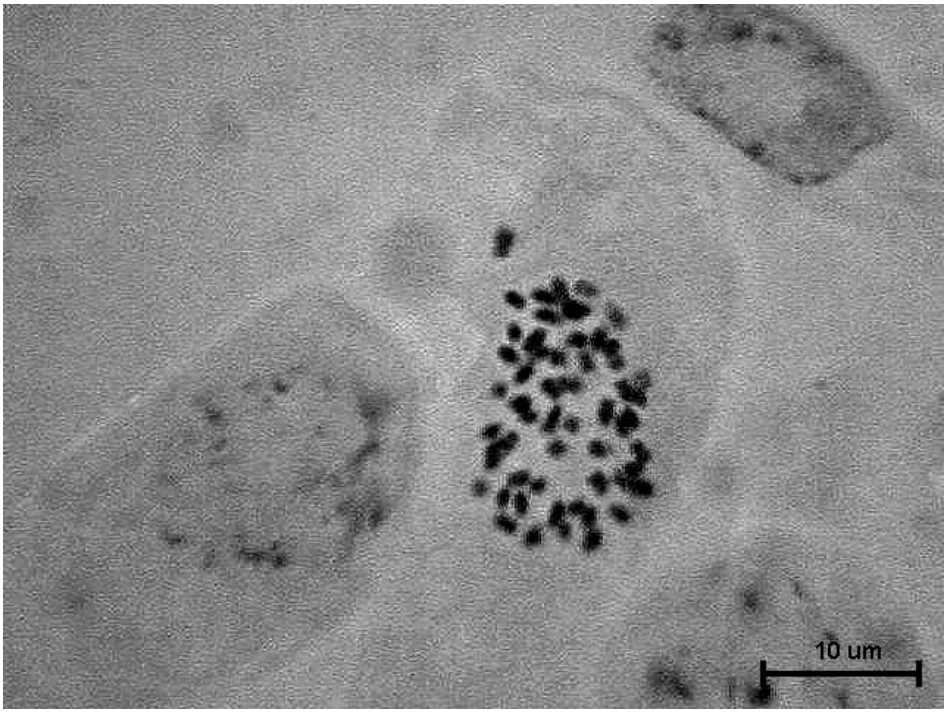

Fig. 3. – Microphotograph of the somatic chromosomes (2n = 3x = 51) of *Sorbus milensis* (type tree) (photo V. Jarolímová).

Phenotypic variation and species-specific characters

The population of *S. milensis* is morphologically homogenous both in vegetative and generative characters (F_1 segregation was not recorded). The plants are fertile, although the proportion of flowering individuals as well as fruit yield may vary markedly between years. Similar fluctuation in flowering and fruit set was also observed in other hybridogenous species of *Sorbus* (Kovanda 1996a).

Fig. 4. – Adult fruiting individual of *Sorbus milensis* at the type locality (photo M. Lepší 2004).

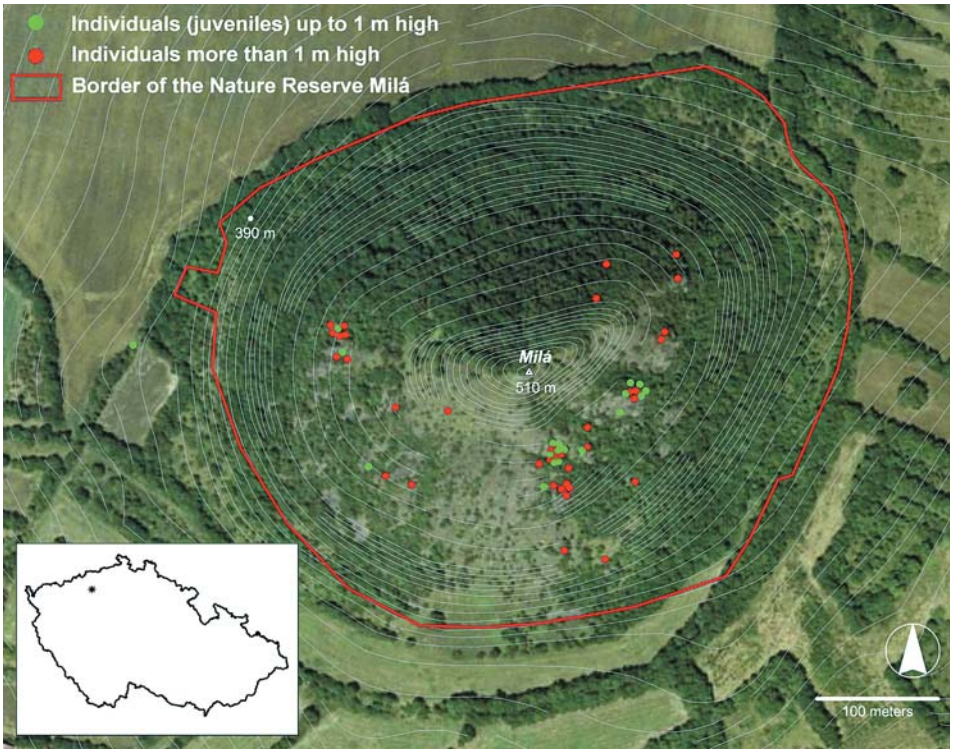

Fig. 5. – Distribution map of *Sorbus milensis*.

With respect to morphology, *S. milensis* belongs to *S. latifolia* agg. (parental combination *S. aria* s. lat. × *S. torminalis*). As two species from the *S. aria* group (i.e., *S. aria* s. lat. and *S. danubialis*) co-occur at the locality, the exact parentage is difficult to infer. The second putative parental species, *S. torminalis*, is currently not present on Milá hill (Veselý 1969, Tyrner & Bárta 1978, Hamerský 1993, Wittenberger 1996) but may have occurred there in the past. The nearest recently recorded locality for *Sorbus torminalis* is on the adjacent Oblík hill (ca 4.5 km away; K. Kubát, in litt. 2007).

Currently the *S. latifolia* agg. encompasses five endemic species in Bohemia: *S. bohemica*, *S. eximia*, *S. gemella*, *S. rhodanthera* and the newly recognized *S. milensis*. The latter taxon morphologically resembles *S. rhodanthera*, but its calyxes are narrower and longer, fruits and anthers are longer, anthers are pale rose (rose in *S. rhodanthera*) and leaves are rather shallowly lobed (Table 2). *Sorbus gemella* has, unlike *S. milensis*, orange fruits, yellow and shorter anthers and shorter and narrower corollas. *Sorbus eximia* differs by orange fruit, yellow and shorter anthers, shorter leaf lobes and a smaller number of lateral leaf veins. Fruit and leaves of *S. bohemica* are smaller, laminas are elliptical to ovate, and anthers are smaller and yellow.

Geographic distribution

Sorbus milensis is confined to the basaltic Milá hill (510.1 m a.s.l.) near the village of Milá (quadrant 5548d of the Central European grid mapping; Ehrendorfer & Hamann 1965), ca 9.5 km NNW of the town of Louny in the České středohoří (NW Bohemia). According to the 2002 tree census, there were 57 individuals of different ages (including 19 juveniles under 1 m in height) (Fig. 5). The locality is situated in the colline vegetation belt in the phytogeographical sub-district of Lounské středohoří Mts, belonging to the Lounsko-labské středohoří Mts district (Skalický 1988). It lies in a warm climatic region (Quitt 1971) with a relatively continental climate. The mean annual temperature is about 8 °C and mean annual precipitation reaches 500 mm (Tolasz et al. 2007). The altitudinal range varies from 385 to 490 m a.s.l. As the landscape in the Lounské středohoří Mts has been intensively modified by agriculture, non-forest vegetation prevails. While man-made fields dominate the flat terrain, scattered neovolcanic hills are covered by abandoned grasslands. The vegetation on the hills (especially their southern slopes) is steppe-like and harbours many relict submeridional and continental plants. Trees or shrubs usually occur only on the northern slopes of the hills.

Ecology

Sorbus milensis inhabits mainly rocky sites and ravines, but occurs also on screes and steep stony slopes with eutrophic soils on basalt. Most individuals grow on the south-east facing slope of the hill (Fig. 5).

This species was recorded in three vegetation types. It occurs most frequently in thermophilous scree forests (*Tilio-Acerion* alliance) with abundant *Fraxinus excelsior* and light-demanding and thermophilous species of the *Quercetalia pubescenti-petraeae* order and the *Festucion valesiacae* alliance. It was also recorded in a relic shrub community on rocky outcrops (*Junipero communis-Cotoneastretum integerrimae*, *Prunion spinosae* alliance). *Sorbus milensis* was rarely found growing on the southern slope in the xerothermophilous grassland community of *Erysimo crepidifolii-Festucetum valesiacae* (*Festucion valesiacae* alliance).

Conservation status

Milá hill is a nature reserve consisting of well-developed xerothermophilous grassland and rocky communities (Hamerský 1993). The main threat to the *S. milensis* population stems from the low number of individuals. In addition, seedlings are rare, perhaps due to grazing by mouflons and low recruitment in shaded habitats. The majority of individuals occur on open rocks, which for most game animals are inaccessible. The spread of *Fraxinus excelsior*, also into rocky habitats, represents another threat to *S. milensis*. Effective conservation measures should thus be implemented and may involve creating openings in dense canopy, reducing the density of game, and/or fencing off vital groups of adult individuals. Considering the endemic status of *S. milensis*, the small number of individuals and risk of further population decline, this species should be classified as a critically endangered plant (CR sensu Holub & Procházka 2000) of the Czech flora.

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Souhrn

V příspěvku je popsán nový apomiktický triploidní (2n = 3x = 51) druh jeřábu *Sorbus milensis* M. Lepší, K. Boublík, P. Lepší et P. Vít (jeřáb milský), který náleží do skupiny *Sorbus latifolia* agg. (obr. 1, 4). Vznikl hybridizací druhu *S. torminalis* a jednoho ze zástupců skupiny *S. aria* s. lat. (*S. aria* s. lat. nebo *S. danubialis*). Je stenoendemitem vrchu Milá, který leží v Českém středohoří ca 9,5 km SSZ od města Louny (obr. 5). V roce 2002 bylo zaznamenáno celkem 57 exemplářů – 38 dospělých a 19 juvenilních. Jeřáb milský obsazuje čedičové skály, rokle, sutě a kamenité půdy strmých svahů. Nejčastěji roste v suťových lesích ze svazu *Tilio-Acerion* a v xerofilních křovinách ze svazu *Prunion spinosae*. Výjimečně byl zaznamenán v úzkolistých suchých trávnících řazených do svazu *Festucion valesiacae*. Analyzovaní jedinci vykazovali velmi malou vnitrodruhovou morfologickou a genetickou variabilitu a byli karyologicky uniformní. Jeřáb milský je relativně dobře morfologicky a geneticky diferencovaný od ostatních hybridogenních jeřábů vyskytujících se v Čechách. Je ohrožen vysokými stavy zvěře a expanzí jasanu. Zmlazování a odrůstání semenáčků je velmi vzácné. K udržení a posílení endemické populace je zapotřebí buď snížit stavy zvěře, nebo oplotit širší okolí dospělých exemplářů. Vhodným zásahem by bylo i prosvětlování lesů (především výřez jasanu) v kombinaci s výše zmíněnými opatřeními. Navrhujeme jeřáb milský zařadit do červeného seznamu taxonů ČR do kategorie kriticky ohrožených druhů (C1 podle Holub & Procházka 2000).

Druh má následující diagnostické znaky: čepel listů fertilních brachyblastů je zpravidla kosočtverečná až široce vejčitá, vzácně i oválná, (8,2–) 9,0–10,3 (–11,3) cm dlouhá a (6,0–) 6,8–7,8 (–9,6) cm široká, mělce laločnatá – zářezy mezi druhým a třetím lalokem jsou jen (0,3–) 0,6–0,7 (–0,9) cm hluboké. Prašníky jsou světle růžové. Plody jsou (12–) 13–14 (–16) mm dlouhé a (13–) 14–15 (–16) mm široké, téměř kulovité a v době zralosti oranžově červené (obr. 4).

Nejpodobnějším českým druhem je *S. rhodanthera*, který má na rozdíl od *S. milensis* tmavěji růžové prašníky, větší plody a hlouběji laločnaté listy. *Sorbus eximia* a *S. gemella* mají oranžové plody a žluté prašníky a *S. bohemica* má výrazně menší eliptické až vejčité listy a žluté prašníky.

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Nová jména rostlin zveřejněná v tomto čísle

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