Sorbus pauca species nova, the first endemic species of the *Sorbus hybrida* group for the Czech Republic

Sorbus pauca species nova – první endemický druh z okruhu *Sorbus hybrida* pro Českou republiku

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We describe a newly distinguished apomictic tetraploid (2n = 4x = 68) species *Sorbus pauca* M. Lepší et P. Lepší. We classify this new species as a member of the *Sorbus hybrida* group because we presume that it has originated from a cross between *S. danubialis* and *S. aucuparia*. *Sorbus pauca*, a stenoendemic that occurs on Bezděz and Malý Bezděz hills (Doksy region, northern Bohemia), is the first species of the *S. hybrida* group to be described for the Czech Republic. Multivariate morphometrics and elliptic Fourier analyses reveal that it is distinct from the morphologically close *S. danubialis*. The new taxon is phenotypicaly homogenous, morphologically well separated from the sympatrically occurring *S. danubialis* and European taxa of *S. hybrida* agg. It does not show any karyological variation. Apomixis was detected as the species' sole mode of reproduction. The two known populations of *S. pauca* consist of 14 adult individuals. The species is restricted to rocky grasslands, rocky scrub and open woodlands on rocks with *Festuca pallens* and *Cotoneaster integerrimus*.

Keywords: apomixis, DAPI flow cytometry, geometric morphometrics, hybridization, karyology, multivariate morphometrics, *Rosaceae*, subg. *Soraria*, taxonomy

Introduction

European hybridogenous species of the genus *Sorbus* L. comprise four main species groups, which arose from crosses between polyploid members of the *Sorbus aria* aggregate and five basic diploid *Sorbus* species (Kutzelnigg 1995, Rich et al. 2010). These species groups differ in their morphology, distribution and ecological demands, which are related to the characters of their parental taxa.

The *Sorbus sudetica* group (diploid parent *S. chamaemespilus*) encompasses alpine species that are confined to European mountains. In contrast, the *S. latifolia* group (diploid parent *S. torminalis*) includes thermophilous species, which are absent from mountains and northern parts of Europe. The *Sorbus aria* group (diploid parents *S. aria* s. str. and in southern Europe possibly also *S. umbellata*) consists of many thermophilous species, but it also occurs in Scandinavia and certain mountain areas in Europe. Finally, the *S. hybrida* group (diploid parent *S. aucuparia*) is abundant in northern Europe and European mountains but occurs only rarely in warm regions of central Europe (Kutzelnigg 1995).

In the Czech Republic, the distribution of endemic, hybridogenous *Sorbus* species follows this pattern. Only *S. sudetica*, which occurs at high altitudes in the Krkonoše Mts, is a descendant of *S. chamaemespilus* and an unknown member of the *S. aria* group, both of which are now extinct in this mountain range (Kovanda 1992). Other taxa are confined to lower altitudes. The *Sorbus latifolia* group is the most abundant and the most studied group within the Czech Republic and currently includes 11 taxa (Kubát et al. 2002, Lepší et al. 2008, 2009, Velebil 2012, Vít et al. 2012). According to our unpublished data, their tetraploid parents are *S. danubialis* and a taxonomically unclear member of the *S. aria* group. The *S. aria* group has not yet been adequately examined from a taxonomical perspective and some taxonomic novelties are likely to occur in the lowlands of Moravia.

There are no native species of the *S. hybrida* agg., in the Czech Republic, but two species have been reported as garden escapes: *S austriaca* (Lepší et al. 2011, Pyšek et al. 2012) and *S. mougeotii*, which was initially described by Kovanda (1996) from Prague as *S. quernea* (Lepší et al. 2013). This paper provides a taxonomic evaluation of a unique population of the *S. hybrida* group discovered in the Bezděz hills in 2008 and described here as a new species, *S. pauca*.

Material and methods

Plant material and field work

Samples for morphometric analyses, flow cytometry and herbarium specimens were collected in 2009 and 2011, following the recommendations of Meyer et al. (2005), Rich et al. (2010) and papers published by Lepší et al. (2008, 2009). The two co-occuring species, *S. pauca* and *S. danubialis,* were studied. Flowering and fructiferous parts were collected in mid-May and September, respectively.

Nine relevés with the presence of *S. pauca* were recorded using the Braun-Blanquet approach, located using Garmin eTrex instruments (WGS-84) and stored in the Czech National Phytosociological Database (CNFD) under relevé numbers 203585–203591, 335307–335308 (Chytrý & Rafajová 2003). Furthermore, 78 relevés from the Bezděz hills were added from CNFD, which describe the habitat preferences of both the supposed parental species (*S. aucuparia* and *S. danubialis*). Considering the lengths of the gradient (over 5.5 SD), the unimodal ordination method DCA was chosen to explain variability in species composition of relevés within the ordination space. The analysis was done using CANOCO for Windows 4.5 (Lepš & Šmilauer 2003).

A revision of relevant *Sorbus* material kept in the following herbarium collections was undertaken: BRNM, BRNL, BRNU, CB, CHEB, CHOM, Herbarium of Museum of Ústí nad Labem, HOMP, HR, LIM, LIT, MP, PL, PR, PRA, PRC, ROZ, SOKO, ZMT. For abbreviations of public herbaria, see Holmgren et al. (1990). Species nomenclature was unified according to Kubát et al. (2002) except for *Sorbus* names, which follow Kutzelnigg (1995), otherwise authorities are provided.

Multivariate and elliptic Fourier analyses

Multivariate morphometric and geometric morphometric analyses were used to reveal species-specific characters in two sympatrically occurring species: *S. pauca* (12 individuals) and *S. danubialis* (10 individuals). Only well developed branches of mature individuals were studied. In total, 24 leaves from *S. pauca* and 15 from *S. danubialis* were used for morphometric analyses. The determination of individuals analysed was based on the shape of lobes or main teeth on leaves – *S. pauca* obtuse lobes, *S. danubialis* acute lobes or teeth. A set of 18 quantitative characters was chosen on the basis of published determination keys and floras (e.g. Kutzelnigg 1995, Meyer et al. 2005, Rich et al. 2010), including those used in our previous studies (Lepší et al. 2008, 2009, Vít et al. 2012). The characters are: ANG – angle between the third lateral vein from the laminar base and the midrib, ANT – length of anther, FL – length of fruit, FW – width of fruit, FWFL – ratio width of fruit/length of fruit, INC – incision between the second and the third lobe from the laminar base, length of lamina, LLLW – ratio length of lamina/width of lamina, LOW – width of the third lobe from the laminar base, LW – width of lamina, NL – number of lenticels per 25 mm^2 on fruit, NV – number of lateral laminar veins, PET – length of petiole, PL – length of petals, PW – width of petals, SL – length of sepals, SW – width of sepals, WP – widest width of lamina from the base. Two measures of each character per individual were recorded and the arithmetic mean was calculated and used as the value for the operational unit for an individual in all morphometric analyses.

Pearson correlation coefficients were calculated for pairs of characters for each species and for the whole data set in order to reveal the relationship among characters. It was necessary to exclude laminar length from subsequent analyses because of its high correlation with laminar width ($r > 0.95$). Principal component analyses (PCA) were performed to provide an insight into the overall pattern of morphological variation and show a potential separation of *S. danubialis* and *S. pauca*. Prior to PCA, the data were standardized to have a zero mean and unit standard deviation. Linear discrimination analyses (LDA), which maximize differences between a priori defined groups, were used to test the discriminating power of morphometric characters, following the methodology described by Lepš & Šmilauer (2003) using forward selection of characters with non-parametric Monte Carlo permutation tests (999 permutations; only axes with P-level < 0.05 were considered).

A cross-validated linear discriminant analysis based on probabilities using only characters selected as discriminating variables by the previous analysis was performed in R, version 2.12.2 (R Development Core Team 2011) using the lda function in the MASS package (Venables & Ripley 2002). PCA and LDA were carried out using Canoco (Lepš & Šmilauer 2003). Box-and-whisker plots of selected morphological characters of each species were carried out in Statistica version 9.1 (StatSoft, Inc. 2010). Univariate statistics (minimum, maximum, quartiles) of the quantitative characters of all the individuals of *S. pauca* collected were calculated and used in the description of the species.

Elliptic Fourier analysis was used to find differences in leaf shape of *S. pauca* and *S. danubialis*, based on the method of elliptic Fourier approximation (Kuhl & Giardina 1982) incorporated in the SHAPE 1.2 software package (Iwata & Ukai 2002). This procedure is described in Vít et al. (2012).

Karyology and DNA ploidy level estimation

For the purpose of counting chromosomes, four specimens from Bezděz and Malý Bezděz hills were collected in March 2010 and subsequently analysed using the procedure described in Lepší et al. (2008). DAPI flow cytometry was used to assess DNA ploidy levels (Suda et al. 2006) in *S. pauca* and *S. danubialis*. Bulked samples from 13 individuals of *S. pauca* and 10 of *S. danubialis* were analysed (i.e. three or four individuals simultaneously) following the methods of Lepší et al. (2008).

Mode of reproduction

To determine the mode of reproduction, embryo/endosperm DNA ploidy levels of seeds were analysed using the flow cytometric seed screening method of Matzk et al. (2000). In total, we analysed 40 seeds originating from 13 different individuals. Twelve seeds were analysed individually and then bulked samples of up to five seeds were used. Sample preparation followed the simplified two-step procedure (Doležel et al. 2007). Only the apical part (2–3 mm) of each seed, which contains the embryo, was used for nucleus isolation. The remainder of the seed, which contains mainly cotyledon tissue, was excluded. The apical part was cut up along with the internal standard (*Bellis perennis* leaf tissue) in 0.5 ml of ice-cold Otto I buffer. The suspension was filtered through a 42-µm nylon mesh and incubated for at least 10 minutes at room temperature. After incubation, 1 ml of the staining solution (Otto II buffer supplemented with 2 µl/ml of 2-mercaptoethanol and 4 µg/ml of the DAPI fluorochrome) was added. Samples were run on a Partec PA II flow cytometer after up to 10 minutes of staining and the fluorescence intensity of 5000 particles recorded. Resulting fluorescence histograms were analysed using FloMax 2.6 software (Partec GmbH, Germany) and the ratios between the mean fluorescence of the embryo, the endosperm and the internal standard recorded.

Results

Multivariate morphometric analyses

PCA revealed a distinct morphological difference between *Sorbus pauca* and *S. danubialis* (Fig. 1). The strongest contribution to the morphological distinctiveness of both species (i.e., the most strongly correlated characters with the first component axis) was the width of the lamina (LW), length of petiole (PET), incision between the second and third lobe from the laminar base (INC) and the length of lamina/width of lamina ratio (LLLW).

The LDA analysis also confirmed that these species are morphologically different. No overlap in the canonical scores of the species was detected. A forward selection procedure identified two characters (LLLW and INC) with a significant conditional effect and six other characters with significant marginal effects (LW, ANG – angle between the midrib and the third lateral vein from the laminar base, PET, FWFL – ratio width of fruit/length of fruit, LOW– width of the third lobe from the laminar base and NV – number of lateral veins per leaf). The species were well separated by the values of the two characters LLLW and INC with only a slight overlap in these values (Fig. 2). The cross-validated discriminant analysis using these two characters resulted in correct classification in all cases.

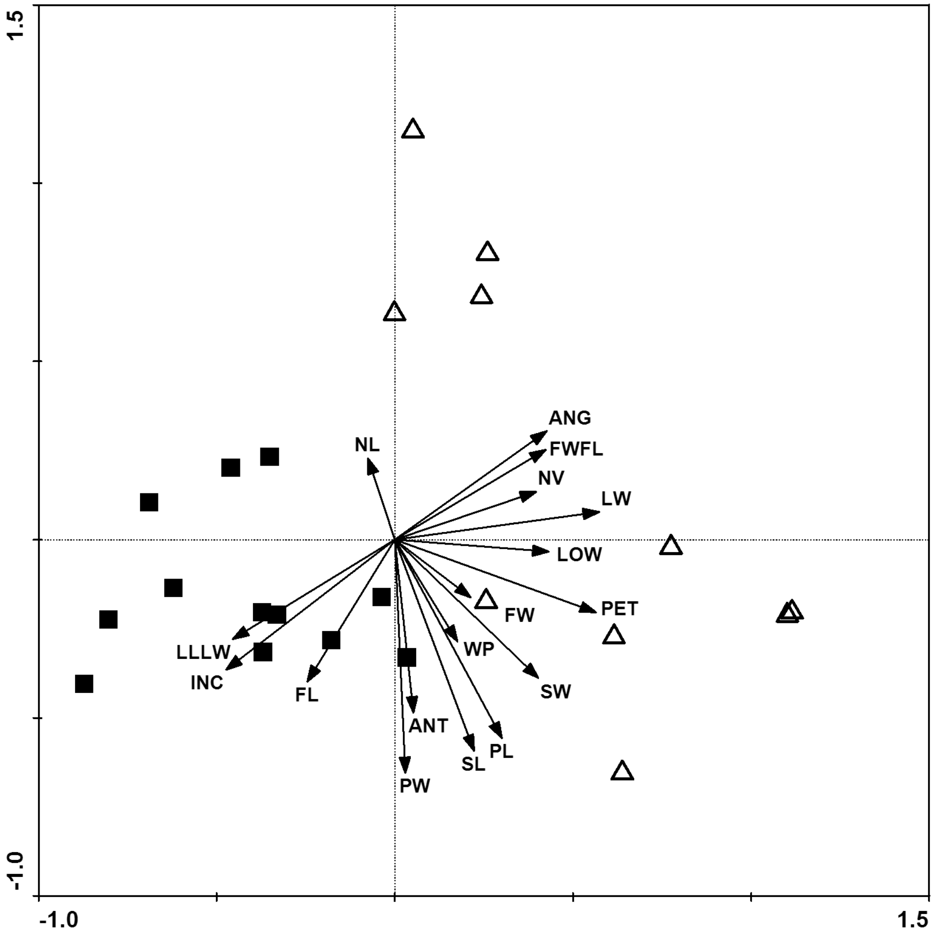

Fig. 1. – PCA of individuals of *Sorbus pauca* and *S. danubialis* using 17 characters. ■ *S. pauca*, △ *S. danubialis*. The first and the second ordination axes are depicted and account for 30.3% and 19.0% of the variation, respectively. See text for character codes.

Elliptic Fourier analysis of leaf laminas

Principal component analysis (PCA) performed on standardized Fourier coefficients revealed distinct differences between the species studies (Fig. 3). A trend in shape associated with the first principal component separates the two species based on the width of the lamina; *S. pauca* has oblong obovate leaves, whereas those of *S. danubialis* are more or less round rhomboidal. Other axes did not facilitate the differentiation between the two taxa (Fig. 3). Only the first two PCA axes were found to improve significantly the discriminant power of the LDA during forward selection. A cross-validated discriminant analysis, which was performed on the principal component scores of the first and second axis, confirmed the classification in all cases.

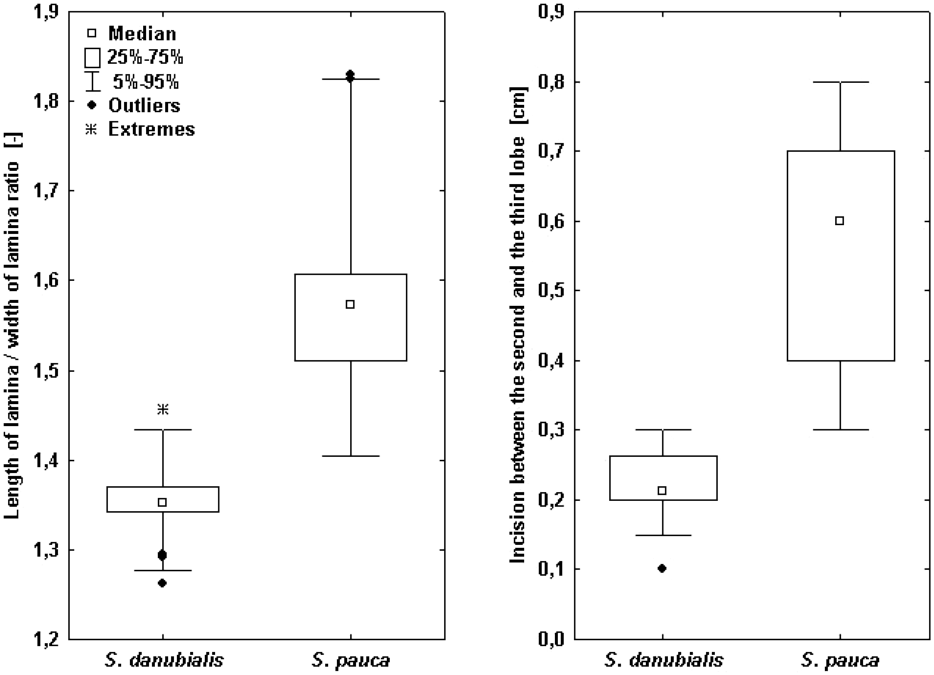

Fig. 2. – Box-and-whisker plots of LLLW (leaf length/width ratio) and INC (incision between the second and the third lobe from the lamina base) characters of *Sorbus danubialis* and *S. pauca*. Only these two characters, which were identified by a forward selection procedure, have significant discriminating power in the linear discriminant analysis.

Chromosome variation and ploidy level

Somatic cells of *S. pauca* have a tetraploid chromosome number (2n = 4x = 68, Fig. 4). No intra-specific variation within *S. pauca* and *S. danubialis* was detected by DAPI flow cytometry and both species are tetraploids. The sample/standard ratio was 0.78 for *S. pauca* (average coefficient of variation for samples: 3.36 and the standard: 2.42) and 0.74 for *S. danubialis* (average CV of samples: 2.77 and standard: 1.82).

Mode of reproduction

The DNA ploidy level of the embryo is tetraploid in all the seeds analysed, the embryo/standard ratio of DAPI fluorescence was 0.78. The endosperm/embryo ratio of DAPI fluorescence was 2.95–3.03, which corresponds closely with the value of 3.00 expected for a 2C (tetraploid) embryo and 6C (dodecaploid) endosperm, most likely consisting of two unreduced maternal chromosome complements and one unreduced paternal chromosome complement. We therefore conclude that *S. pauca* is an apomictic tetraploid with an endosperm formed through pseudogamy via unreduced (tetraploid) pollen.

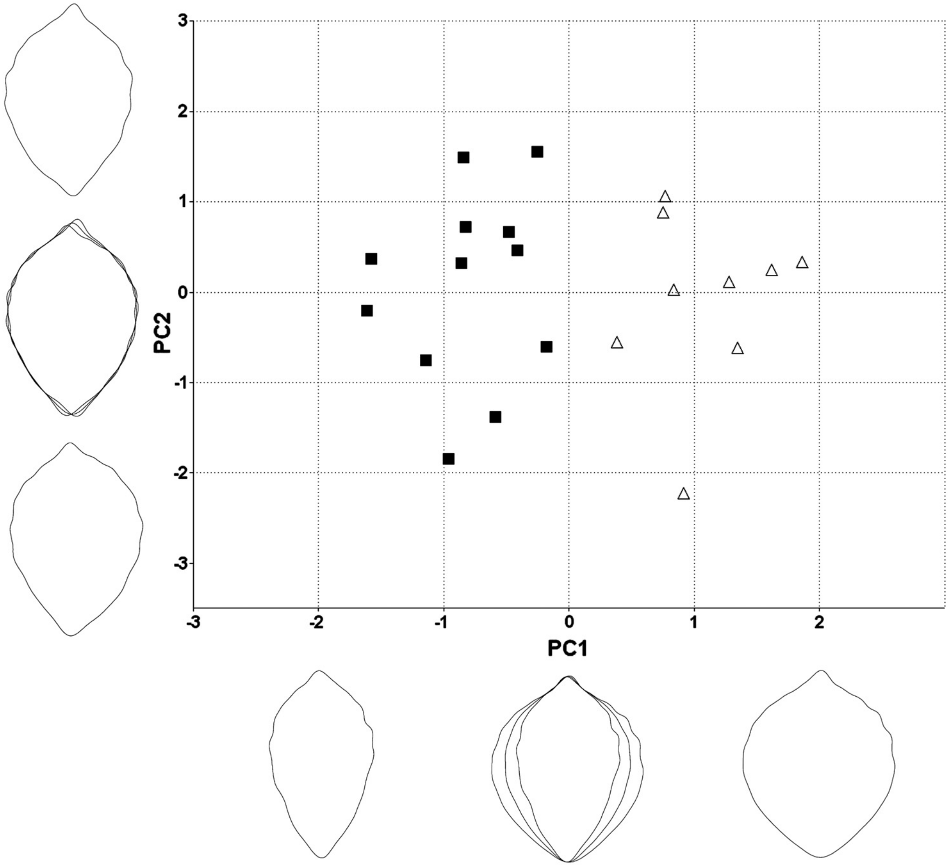

Fig. 3. – PCA of Fourier coefficients describing the total leaf lamina shape of ■ *Sorbus pauca* and △ *S. danubialis*. The first and second ordination axes are displayed and account for 71.1% and 7.6% of the overall variation, respectively. Reconstructed contours corresponding to the –2 and +2 SD positions on both axes are visualized along the axes (the scale of the plot is in SD units). In the middle, these two contours overlap the mean leaf shape (corresponding to the [0.0] point of the plot).

Sorbus pauca M. Lepší et P. Lepší **spec. nova** (Figs 5–7)

Diagnosis: Frutices humiles usque 4 m alti; foliis (in brachyblastis sterilibus) simplicibus, laminis ambitu conspicue polymorphis, i. e. obovatis, oblonge obovatis vel ellipticis et saepe in parte inferiore usque centrali paulo incisis, irregulariter pinnato-lobatis, in parte superiore tantum duplicato-dentatis, margine undulatis, parvis, (62–) 70–76 (–97) mm longis et (38–) 45–50 (–59) mm latis, (1.4–) 1.5–1.6 (–1.8) plo longiores quam latiores, basi cuneatis, apice plus minusve acutis usque acuminatis, subtus griseo-viride tomentosis, lobis parvis, obtusis, variabiles in magnitudine et forma, contingens usque partim coincidens, incisionibus inter secundis et tertis lobis (2–) 4–7 (–10) mm, petiolis (9–) 10–12 (–17) mm longis; fructibus subglobosis, (11–) 12 (–13) mm longis et (12–) 13–14 mm latis, (0.85–) 0.91–0.93 (–0.98) plo longiores quam latiores, maturitate rubris, cum (1–) 2–3 (–5) lenticellis parvis ad 25 mm^2. Numerus chromosomatum tetraploideus 2n = 68. Planta apomicta.

Holotypus: Bohemia septentrionalis, distr. Česká Lípa, pagus Bezděz (5454c): in rupe phonolithica sub cacumine collis Bezděz; 580 m s. m., 50°32'23.3"N, 14°43'18.0"E; raro; frutex ca. 2.5 m altus; 23. 7. 2011 leg. M. Lepší et P. Lepší; CB, No. 79599 (Fig. 5). – Isotypus: PR, 79599a.

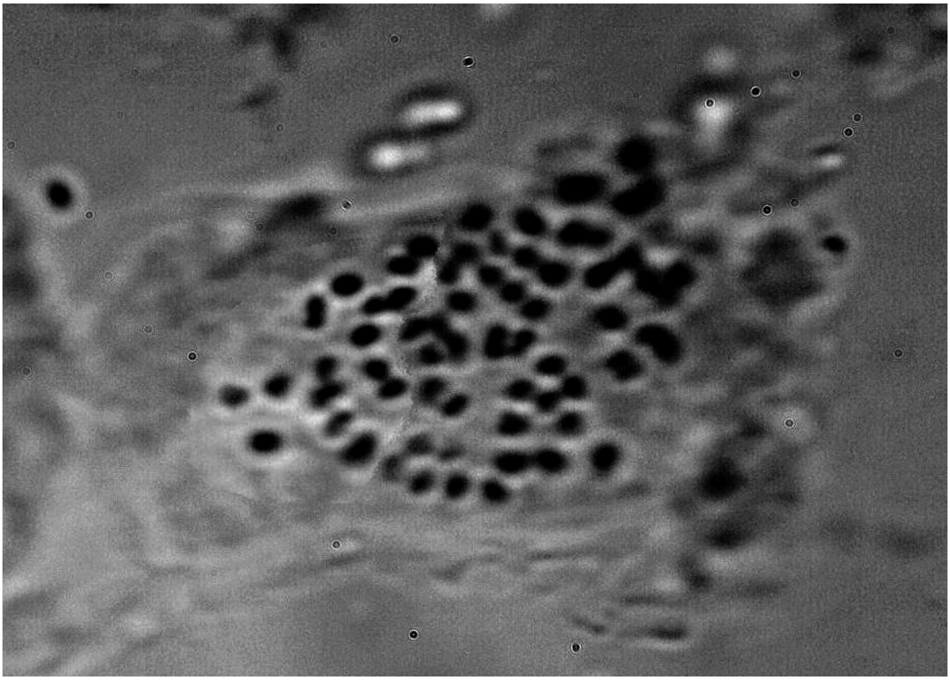

Fig. 4. – Microphotograph of the somatic chromosomes (2n = 4x = 68) of *Sorbus pauca* from Malý Bezděz hill (photo V. Jarolímová).

Description: Shrubs up to 4 m high. Bark grey, with scattered lenticels. Twigs brownish-grey; young shoots brown, sparsely tomentose when young and almost glabrous at maturity, with elliptical or sub-rotund pale brown to ochraceous lenticels. Buds 5.5–9.0 mm long and 3.0–4.5 mm wide, ovoid; scales brownish green, with brown sparsely tomentose margins. Leaves (in the centre of short sterile shoots) simple; laminas conspicuously polymorphic, obovate, oblong-obovate or elliptical, with sides in the lower part often concave, somewhat glossy, dark green above, greyish-green beneath, undulate at margins, more or less acute to acuminate at apex, cuneate and partly serrate at base, almost glabrous on upper surface, evenly tomentose on lower surface, (62–) 70–76 (–97) mm long and (38–) 45–50 (–59) mm wide, (1.4–) 1.5–1.6 (–1.8) times as long as wide, widest at (29–) 33–40 (–47)% of the laminar length (from the tip), irregularly shallowly lobed, double dentate apically with more or less outwardly directed teeth; lobes small, obtuse, often overlapping each other, serrate or doubly serrate with acute to obtuse, outwardly directed teeth terminating the main veins, other teeth usually smaller, acute, forwardly directed; sides of lobes more or less arcuate; the third lobe (from the base) (7–) 8–11 (–14) mm broad; some opposite lobes unequal in size and shape owing to asymmetrically pinnate (curved, forked or even crossed lateral veins); incision between the second and the third lobe (2–) 4–7 (–10) mm; lobes broader than 10 mm (0–) 1–2 (–4) on each side; lateral veins (7–) 9 (–11) on each side, at an angle of (27–) 34–37 (–44)° to midrib at centre of the leaf; petioles (9–) 10–12 (–17) mm long, tomentose. Inflorescences convex; branchlets more or less tomentose. Hypanthium turbinate, tomentose. Sepals (2.4–) 2.9–3.3 (–4.1) mm long and (2.4–)

HERBARIUM MUSEI REGIONALIS BOHEMIAE MERIDIONALIS
ČESKÉ BUDĚJOVICE

Flora: Bohemia septentrionalis

HOLOTYPUS!
No. **CB 79599**

Sorbus pauca M. Lepší et P. Lepší

Habitat

Distr. Česká Lípa, pagus Bezděz (5454c): in rupe phonolithica sub cacumine collis Bezděz; 580 m s. m., 50°32'23.3"N, 14°43'18.0"E; raro; frutex ca. 2.5 m altus.

Die: 23. 7. 2011

Legit: M. Lepší et P. Lepší

Fig. 5. – Holotype of *Sorbus pauca*.

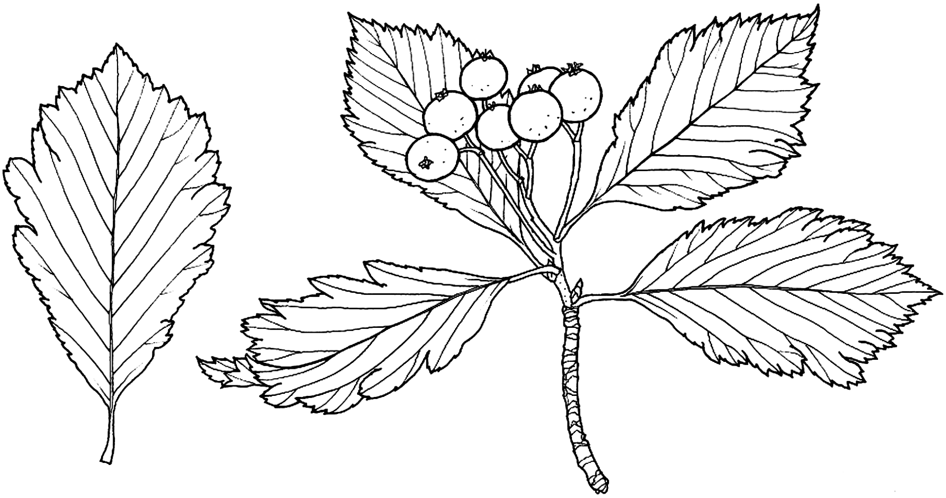

Fig. 6. – *Sorbus pauca*: short fructiferous shoot (right) and leaf from the centre of a short sterile shoot (left). Drawing by A. Skoumalová.

Fig. 7. – Short fertile shoot of *Sorbus pauca* growing on Malý Bezděz hill (photo P. Lepší 2009).

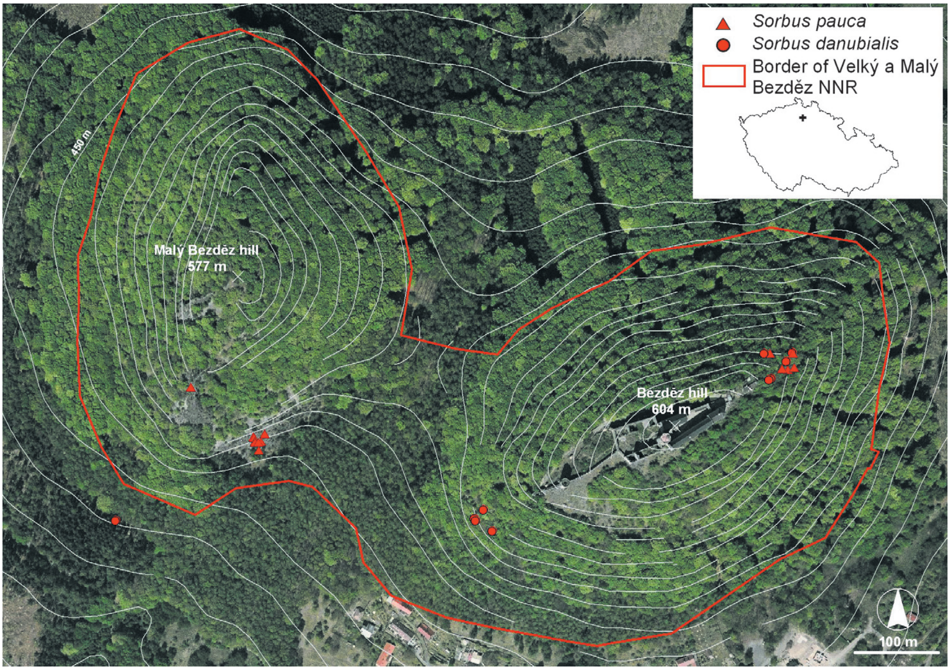

Fig. 8. – Map showing the distribution of *Sorbus pauca* and the individuals of *S. danubialis* sampled (the use of the map was approved by the Ministry of the Environment of the Czech Republic).

2.9–3.3 (–3.5) mm wide, triangular, acuminate, tomentose on both surfaces, patent, persistent, dry, erect. Petals (5.7–) 6.5–7.4 (–8.4) mm long and 4.9–5.3 (–5.7) mm wide, broadly ovate to broadly elliptical, concave, whitish, patent, sparsely hirsute at base of upper surface, with a short claw. Stamens ca 20; filaments whitish; anthers pale yellow to pale rose, 1.3–1.5 (–1.7) mm long. Ovary semi-inferior. Styles 2 (–3), greenish-cream, connate and hairy at base. Stigma greenish-cream, more or less flat. Fruit (11–) 12 (–13) mm long and (12–) 13–14 mm wide, (0.85–) 0.91–0.93 (–0.98) times as long as wide, sub-globose, red at maturity, sparsely tomentose to almost glabrous, matt, with (1–) 2–3 (–5) ochraceous lenticels per 25 mm^2; mesocarp heterogeneous; endocarp cartilaginous. Seeds fuscous. Somatic chromosome number $2n = 68$ (tetraploid). Reproduction apomictic. Flowering V.

Distribution and population size

Sorbus pauca occurs on Bezděz and Malý Bezděz hills near the town of Doksy in northern Bohemia (Fig. 8). At these sites, which are ca 0.6 km apart, five and nine mature individuals were found, respectively. We did not record any seedlings at the localities because their determination is problematic. The age of older individuals cannot be determined because of their clonality (re-sprouting from the base). The population on Bezděz hill occurs on a large rock with relict vegetation. Nine individuals on Malý Bezděz hill occur in an abandoned quarry, except for one shrub growing on rock at the summit. The altitudinal range of the species spans from 440 to 580 m a.s.l. In terms of Czech phytogeography and climatol-

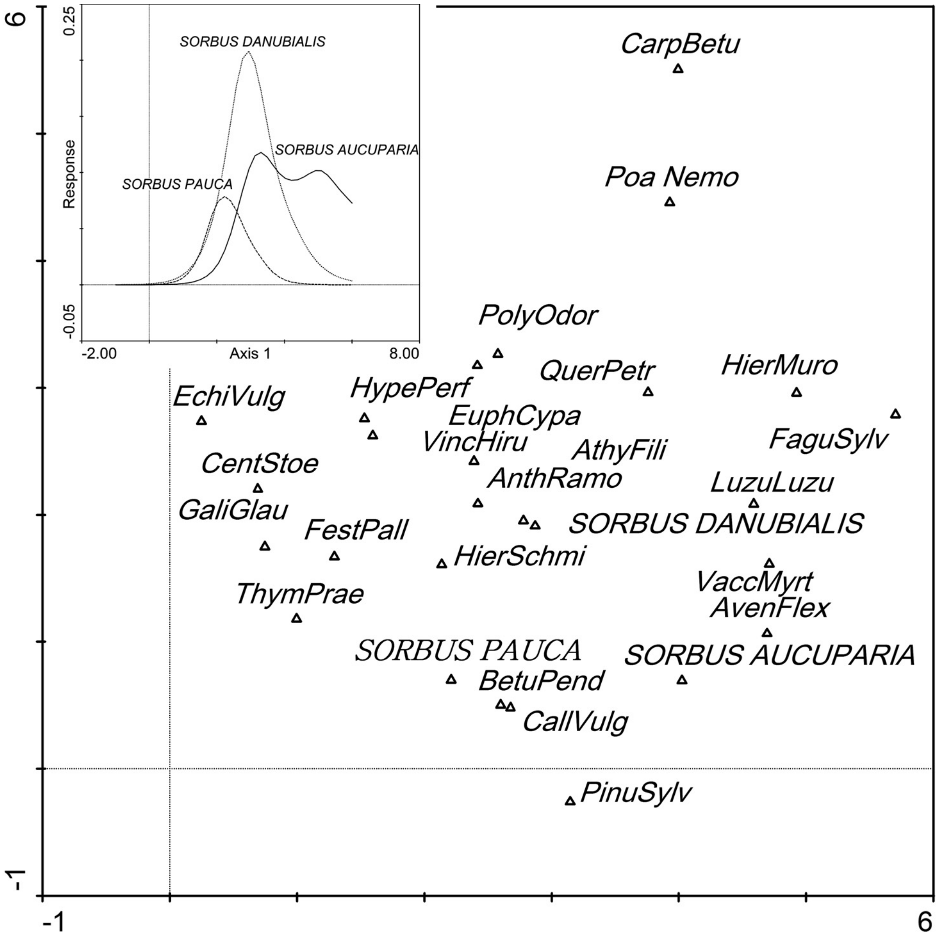

Fig. 9. – DCA diagram depicting the position of species best fitting the ordination axes. The diagram was constructed using 18 of our own and 60 published relevés of vegetation where *Sorbus pauca* or its parental species occur at the study locality (Bezděz and Malý Bezděz hills). The first and second DCA axes account for 10.4% and 5.2% of the variability in species composition, respectively. The picture in the upper left corner shows the species response curves for the three *Sorbus* species based on the generalized additive model (GAM) along the first DCA axis with Poisson distribution. The abbreviations correspond with these names: *Anthericum ramosum*, *Athyrium filix-femina*, *Avenella flexuosa*, *Betula pendula*, *Calluna vulgaris*, *Carpinus betulus*, *Centaurea stoebe*, *Echium vulgare*, *Euphorbia cyparissias*, *Galium glaucum*, *Hieracium murorum*, *H. schmidtii*, *Fagus sylvatica*, *Festuca pallens*, *Hypericum perforatum*, *Luzula luzuloides*, *Pinus sylvestris*, *Poa nemoralis*, *Polygonatum odoratum*, *Quercus petraea*, *Thymus praecox*, *Vaccinium myrtillus*, *Vincetoxicum hirundinaria.*

ogy, the hills belong to the supracolline vegetation belt (Chytrý 2012), the phytogeographical district Ralsko-bezdězská tabule (Skalický 1988) and a moderately warm climatic region (Quitt 1971) with a mean annual temperature of about 7–8 °C and mean annual precipitation of 600–650 mm (Tolasz et al. 2007).

Herbarium specimens:

Czech Republic. Northern Bohemia, 52. Ralsko-bezdězská tabule/table, CEBA 5454c: 1. Bezděz hill: Bezděz village, a protruding rock ridge on NE slope of Bezděz hill, low shrubs, three individuals, 50°32'23.1"N, 14°43'18.2"E, 560 m a.s.l. (leg. M. Lepší & P. Lepší 27. 8. 2009, CB 73616, CB 73617). – Bezděz village, a rocky slope connected with the summit castle area of Bezděz hill, one shrub, 50°32'23.9"N, 14°43'17.3"E, 560 m a.s.l. (leg. M. Lepší & P. Lepší 26. 3. 2010, CB 79422). **2. Malý Bezděz hill:** Bezděz village, lower parts of walls of abandoned quarry on the SE slope of Malý Bezděz hill, 5 shrubs ca 1 m high, 50°32'18.3"N, 14°42'51.6"E, 440 m a.s.l. (leg. M. Lepší & P. Petřík 20. 8. 2009, CB 73768). – Bezděz village, the upper parts of walls of abandoned quarry on the SE slope of Malý Bezděz hill, several shrubby individuals, 50°32'18.9"N, 14°42'52.1"E, 450 m a.s.l. (leg. M. Lepší & P. Lepší 28. 8. 2009, CB 73618). – Bezděz village, the bottom of an abandoned quarry on the SE slope of Malý Bezděz hill, 1 shrub ca 3 m high, 50°32'18.3"N, 14°42'51.6"E, 440 m a.s.l. (leg. M. Lepší & P. Petřík 20. 8. 2009, CB 73767; leg. M. Lepší & P. Lepší 23. 3. 2010, CB 79421; leg. P. Lepší & M. Lepší 16. 5. 2011, CB 79600, PRC, PRA, LI).

History of finds

We did not find any herbarium specimens belonging to *S. pauca* in any of the herbarium collections studied. Klika (1937) reports *S. franconica* (a hybridogenous species of the presumed parental combination *S. aria* agg. × *S. torminalis*) from Bezděz hill, but both the specimens that this record is based on refer to *S. danubialis* (Lepší et al. 2009). The first known record of this species was therefore, reported by J. Sádlo in 2008.

Ecology

A striking feature of the localities investigated is the spatial heterogeneity of habitats caused by local differences in the relief and diversity of microclimates, substrate properties and current vegetation. This heterogeneity allows the *Sorbus* species studied to co-occur despite their different habitat requirements.

Populations of *Sorbus pauca* inhabit dry phonolite cliffs and stony slopes. The species occurs mainly in sparse xerophilous vegetation consisting of scattered woody plants such as *Betula pendula*, *Calluna vulgaris*, *Cotoneaster integerrimus*, *Juniperus communis* and *Pinus sylvestris*, and low herbaceous plants such as *Festuca pallens*, *Hieracium schmidtii*, *Thymus praecox* and *Vincetoxum hirundinaria* (see Electronic Appendix 1). In phytosociologal terms, the vegetation belongs to the units *Alysso-Festucion pallentis* Moravec in Holub et al. 1967 (grassland), *Berberidion vulgaris* Br.-Bl. ex Tüxen 1952 (scrub) and *Festuco-Pinion sylvestris* Passarge et Hofmann 1968 (sparse woodland). Both parental species of *S. pauca* also occur abundantly in these habitats, but their environmental range is broader (Fig. 9). Whereas *S. danubialis* resembles *S. pauca* in its range of habitats, the second ancestor, *S. aucuparia*, is more mesophilous and tolerant of shade and plentiful nutrients.

Discussion

Phenotypic variation, diagnostic characters and the origin of this species

Individuals of *S. pauca* are morphologically homogeneous in both vegetative and generative characters and produce fully developed seeds. Plants intermediate between *S. pauca* and supposed parental species have not been recorded.

Considering the principal morphological features of this species (i.e. oblong-obovate leaves with obtuse lobes), we conclude that it belongs to the *S. hybrida* group and its most probable ancestors are *S. danubialis* of the *S. aria* group and *S. aucuparia*, which are the only native *Sorbus* species occurring at present at the localities studied and in the adjacent area of northern Bohemia.

Sorbus pauca differs markedly from other European species of the *S. hybrida* group in having small undulating leaves with irregular shallow (but distinct) lobes and average sized fruit with scattered lenticels. The leaves of related species is either partly pinnate (e.g. *S. borbasii* Jávorka, *S. hybrida*, *S. meinichii* Hedl., *S. pseudofennica* E. F. Warb.) or deeply lobed (e.g. *S. arranensis* Hedl., *S. austriaca*, *S. dacica*, *S. pulchra* N. Mey., *S. scepusiensis* Kovanda or *S. schwarziana* N. Mey.). Other species with comparably shallow lobes have larger leaves or smaller fruit (e.g. *S. anglica* Hedl., *S. cuneifolia* T. C. G. Rich, *S. minima* (Ley) Hedl., *S. mougeotii*). The few taxa reported from Hungary and adjacent regions (such as *S. buekkensis*, *S. hungarica*, *S. javorkae* or *S. velebitica*) have indistinctly lobed leaves and therefore seem to belong to the *S. aria* group rather than the *S. hybrida* group.

Sorbus pauca more closely resembles *S. danubialis* than *S. aucuparia* in the above mentioned characters. *Sorbus danubialis* is characterized by rhomboidal to round rhomboidal leaves, with a coarsely double serrate to indistinctly lobed margin to the upper part of the lamina, with acute lobes or main teeth and average sized fruit with scattered lenticels. Many species of the *S. hybrida* group resemble *S. aucuparia* in having pinnatifid to pinnatisect leaves and small fruit with few or no lenticels. We assume that the absence of these characters reflects an increased portion of genetic information from *S. danubialis* in the genome of the new species.

The ploidy level of *S. pauca* seems to support this concept. *Sorbus pauca* most probably arose from two hybridization events. A triploid hybrid could have arisen from a cross between the tetraploid *S. danubialis* and diploid *S. aucuparia* and subsequently a reduced diploid gamete of this triploid ancestor fused with a reduced diploid gamete of *S. danubialis* giving rise to a tetraploid species. Another hypothetical option concerns direct hybridization of an unreduced gamete of *S. aucuparia* with a reduced gamete of *S. danubialis*, which, considering the lack of information about the production of unreduced gametes in *S. aucuparia* is less probable. Also the morphology of *S. pauca* indicates that the contribution from *S. aucuparia* is less than one half of the genome.

The historical context of the occurrence of this species

In addition to *Sorbus pauca*, a considerable number of endemic plants occur in the Doksy region. *Dactylorhiza bohemica* (Businský 1989) and *Pinguicula vulgaris* subsp. *bohemica* (Kubát et al. 2002) occur in local fens (the latter also previously occurred in fens by the river Elbe ca 45 km away). Pale-flowered and small-leaved populations of *Pulsatilla pratensis* occurring in dry calcic soils in pine forests were named as var. *albida* (Domin) Skalický (Skalický 1988). *Potentilla psammophila* Soják is a recently missing hybridogeneous species of the *P. collina* group (Soják 2009). Local populations of *Minuartia cespitosa* differ considerably from other populations in terms of the physiology of the individual plants, which can grow in calcic soils, whereas those of other populations are associated with metaliferous soils where calcium carbonate is almost absent (Brooks

1987). In addition, two species forming peculiar local populations are restricted to hills of volcanic origin, including Bezděz hill, namely *Cardaminopsis petraea* (rosy flowered plants with a pilose stem, Měsíček et al. 1992) and *Viola tricolor* subsp. *polychroma* (plants with large blue-violet flowers lacking a yellow tint, Kirschner & Skalický 1990).

This endemism might be due to the stability of the vegetation in the area in which the biome of boreal forests (lowland taiga) persisted since the early Holocene (Chytrý 2012, Novák et al. 2012). In the landscape surrounding the Doksy region, the vegetation has undergone major changes. Relatively stable conditions, however, may have persisted on the rocks of the Bezděz hills and some other volcanic hills in the region. Our hypothesis that the rocks were not forested throughout the Holocene is supported by the local occurrence of presumably relict species such as *Allium strictum*, *Arctostaphylos uva-ursi*, *Aster alpinus*, *Calamagrostis varia*, *Dianthus gratianopolitanus*, *Festuca pallens* and *Hieracium schmidtii*. These species are unable to spread either under a forest canopy or in open habitats of a cultural landscape, and their survival is dependent on harsh conditions on open rocks.

The picturesque cultural history of the Bezděz hills (Žemlička 1980, Durdík 1999) sharply contrasts with the relic character of their rocky slopes and the general stability of the vegetation in the region. For at least eight centuries, stable conditions in natural habitats alternated in space and time with human-made stages of disturbances, abandonment and repeated afforestation.

Summits of hills were undoubtedly utilized as partly deforested lookouts since ancient times. The establishment of the monarch's church of St. Aegidius in the foothills implies an early colonization during the middle ages. During the 13th century, construction of the king's castle Bezděz, a fortification on Malý Bezděz hill and a generous but utterly failed attempt to turn the village into a town resulted in extensive deforestation and, without doubt, also soil erosion on both hills although the steepest slopes were probably never deforested. Both hills were grazed to different extents by goats and sheep until the first half of the 20th century. Between the 16th and 18th centuries, several attempts to restore the castle, which was later used as a monastery, were interrupted by repeated fires and vandalism. The ruins were repaired in several stages after the beginning of the 19th century (Flegl 1983). A phonolite quarry, where *S. pauca* currently occurs, was opened on the south-eastern slope of Malý Bezděz hill no later than the beginning of the 19th century. In the last hundred years, hill slopes covered by sparse vegetation, with clusters of trees and shrubs, developed into a closed forest with some rocky patches. It is possible that this decline in the extent of open rocky habitats is the main cause of the current scarcity of *S. pauca*. Though *S. pauca* has survived the intensive human pressure at the Bezděz hills, it is absent on roughly twenty similar, but more natural, volcanic hills in a radius of 20 km. It is therefore probable that the current distribution of *S. pauca* is not a remnant of a previous (e.g. early Holocene) wide distribution. Rather, it might be a historically young species that never managed to spread. Like its parents, the species is a weak competitor against trees and only manages to survive owing to human-made disturbances, which reduce the competition and release nutrients. Conversely, the population of this species could have been reduced considerably by the construction of the castle or intensive grazing in the past. Besides its biological value (e.g. as an example of stenoendemism, see Kaplan 2012), this species might also have a cultural value as a possible unforeseen consequence of the effect of humans on the Bezděz hills.

Conservation

All specimens were found within the area of the Velký a Malý Bezděz National Nature Reserve, which ensures their protection. Current human activities do not seem to threaten directly this species except for the occasional cutting of shrubs. Conversely, the population is so sparse that it may be endangered even by a random event such as a rock fall or a long term process such as succession resulting in the growth of tall shrubs or trees. Therefore the reproduction and establishment of the species should be supported by partial removal of surrounding woody vegetation. The species should be included among the critically endangered plants of the Czech flora (C1; sensu Grulich 2012) and critically endangered species (status criteria B2a; D) according to the IUCN (2001) and it seems to be a very good candidate for *ex situ* conservation in a seed bank and/or a botanical garden.

See www.preslia.cz for Electronic Appendix 1

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Souhrn

Nově rozeznaný jeřáb bezdězský (*Sorbus pauca*) je endemitem dvou blízkých vrchů, Bezdězu a Malého Bezdězu v Ralsko-bezdězské pahorkatině. Je to hybridogenní apomiktický tetraploidní (2n = 4x = 68) druh z okruhu *S. hybrida* agg., do nějž jsou řazeni potomci křížení *S. aria* agg. a *S. aucuparia*. Z tohoto okruhu byly doposud v České republice známy dva nepůvodní druhy, *S. austriaca* a *S. mougeotii*, ale žádný druh původní. Na příslušnost k okruhu *S. hybrida* ukazuje podlouhle obvejčitý obrys a zaokrouhlené (tupé) laloky listové čepele. Od ostatních evropských druhů tohoto okruhu se *S. pauca* odlišuje poměrně drobnými listovými čepelemi s nepravidelně vyvinutými a mělkými, avšak dobře patrnými laloky a středně velkými plody s roztroušenými lenticelami. Druhým rodičovským taxonem je pravděpodobně *S. danubialis*, který se vyskytuje na obou lokalitách společně s novým druhem. *Sorbus danubialis* má na rozdíl od *S. pauca* kosočtverečné až zaokrouhleně kosočtverečné čepele listů, které jsou v horní polovině zastřihovaně dvojitě pilovité, někdy až mělce laločnaté, laloky či hlavní zuby jsou špičaté nikoliv tupé. Vysoká podobnost obou druhů nás vede k hypotéze, že *S. pauca* vznikl zpětným křížením hybrida *S. aucuparia* × *S. danubialis* s druhem *S. danubialis*. *Sorbus pauca* je morfologicky i karyologicky homogenní a morfologicky dobře vymezený od *S. danubialis* i od ostatních v Evropě doposud popsaných taxonů jeřábů. Druh je vázán na skalnaté biotopy (trávníky, křoviny, lesy) s *Festuca pallens* a *Cotoneaster integerrimus*. Z lokality jsou známy dvě populace se 14 plodnými jedinci jeřábu bezdězského. Vzhledem k jeho malé početnosti a tudíž pravděpodobnému ohrožení ho navrhujeme zařadit do kategorie kriticky ohrožených druhů cévnatých rostlin (C1 podle Grulich 2012).

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