There is no diploid apomict among Czech *Sorbus* species: a biosystematic revision of *S. eximia* and discovery of *S. barrandienica*

Mezi českými jeřáby se nevyskytuje diploidní apomikt – biosystematická revize *Sorbus eximia* a objevení *S. barrandienica*

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Sorbus eximia Kovanda, a hybridogenous species that originated from the parental combination *S. torminalis* and *S. aria* s.l., is thought to be an apomictic species, which includes diploid and tetraploid individuals. The present study confirmed the existence of only triploid individuals. A new tentatively apomictic triploid (2n = 3x = 51) species from the *S. latifolia* group: *S. barrandienica* P. Vít, M. Lepší et P. Lepší is described based on a revision of *S. eximia*. This species is assumed to have originated from a cross between *S. danubialis* or *S. aria* s.l. and *S. torminalis*. A wide palette of biosystematic techniques, including molecular (nuclear microsatellite markers) and karyological analyses (chromosome counts, DAPI flow cytometry) as well as multivariate morphometric and elliptic Fourier analyses, were used to assess the variation in this species and justify its independent taxonomic status. Allopatric occurrences of both species were recorded east of the town of Beroun in the Český kras, central Bohemia (Bohemian Karst). A distribution map of the two species is provided. *Sorbus eximia* occurs at four localities (the total number of adults and juveniles is 100 and 200, respectively) in basiphilous thermophilous oak forests (*Quercion pubescenti-petraeae*), mesic oak forests (*Melampyro nemorosi-Carpinetum*), woody margins of dry grasslands (*Festucion valesiacae*) and pine plantations. *Sorbus barrandienica* has so far been recorded at 10 localities (ca 50 adults). Recent field studies failed to verify two of these localities. It is mainly found growing on the summits of hills, usually in thermophilous open forests (*Primulo veris-Carpinetum*, *Melampyro nemorosi-Carpinetum*, *Quercion pubescenti-petraeae*) and woody margins of dry grassland. Its populations exhibit minimal genetic variation and are phenotypically homogeneous and well separated from other Bohemian hybridogenous *Sorbus* species. The epitype of *S. eximia* is designated here, and a photograph of the specimen is included. Photographs of the type specimens and in situ individuals, and line drawings of both species are presented.

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

Introduction

In recent years, taxonomic research on agamospermous groups in the Czech Republic has led to the description of many new taxa. This especially applies to the genera *Taraxacum* (e.g. Øllgaard 2003, Vašut & Trávníček 2004, Vašut et al. 2005, Trávníček et al. 2008) and

Rubus (e.g. Zieliński & Trávníček 2004, Trávníček et al. 2005, Trávníček & Zázvorka 2005, Žíla & Weber 2005, Lepší & Lepší 2006, 2009, Žíla 2009). A recent increase in taxonomic novelties is also recorded in the genus *Sorbus* s.l. Three new species of the *S. latifolia* group – *S. milensis*, *S. albensis* and *S. portae-bohemicae* – have been described from the České středohoří hills in northern Bohemia. The finding of scattered plants recognized as *S. milensis* during extensive floristic research prompted a subsequent detailed taxonomic revision of *S. bohemica*, which in addition resulted in distinguishing two new species, *S. albensis* and *S. portae-bohemicae* (Lepší et al. 2008, 2009). Over the last few years, taxonomic research has focused on another apomictic species – *S. eximia* Kovanda.

Sorbus eximia was described in 1984 as a hybridogenous species endemic to the Český kras karst (Kovanda 1984). Subsequent chemotaxonomical research indicated that it most probably originated from a back-cross between the F1 hybrid *Sorbus torminalis* × *Sorbus aria* s.l. and *Sorbus aria* s.l. (Challice & Kovanda 1986). The species was subsequently studied embryologically and was presented as an apomict comprising two cytotypes: diploid and tetraploid, combined with apospory and diplospory (Jankun & Kovanda 1988). The most surprising result of that study is the discovery of apomixis at the diploid level. Apomixis in *Maloideae* is, with a few exceptions, associated with polyploidy (Campbell & Dickinson 1990). The only reported cases of diploid apomicts are some individuals of *Crataegus calpodendron* (Ehrh.) Medikus, one individual of an apple cultivar, *Sorbus eximia* and possibly *S. subfusca* Boiss. (Campbell et al. 1991). Jankun & Kovanda (1988) found diploid individuals of *S. eximia* at a single locality nearby the settlement of Koda and observed that diploids had leaf laminas more obtusely and more shallowly lobed than tetraploids.

Between 2004 and 2005, a revision of the morphological, karyological and genetic variation in *S. eximia* over its entire distribution area revealed new facts (Vít 2006) that affirmed the karyological results obtained by Jankun & Kovanda (1988). By contrast, morphometrics and molecular techniques confirmed the unique character of the Koda population (Vít 2006), which called for a new taxonomical evaluation of the species. Our additional studies of the type material indicated that the name *S. eximia* relates to the Koda morph and that the remainder of the known populations belong to a new taxon yet to be described. Detailed field work in 2009 and a revision of voucher specimens in major Czech herbaria revealed or confirmed four localities for *S. eximia* and 10 for the new apomictic taxon.

This paper presents a formal description of the newly delimited taxon based on the results of field observations, molecular analyses, karyology, multivariate morphometric and elliptic Fourier analyses. Furthermore, an epitype of *S. eximia* is designated, and its description is revised and emended here. An updated distribution map and a list of revised herbarium specimens of both taxa are also provided.

Material and methods

Plant material and field work

Mature and well developed individuals were selected for the study of phenotypic and genetic variation. For molecular analyses (nuclear microsatellite markers), 12 individuals of *S. eximia* and 10 of *S. barrandienica* were sampled. For the multivariate morphometric

analyses, 45 individuals of *S. eximia* and 19 of *S. barrandienica* were used (see Table 1 for locality details). In addition, six other hybridogenous taxa of the *S. latifolia* group occurring in Bohemia, which are closely related to the *Sorbus* species currently under study, were included in the multivariate morphometric analyses in order to assess phenotypic variation within the group and determine species-specific characters. These were *S. albensis* (84), *S. bohemica* (111), *S. gemella* (10), *S. milensis* (15), *S. portae-bohemicae* (13) and *S. rhodanthera* (12) (see Lepší et al. 2008, 2009 for locality details). Elliptic Fourier analysis was carried out to reveal species-specific characters of leaves of *S. eximia* (96 leaves analysed) and *S. barrandienica* (93) and also of the holotype of the name of *S. eximia* (see Table 1 for locality details). Specimens were collected during 2004–2009 following the recommendations of Kutzelnigg (1995) and Meyer et al. (2005), for details see Lepší et al. (2009). To describe the phytosociological affinities of *S. eximia* and *S. barrandienica*, relevés were recorded in subjectively selected plots using the Braun-Blanquet approach. The relevés are stored in the Czech National Phytosociological Database (Chytrý & Rafajová 2003) under the numbers 348308, 203571–203584. Altitudes and geographic coordinates (WGS-84) were determined using Garmin eTrex and GPSmap 60CSx instruments.

Table 1. – Details of the localities of *Sorbus* species included in the morphometric, molecular and ploidy level analyses.

Locality	Geographic coordinates	Altitude (m a.s.l.)	Number of individuals analysed			
			Nuclear microsatellite markers	Classical morphometry	Elliptic Fourier analysis	Ploidy level
Taxon *S. eximia*						
Koda hill near Srbsko	49°56'03.6"N, 14°07'09.5"E	360–370	12	45	15	73
Kotýz prehistoric settlement near Tmaň	49°54'57.5"N, 14°02'56.5"E	390	–	–	10	–
Taxon *S. barrandienica*						
Paní hora hill near Bubovice	49°57'43.1"N, 14°09'52.3"E	410	3	8	5	5
Mokrý vrch hill near Bubovice	49°57'22.2"N, 14°09'44.2"E	390	–	–	1	–
Doutnáč hill near Srbsko	49°57'23.5"N, 14°09'09.5"E	430	–	1	3	1
Haknová hill near Karlštejn	49°56'15.7"N, 14°11'55.7"E	410	5	5	2	5
Plešivec hill near Karlštejn	49°56'04.2"N, 14°11'24.5"E	340	2	5	–	2

A taxonomic revision of the relevant *Sorbus* material kept in the following herbarium collections was undertaken: BRNM, BRNL, BRNU, CB, CHEB, CHOM, Herbarium of the Museum of Ústí nad Labem, HOMP, HR, LIM, LIT, MP, PL, PR, PRA, PRC, ROZ, SOKO and ZMT. For abbreviations of public herbaria, see Holmgren et al. (1990). Revised herbarium specimens were sorted by locality and then according to the year of collection. Information in Czech on herbarium labels was translated into English. Each locality was numbered and named. Coordinates missing on herbarium sheets were obtained using on-line maps (http://www.mapy.cz). Locality numbers were used for displaying localities on the distribution map. Names of the most frequent collectors are abbreviated: ML = M. Lepší, PL = P. Lepší, PV = P. Vít. Species nomenclature is unified according to Kubát et al. (2002) except for, *S. albensis*, *S. portae-bohemicae*, *S. milensis* and *S. latifolia*, which follow Kutzelnigg (1995) and Lepší et al. (2008, 2009). Phytosociological nomenclature follows Chytrý et al. (2001).

Digitalization and elliptic Fourier analysis

Detailed elliptic Fourier analysis was applied to elucidate the variation in leaf shape. Leaves for analysis were predominantly selected from the middle part of short sterile shoots, because most of the *Sorbus* studied were sterile in 2009. Several fertile short and terminal shoots were also included in the analysis to span the leaf variation of the type specimen of *S. eximia,* which only has fertile shoots. Well developed, mature and intact leaves were collected, carefully flattened and dried, and subsequently scanned at 300 dpi using Epson scan 1.11E software. The method of elliptic Fourier approximation (Kuhl & Giardina 1982) incorporated in the SHAPE 1.2 software package (Iwata & Ukai 2002) was employed to describe the variation in leaf shape of both hybridogenous species. The chain-coded contour of each leaf was approximated using the first 20 harmonics, and the elliptic Fourier descriptors (EFDs) normalized to avoid variations related to size, rotation and starting point of the contour trace. Subsequently, principal component (PC) scores for each specimen were calculated from the standardized EFDs, and the shape variation associated with each PC was visualized using the procedure described by Furuta et al. (1995).

A cross-validated linear discriminant analysis using principal component scores (from the above mentioned PCA analysis) as discriminating variables was performed in R, version 2.0.0 (R Core Development Team 2004) using the MASS package (Venables & Ripley 2002). Only the scores of selected PCA axes were used for the discriminant analysis. These axes were selected by a forward selection algorithm in the CVA analysis in Canoco (Lepš & Šmilauer 2003), using the Monte Carlo permutation test (999 permutations; only axes with P level < 0.05 were considered).

Karyology

Three samples each of short, two-year old branches with well-developed leaf buds of each species were collected from the type localities of *S. eximia* and *S. barrandienica* in February 2006. Actively growing vegetative tissue was pre-treated in a saturated water solution of p-dichlorbenzen (2–3 hours at RT) 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. 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.

Estimate of the DNA ploidy level

DAPI flow cytometry was applied to assess the variation in relative genome size and to infer DNA ploidy levels (Suda et al. 2006) in *S. eximia* and *S. barrandienica*. A group of individuals were analyzed individually, then bulked samples were analysed (i.e. five individuals simultaneously) from 73 different trees of *S. eximia* and 13 trees of *S. barrandienica*. *Bellis perennis* (2C = 3.38 pg; Schönswetter et al. 2007) was selected as a suitable internal reference standard (with genome size close to, but not overlapping that of the *Sorbus* species). Nuclei were isolated using a modified two-step procedure (Doležel et al. 2007), stained with DAPI fluorochrome and analysed following the method of Lepší et al. (2008).

Morphometric data and analyses

Seventeen quantitative characters were measured and scored for all of the hybridogenous apomictic *Sorbus* species studied (for a summary of the characters measured, see Lepší et al. 2008). Two new characters were included: "style length" and "length of the fused part of the style". This character set was chosen on the basis of published determination keys, floras and our own observations. The dataset was analysed using the SAS package (version 9.1; SAS Institute, Cary, NC, USA) with CANDIS and DISCRIM procedures, following the methodology described in Klecka (1980). For details see Lepší et al. (2008).

Nuclear microsatellite markers (SSR)

Total genomic DNA was extracted from silica-dried leaves (22 samples in total) following the CTAB protocol (Doyle & Doyle 1987) with minor modifications as described by Pfosser et al. (2005). Microsatellite primers developed for the genera *Sorbus* (Mss1, Mss5, Mss6, Ms6g and Ms14; Oddou-Muratorio et al. 2001, Nelson-Jones et al. 2002) and *Malus* (CH02D11 and CH01H10; Gianfranceschi et al. 1998) were used for the determination of intraspecific genetic variation, following the methodology provided by the original authors. For details see Lepší et al. (2008). 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). Based on the different ploidy levels of samples analysed (both species studied are triploids, putatively parental taxa are diploid and tetraploid), the microsatellite pattern was scored as "allele phenotypes" (Becher et al. 2000). The data set was converted to a binary matrix and analysed with procedures recommended for dominant markers (i.e. PCoA). Intraspecific variation was measured using the Arlequin ver. 3.01 computer programme (Excoffier et al. 2005), which computes the average gene diversity of all loci (AGD, Nei 1987).

Results

Typification of Sorbus eximia

The type specimen of *Sorbus eximia* consists only of a fertile terminal shoot and a short fertile side shoot, both bear untypical or (partly) damaged leaves (Fig. 1). The shape of the

laminas, the shallow incision between lobes and the results of elliptic Fourier analysis indicate that the type specimen is more likely to belong to the Koda type than the second taxon. The determination, however, is not certain. Information on the label does not help much in this sense because the locality is quite broad: slopes of a hill by the village of Srbsko, which may include both the locality of the Koda population and the distribution area of the second taxon.

Because the determination of the type specimen is ambiguous we consider it advisable to select an interpretative epitype. – Holotype: Herbar. Beck., Böhmen Berghänge bei Srbsko, Kalk, leg. [Beck] 17. 8. 1918, PRC (Fig. 1). – Epitype: Bohemia centralis, distr. Beroun, pagus Srbsko (6050d): ca 300 m situ sept.-orientali a pago Koda, in rupibus in declivibus meridionalibus cotae 393 m, solo calcareo; 360 m s.m., 49°56'03.8"N, 14°07'13.6"E; disperse; leg. M. Lepší 2. 8. 2007 (**epitype designated here:** CB, No. 65278, Fig. 2).

When Kovanda described *S. eximia* he had only three specimens of the Koda taxon and had not seen it in the field. On the other hand, he observed the second taxon (*S. barrandienica* described herein) at three localities in the field and cited nine specimens clearly belonging to it. In addition, the pen drawing of a flowering shoot in the original paper (Kovanda 1984) belongs to the new species of *Sorbus*. Consequently, we can assume that the original diagnosis is based on both taxa but mainly on the formally undescribed taxon, at least for the flowers, which are not present on the specimens of the Koda type. It is apparent that a new description of *S. eximia* is needed.

Sorbus eximia Kovanda, Preslia 56: 170, 1984 emend. P. Vít, M. Lepší et P. Lepší
(Figs 1–4)

D e s c r i p t i o n: Trees (or shrubs) up to 16 m high. Trunk up to 1.1 m in circumference. Bark grey, smooth when young, with vertical fissures (particularly at the trunk base) at maturity, with scattered (4–) 8–11 (–16) mm long and (4–) 6–9 (–16) mm wide lenticels. Twigs brownish-grey; young shoots brown, sparsely tomentose when young and almost glabrous at maturity, with numerous elliptical or subrotund pale brown to ochraceous lenticels. Buds 6–14 mm long and 3–6 mm wide, narrowly ovoid to turbinate; scales green, with narrow brown sparsely tomentose margins. Leaves (on short fertile shoots) simple; laminas more or less broadly ovate to broadly elliptical, cochleariform to more or less flat, somewhat glossy, pale to dark green above, yellowish-greyish-green beneath, usually not undulated at margins, more or less broadly rounded acute to obtuse at apex, usually rounded or broadly cuneate and partly serrate at base, almost glabrous on upper surface, evenly tomentose on lower surface, (7.5–) 8.6–9.3 (–11.3) cm long and (5.0–) 6.7–7.5 (–9.4) cm wide, widest at (39–) 51–58 (–64)% of the lamina length (from the tip), double serrate to regularly shallowly lobed (serrate to double serrate apically); lobes serrate or doubly serrate with sharply acuminate teeth terminating the main veins, other teeth smaller, acuminate; sides of lobes more or less arcuate; the third lobe (from the base) (1.0–) 1.1–1.3 (–1.7) cm broad; incision between the second and the third lobe (0.25–) 0.40 (–0.55) cm; lobes broader than 1 cm (2–) 3–4 on each side; main veins terminating in lobes or teeth (6–) 7–8 (–9) on each side; petioles (1.5–) 1.9–2.1 (–2.6) cm long, more or less tomentose. Inflorescences with (16–) 25–42 (–70) flowers, (5.5–) 6.0–9.5 (–10.5) cm in diameter, convex; branchlets more or less tomentose. Hypanthium turbinate, tomentose. Sepals (1.7–) 2.1–2.5 (–3.0) mm long and (2.2–) 2.7–2.9 (–3.3) mm wide, triangular,

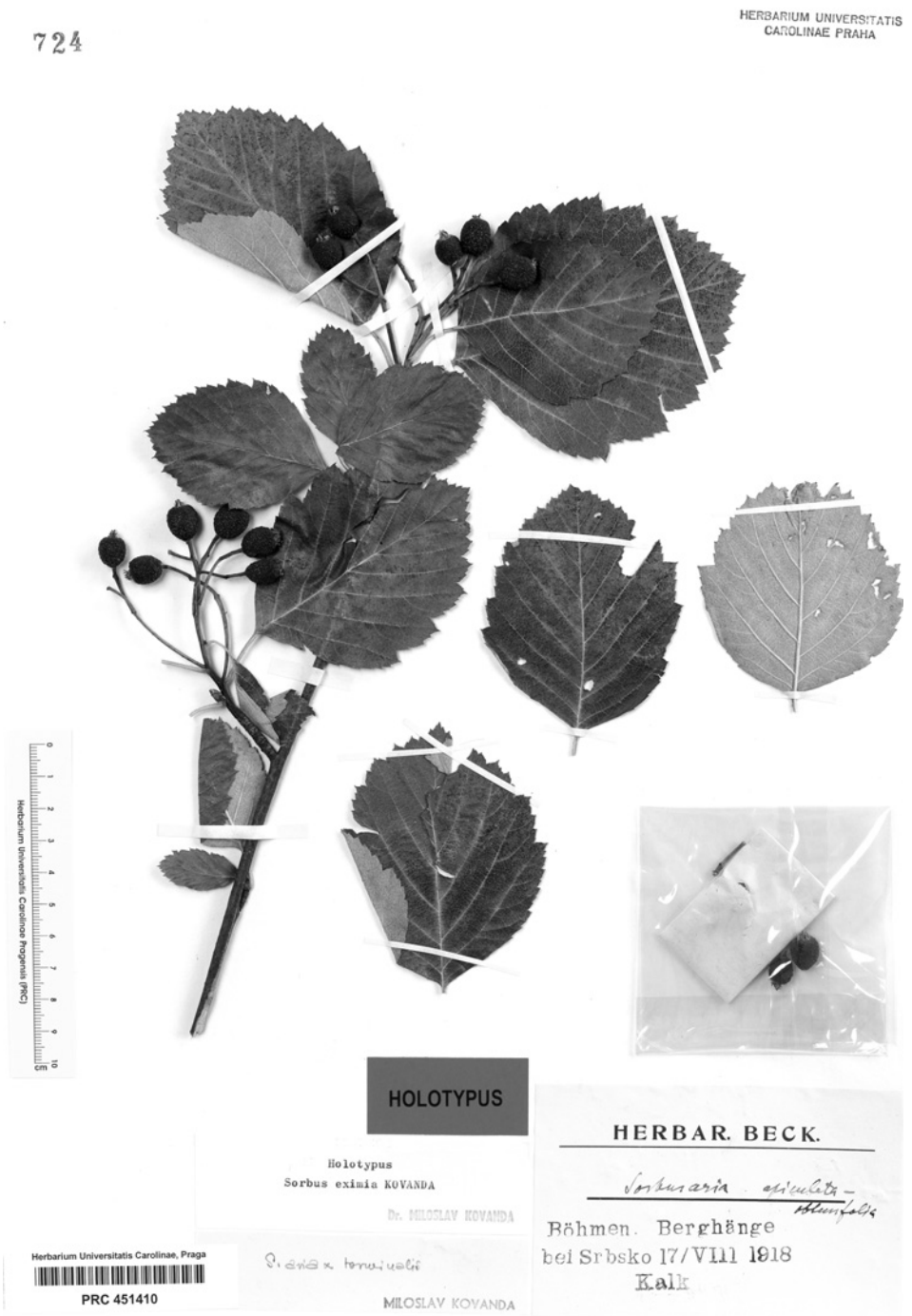

Fig. 1. – Holotype of *Sorbus eximia* Kovanda.

Fig. 2. – Epitype of *Sorbus eximia* Kovanda.

acuminate or acute, densely tomentose on both surfaces, patent, reclinate after anthesis, persistent, dry, erect. Petals (5.1–) 5.7–6.2 (–6.8) mm long and (3.8–) 4.6–4.9 (–5.2) 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, (1.0–) 1.2–1.4 (–1.6) mm long. Ovary semi-inferior. Styles (1–) 2, greenish-cream, (3.0–) 3.4–3.8 (–4.6) mm long, hairy at the base, connate up to (27–) 44–49 (–59)%. Stigma greenish-cream, more or less flat, 0.6–0.7 (–0.8) mm wide. Fruits (11–) 12–13 (–15) mm long and (11–) 12–13 (–15) mm wide, subglobose, often as wide as long or wider than long, orange to orange-red at maturity, glabrous or almost glabrous, glossy, with (8–) 16–32 (–64) ochraceous lenticels per 0.25 cm^2, mesocarp heterogeneous; endocarp cartilaginous. Seeds fuscous. Somatic chromosome number 2n = 51 (triploid). Reproduction tentatively apomictic. Flowering V.

There are pen drawings of a flowering shoot and a leaf in the Flora of the Czech Republic (Kovanda 1992). The same drawing of the leaf is used in the Key to the flora of the Czech Republic (Kubát et al. 2002).

Diagnostic characters

Leaf laminas are broadly ovate to broadly elliptical, (7.5–) 8.6–9.3 (–11.3) cm long and (5.0–) 6.7–7.5 (–9.4) cm wide, often cochleariform, more or less rounded acute to obtuse at apex, usually rounded or broadly cuneate at base, double serrate to regularly shallowly lobed; incision between the second and the third lobe terminating the main veins (0.25–) 0.40 (–0.55) cm long, teeth or lobe terminating the main veins sharply acuminate. Anthers are pale yellow. Styles connate up to (27–) 44–49 (–59)% of their length. Fruits are subglobose, often as wide as or wider than long, orange to orange-red at maturity (Fig. 4).

Ecology

Sorbus eximia occurs in open (woody margins of dry grasslands) and (semi)shaded habitats (forests) on base-rich soils on limestone. In forests, it usually grows in the understorey. Exceptionally it may reach the high tree layer or form monospecific stands (such as by the settlement of Koda). It is recorded on slopes of all aspects. Most individuals grow on southeast, south and southwest slopes. It inhabits mainly basiphilous thermophilous oak forests (*Quercion pubescenti-petraeae*) and mesic oak forests (*Melampyro nemorosi-Carpinetum*). It is rarely also found in narrow-leaved dry grassland (*Erysimo crepidifolii-Festucetum valesiacae*). A majority of individuals occur in semi-natural forests or grasslands with a high abundance of relic species, but it is also found in man-made habitats such as plantations of *Pinus nigra*, long-abandoned quarries (in *Sesleria* grassland – *Diantho lumnitzeri-Seslerion*) or at sites of prehistoric settlements (in species-poor dry grasslands – *Festucion valesiacae*). The species grows sympatrically with *S. aria* s.l., *S. danubialis* and *S. torminalis*. *S. aria* s.l. and *S. danubialis* are a little more heliophilous and xerothermophilous, while *S. torminalis* is a more mesophilous species.

Geographical distribution

Sorbus eximia is recorded at four localities in the Bohemian Karst between Prague and Beroun (Central Bohemia). The largest stand of this species, which includes tens of adults

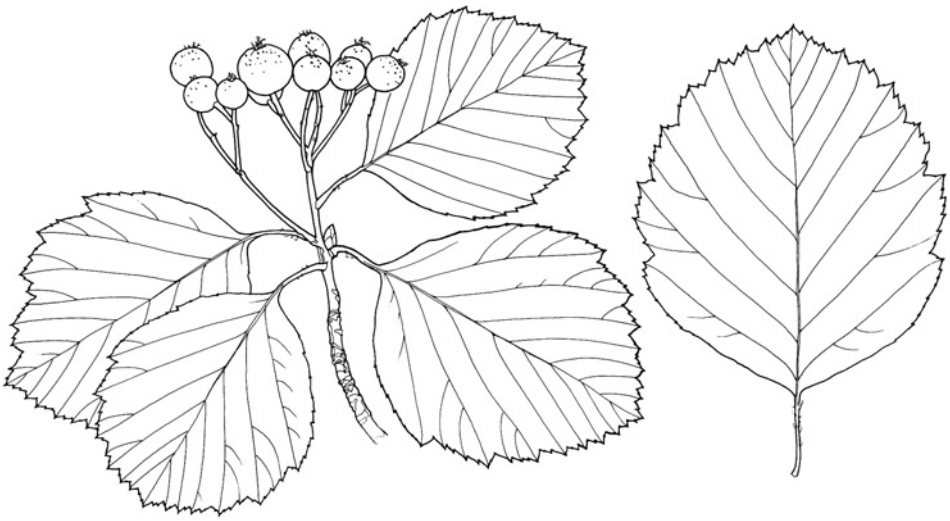

Fig. 3. – *Sorbus eximia*: short fructiferous shoot (left) and leaf from the middle part of short sterile shoot (right). Drawing by A. Skoumalová.

Fig. 4. – Fructiferous short fertile shoot of *Sorbus eximia* at the type locality (photograph taken by P. Lepší, 2009).

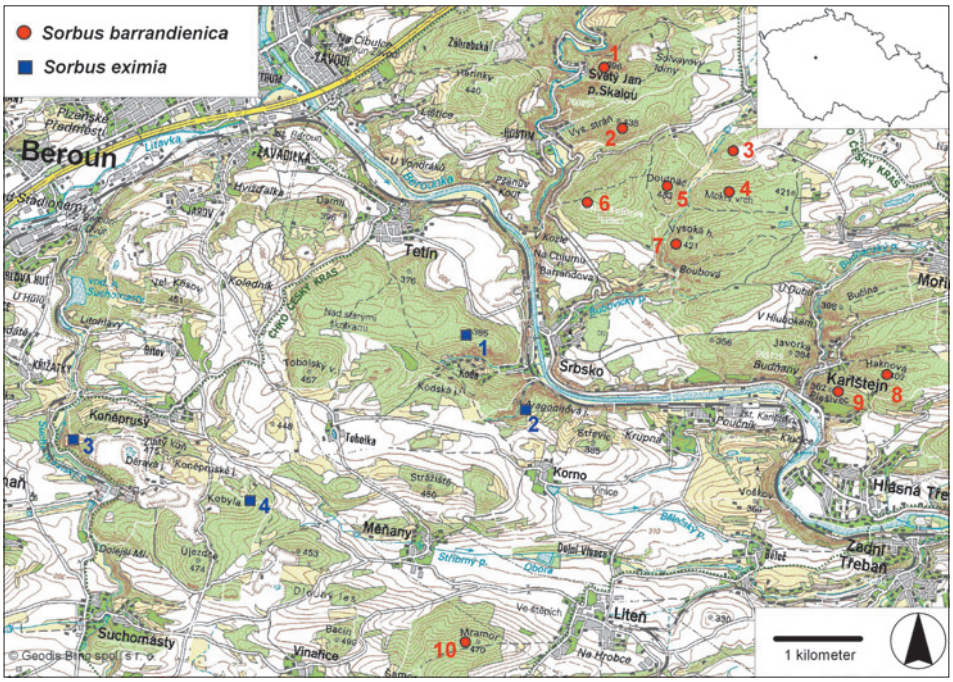

Fig. 5. – Distribution map of *Sorbus eximia* and *S. barrandienica*. The numbers on the map correspond to the locality numbers in the list of revised herbarium specimens and recorded localities (the use of the map was approved by the Ministry of Environment of the Czech Republic).

and ca 200 young trees of different ages, is located by the settlement of Koda near the village of Srbsko within the boundaries of the Koda national nature reserve and covers ca 5 ha. This locality was documented by G. Beck for the first time as far back as in 1918 (holotype in PRC). By contrast, the occurrence of this species nearby Tmaň and Koněprusy was discovered recently in 2009. These two sites harbour a distinctly smaller population, 10 individuals by the village of Tmaň and 20 by the village of Koněprusy. Both of these localities extend over a few tens of square meters. The forth locality in the Císařská rokle gorge is an ex situ conservation plot and houses 11 juveniles. The distance between the localities that are furthest apart is ca 6 km (excluding the locality Císařská rokle) (Fig. 5). *Sorbus eximia* grows in two quadrants (6050d, c) of the Central-European mapping grid (Ehrendorfer & Hamann 1965). The localities are situated in the colline vegetation belt in the phytogeographical district of Český kras (Bohemian Karst) (Skalický 1988). This species grows in a warm and moderately warm climatic region (Quitt 1971) with a mean annual temperature of about 7–9 °C and mean annual precipitation of 500–600 mm (Tolasz et al. 2007). Its altitudinal range spans from 350 m (Koda hill) to 460 m a.s.l. (Kobyla hill). The species has also been planted along the road between the villages of Řevnice and Mořina ca 8 km to the east of its nearest native occurrence (Koda hill).

Herbarium specimens and records:

Czech Republic. Central Bohemia, Bohemian Karst: 1. Koda hill: Herbar. Beck., Böhmen Berghänge bei Srbsko, Kalk (leg. [Beck] 17. 8. 1918, PRC, HOLOTYPE). – Böhmen auf Kalkfelsen bei Koda, nächst Srbsko, häufig (leg. B [=Beck] 1920, PRC). – Koda (leg. J. Klika 4. 10. 1942, PR). – Koda, the plateau (leg. J. Klika 11. 8. 1944, PR 174901). – The S face of the plateau of Koda hill (the reserve) (leg. J. Klika 11. 10. 1944, PR 174899). – On the plateau at the top of Koda hill above the gorge by Koda settlement, growing in association with *Quercus pubescens*, *S. cretica* [= *Sorbus danubialis*], *S. torminalis* and *Carpinus* (leg. J. Klika 5. 7. 1945, two identical specimens in PR). – The plateau of Koda hill by Beroun town (leg. [J. Klika] 2. 4. 1946, PR). – Koda settlement (leg. M. Kovanda 27. 9. 1985, PRA). – Srbsko, rocks and forest-steppe NE of Koda settlement, S slopes at this spot at a height of 393 m, scattered, 6050d: 49°56'03.2"N, 14°07'17.5"E 370 m a.s.l. (leg. ML 2. 8. 2007, PRC 65280/a, CB 65280/b), 49°56'03.8"N, 14°07'13.6"E, 360 m a.s.l. (leg. ML 2. 8. 2007, CB 65278, EPITYPE), 49°56'02.2"N, 14°07'13.6"E, 360 m a.s.l. (leg. ML 2. 8. 2007, CB 65277; leg. PL 19. 9. 2009, CB 71605), 49°56'03.6"N, 14°07'09.5"E, 370 m a.s.l. (leg. ML 19. 8. 2009, CB 71584), 49°56'03.9"N, 14°07'09.5"E, 360 m a.s.l. (leg. PL & ML 10. 5. 2009, CB 71590, CB 71592), 49°56'03.9"N, 14°07'09.6"E, 360 m a.s.l. (leg. PL & ML 10. 5. 2009, CB 71591), 49°56'04.2"N, 14°07'09.9"E, 370 m a.s.l. (leg. PL & ML 10. 5. 2009, CB 71593), 49°56'02.0"N, 14°07'13.3"E, 360 m a.s.l. (leg. PL & ML 10. 5. 2009, CB 71594). – Koda, ca 400 m WNW of the spot at a height of 390 m NE of the settlement, in a oak-hornbeam forest, 6050d, 49°56'09.5"N, 14°07'02.5"E, 375 m a.s.l., scattered (leg. PV, PL, ML & J. Mottl 19. 6. 2009, CB 71595). – Koda, ca 260 m ENE of the spot at a height of 393 m, NE of the settlement, an oak-hornbeam forest, 6050d, 49°56'17.1"N, 14°07'03.4"E, 350 m a.s.l. (leg. PL 21. 6. 2009, CB 71605). This large locality also includes Kovanda's record: Koda forest, N slope (N. of point 390 m), 360–380 m a.s.l. (Jankun & Kovanda 1988). **2. The Císařská rokle gorge:** Srbsko, ca 1 km S of the bridge across the Berounka river, on the right-hand side of the Císařská rokle gorge, 6050d, 49°55'39.5"N, 14°07'56.9"E, 360 m a.s.l., planted in lines, 11 ca 0.5 m high juveniles (leg. PV, PL, ML & J. Mottl 19. 6. 2009, CB 71596). The first record at this locality provided by Schlägelová (2006). **3. Kotýz prehistoric settlement:** Tmaň, the area of Kotýz prehistoric settlement, open thermophilous scrubland, 6050c, 49°54'57.5"N, 14°02'56.5"E, 390 m a.s.l., ca 10 individuals (leg. PL, ML & J. Mottl 19. 6. 2009, CB 71597). A new locality. **4. Kobyla hill:** Koněprusy, SE slopes of Kobyla hill, in the undergrowth of a *Pinus nigra* plantation, 6050d, 49°54'38.5"N, 14°05'05.8"E, 450 m a.s.l., ca 20 individuals of different age (not. J. Mottl 4. 8. 2009, leg. ML 20. 8. 2009, CB 71582). – Koněprusy, the wall of an abandoned quarry on N slope of Kobyla hill, 6050c, 49°54'43.6"N, 14°04'54.4"E, 460 m a.s.l., 1 young tree (leg. ML 20. 8. 2009, CB 71583). A new locality.

Sorbus barrandienica P. Vít, M. Lepší et P. Lepší, **spec. nova** (Figs 6–8)

Descriptio: Arbores usque 12 m alti; foliis (in brachyblastis fertilibus) simplicibus, laminis ambitu fere ellipticis, regulariter pinnato-lobatis (lobis acuminatis, serratis), in parte superiore tantum duplicato-serratis, (8.1–) 8.8–10.3 (–11.6) cm longis et (5.4–) 6.7–7.4 (–8.9) cm latis, ad basin cuneatis usque raro late cuneatis, subintegris vel remote serratis, obscure viridibus, subtus ochro-griseo-viride tomentosis, nervis ab utroque latere (7–) 8 (–9) in numero; petiolis (1.9–) 2.1–2.4 (–2.7) cm longis; corymbothyrsis multifloris, convexis, ramis plus minusve tomentosis. Dentibus calycinis triangularibus, acuminatis usque acutis, (2.3–) 2.5–3.5 (–3.8) mm longis et (2.7–) 2.8–3.0 (–3.2) mm latis, patentibus usque reclinatis, post anthesin reclinatis, dense tomentosis, tempore fructificationis siccis, persistentibus; petalis late ovatis usque late ellipticis, breviter unguiculatis, (6.1–) 6.2–6.9 (–7.5) mm longis et (4.4–) 4.5–4.9 (–5.2) mm latis, albidis, superne ad basin sparse villosis, patentibus; staminibus ca 20, antheris pallide luteis, (1.0–) 1.2–1.3 (–1.5) mm longis; ovario semi-infero; stylis 2 (–3) ad (16–) 29–42 (–57)% coalescentibus, ad basin villosis, albo-viridis, (3.5–) 3.7–3.9 (–4.0) mm longis, stigmatibus plus minusve planis; fructibus subglobosis, (11.5–) 12.0–13.0 (–14.0) mm longis et (11.0–) 12.0–12.5 (–14.0) mm latis, maturitate aurantiacis usque rubris, glabris vel fere glabris, nitidis, cum (4–) 16–32 (–36) lenticellis parvis, ochraceis ad 0.25 cm^2; mesocarpio heterogeneo; endocarpio cartillagineo, seminibus atro-fuscis. Numerus chromosomatum triploideus 2n = 51. Probabiliter planta apomicta. Floret V.

Holotypus: Bohemia centralis, distr. Beroun, pagus Srbsko (6050b): in summo collis Doutnáč, in querceto, solo calcareo; 430 m s.m., 49°57'23.5"N, 14°09'09.5"E; disperse; leg. M. Lepší 2. 8. 2007; CB, No. 65274 (Fig. 6). – Isotypus: PRC, No. 65274/a.

Description: Trees up to 12 m high. Trunk up to 0.55 m in circumference. Bark grey, smooth when young, with vertical fissures (particularly at the trunk base) at maturity, with scattered (3–) 7–11 (–14) mm long and (3–) 7–11 (–14) mm wide lenticels. Twigs

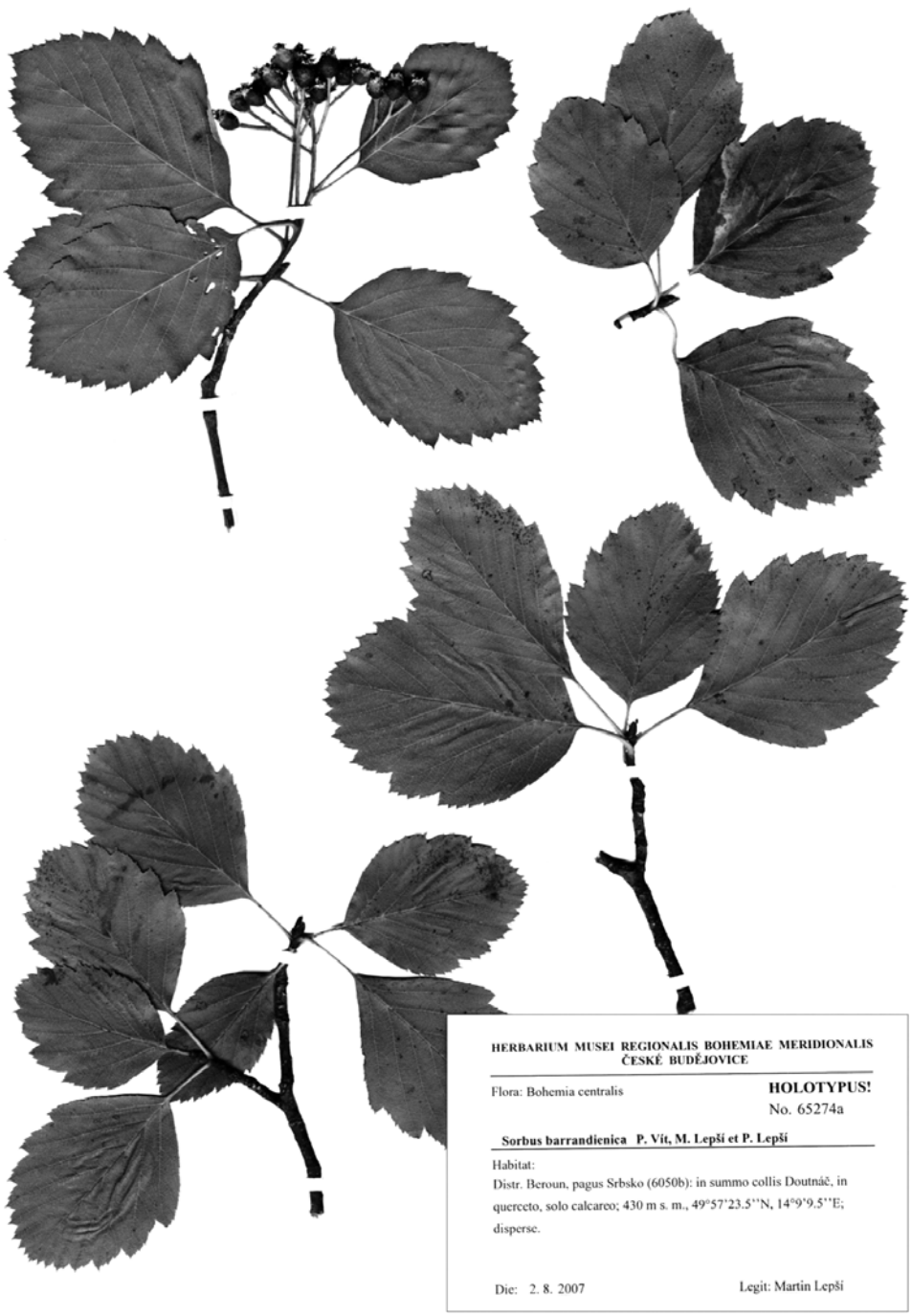

Fig. 6. – Holotype of *Sorbus barrandienica* P. Vít, M. Lepší et P. Lepší.

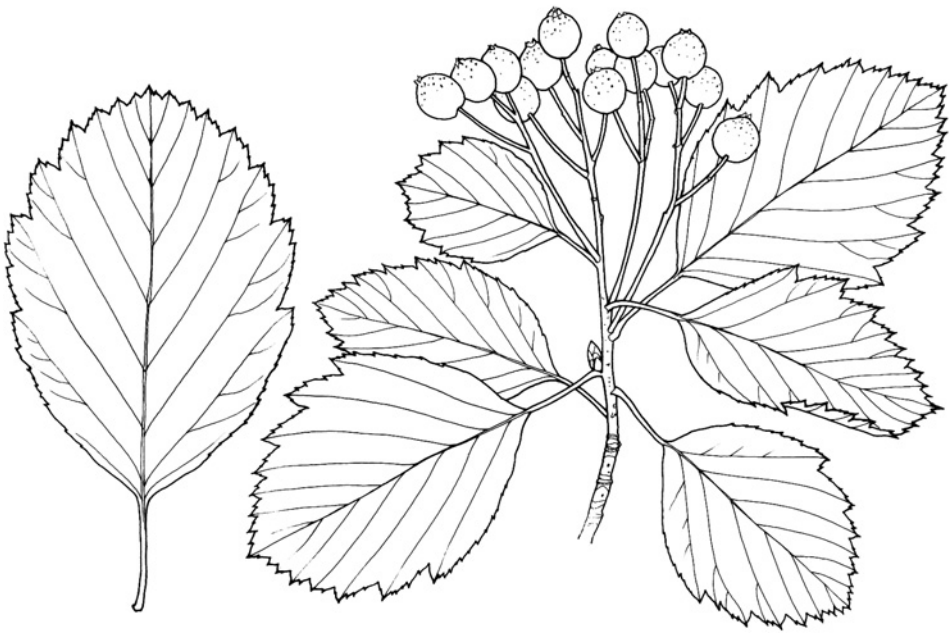

Fig. 7. – *Sorbus barrandienica*: leaf from the middle part of short sterile shoot (left), short fructiferous shoot (right). Drawing by A. Skoumalová.

Fig. 8. – Sterile shoots of *Sorbus barrandienica* growing at Doutnáč hill (photograph taken by P. Lepší 2009).

brownish-grey; young shoots brown, sparsely tomentose when young and almost glabrous at maturity, with elliptical or subrotund pale brown to ochraceous lenticels. Buds 7–14 mm long and 3–6 mm wide, narrowly ovoid to turbinate; scales green, with narrow brown sparsely tomentose margins. Leaves (of short fertile shoots) simple; laminas more or less elliptical, more or less flat, somewhat glossy, dark green above, yellowish-greyish-green beneath, usually flat at margins, more or less rounded acute at apex, usually cuneate rarely broadly cuneate and partly serrate at base, almost glabrous on upper surface, evenly tomentose on lower surface, (8.1–) 8.8–10.3 (–11.6) cm long and (5.4–) 6.7–7.4 (–8.9) cm wide, widest at (16–) 29–42 (–64)% of the lamina length (from the tip), regularly lobed (double serrate apically); lobes serrate or doubly serrate with sharply acuminate teeth terminating the main veins, other teeth smaller, acuminate; sides of lobes more or less arcuate; the third lobe (from the base) (0.95–) 1.15–1.35 (–1.80) cm broad; incision between the second and the third lobe (0.40–) 0.45–0.6 (–0.75) cm; lobes broader than 1 cm (2–) 3–4 on each side; main veins terminating in lobes or teeth (7–) 8 (–9) on each side; petioles (1.9–) 2.1–2.4 (–2.7) cm long, more or less tomentose. Inflorescences with (44–) 50–60 (–66) flowers, (7–) 8–9 (–10) cm in diameter, convex; branchlets more or less tomentose. Hypanthium turbinate, tomentose. Sepals (2.3–) 2.5–3.5 (–3.8) mm long and (2.7–) 2.8–3.0 (–3.2) mm wide, triangular, acuminate or acute, densely tomentose on both surfaces, patent, reclinate after anthesis, persistent, dry, erect. Petals (6.1–) 6.2–6.9 (–7.5) mm long and (4.4–) 4.5–4.9 (–5.2) 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, (1.0–) 1.2–1.3 (–1.5) mm long. Ovary semi-inferior. Styles 2 (–3), greenish-cream, (3.5–) 3.7–3.9 (–4.0) mm long, hairy at the base, connate up to (16–) 29–42 (–57)%. Stigma greenish-cream, more or less flat, (0.6–) 0.7 (–0.8) mm wide. Fruits (11.5–) 12.0–13.0 (–14.0) mm long and (11.0–) 12.0–12.5 (–14.0) mm wide, subglobose, orange to orange-red at maturity, glabrous or almost glabrous, glossy, with (4–) 16–32 (–36) ochraceous lenticels per 0.25 cm^2, mesocarp heterogeneous; endocarp cartilaginous. Seeds fuscous. Somatic chromosome number 2n = 51 (triploid). Reproduction tentatively apomictic. Flowering V.

There is also a pen drawing of a flowering shoot in Kovanda (1984).

Diagnostic characters

Leaf laminas are more or less elliptical, (8.1–) 8.8–10.3 (–11.6) cm long and (5.4–) 6.7–7.4 (–8.9) cm wide, more or less rounded acute at apex, usually cuneate, rarely broadly cuneate at base, shallowly lobed; incision between the second and the third lobe terminating the main veins (0.4–) 0.45–0.6 (–0.75) cm long, teeth or lobe terminating the main veins sharply acuminate. Anthers are pale yellow. Styles connate up to (16–) 29–42 (–57)% of their length. Fruits are subglobose, orange to orange-red at maturity.

Etymology

The name "*barrandienica*" derives from the Barrandien, a geologically and paleontologically conspicuous region located between Prague and Pilsen. This species is recorded growing on the Bohemian Karst, which is known as the most significant part of the Barrandien region. The authors propose the epithet "barrandienský" for the Czech name.

Ecology

Sorbus barrandienica occurs mainly on base-rich soils that develop on limestone. It occurs most frequently on the summits of hills, usually in thermophilous open forests or woody margins of dry grassland, and exceptionally in dry grassland, thermophilous scrub or rocks. In most cases, it grows in thermophilous and mesophilous oak-hornbeam forests (*Primulo veris-Carpinetum* and *Melampyro nemorosi-Carpinetum*) and in transition vegetation between these communities. It is also recorded in basiphilous thermophilous oak forests (*Quercion pubescenti-petraeae*) and their mesophilous derivatives. On one occasion, it was recorded in an acidophilous thermophilous oak forest (*Sorbo torminalis-Quercetum*). In the undergrowth of forests, it is very often sterile and does not produce fruits. When it is overshadowed by taller trees, it usually dies. It is recorded on slopes of all aspects save for eastern-facing slopes. Most localities are situated on south and southwest slopes. It does not occur in man-made biotopes. The species grows sympatrically with *S. danubialis*, *S. aria* s.l. and *S. torminalis* at most localities. *Sorbus danubialis* and *S. aria* s.l. are a little more heliophilous and xerothermophilous, while *S. torminalis* is a more mesophilous species.

Geographical distribution

Sorbus barrandienica was recorded at 10 localities in the Bohemian Karst located between Prague and Beroun (Central Bohemia). The centre of its current distribution is located close to Doutnáč hill between the villages of Srbsko and Bubovice (localities 3, 4, 5, 7). This species is not abundant and is represented by small populations in this area. At other localities there are only a few scattered individuals (localities 2, 6, 8 and 9) or the species has not been seen recently (locality 1, 10). The distance between localities that are furthest apart is ca 8 km. The easternmost locality is close to Karlštejn, both the northernmost and westernmost close to the village of Svatý Jan pod Skalou and the southernmost at a very isolated locality located on Mramor hill at the village of Liteň (Fig. 5). *Sorbus barrandienica* is recorded in five quadrants (6050b, d; 6051a, c; 6151a) of the Central European mapping grid (Ehrendorfer & Hamann 1965). The first record of this species is that of V. Krajina in 1926 near Svatý Jan pod Skalou (PRC). These localities are situated in the colline vegetation belt in the phytogeographical district of Český kras (Bohemian Karst) (Skalický 1988). This species grows in a warm and moderately warm climatic region (Quitt 1971) with a mean annual temperature of about 7–9 °C and mean annual precipitation of 500–600 mm (Tolasz et al. 2007). Its altitudinal range spans from 340 m a.s.l. (Plešivec hill) to 450 m a.s.l. (Mramor hill).

Herbarium specimens and records:

Czech Republic. Central Bohemia, Bohemian Karst: 1. Svatý Jan pod Skalou (49°58'10.2"N, 14°08'04.3"E): Karlštejn, Svatý Ivan (leg. Vladimír Krajina 1. 5. 1926, PRC). – Svatý Jan pod Skalou – rocks above the monastery (leg. Štěpánková 4. 6. 1962, CB). The last record for this locality was in 1985: the summit area of U Kříže hill, near Svatý Jan pod Skalou village, 396 m a.s.l. (no specimen available) (Jankun & Kovanda 1988). A locality with unconfirmed occurrence. **2. Vysoká stráň hill:** Svatý Jan pod Skalou, Vysoká stráň hill, ca 950 m NW of the summit of Doutnáč hill, in a thermophilous oak forest, 6050b, 49°57'47.1"N, 14°08'38.0"E, 390 m a.s.l., 2 fertile and 1 sterile individual (leg. ML & Karel Boublík 28. 7. 2007, CB 65276). The first record for this locality was in 1986: the summit area of Vysoká stráň hill, near Hostim village, 435 m a.s.l. (no specimen available) (Jankun & Kovanda 1988). **3. Paní hora hill:** In colle Paní hora prope pagum Bubovice haud procul ab

oppido Beroun, solo calcareo, alt. 410 m (leg. M. Kovanda 23. 6. 1964, PRA). – Bubovice, the summit area of Paní hora hill, the scrubby margins of dry grassland, scattered, 6050b: 49°57'43.1"N, 14°09'52.3"E, 410 m a.s.l. (leg. ML & PL 19. 6. 2009, CB 71600), 49°57'43.9"N, 14°09'51.3"E, 410 m a.s.l. (leg. PL 19. 9. 2009, CB 71606). **4. Mokrý vrch hill:** Karlštejn, forester's lodge N 5, Mokryj verch [= Mokrý vrch hill] (leg. B. Augstová 6. 6. 1957, PR 5900946; 24. 5. 1958, PR 590377). – Bubovice, SW slopes of Mokrý vrch hill, the woody margins of grassland, 6050b, 49°57'22.2"N, 14°09'44.2"E, 390 m a.s.l., ca 10 individuals (leg. ML 5. 5. 2009, CB 71585). – Bubovice, W slopes of Mokrý vrch hill, the undergrowth of an oak-hornbeam forest, 6050b, 49°57'24.3"N, 14°09'32.5"E, 380 m a.s.l., 1 sterile individual (leg. ML 5. 5. 2009, CB 71586). – Bubovice, ca 200 m SE of the summit of Mokrý hill, the undergrowth of an oak-hornbeam forest, 6051a, 49°57'22.6"N, 14°10'06.9"E, 420 m a.s.l., 2 old dying individuals (leg. ML 10. 5. 2009, CB 71587). – Bubovice, Mokrý vrch hill, ca 0.6 km WNW of the centre of the Malá Amerika quarry, along a forest road, 6051a, 49°57'17"N, 14°10'02"E, 400 m a.s.l. (leg. PL 20. 6. 2009, CB 71602). This locality probably includes also Kovanda's record: the forest margin in the shallow valley ca 0.8 km NW of the Amerika quarry, 300 m a.s.l. (Kovanda 1999). **5. Doutnáč hill:** Doutnáč hill by Srbsko village (sine coll. IX. 1935, PR 174743). – Distr. Beroun, in nemore ad declivia occid.-merid. montis Doutnáč supra vic. Srbsko, 370 m s.m., No 10868 (leg. Domin & Dostál 28. 6. 1939, PRC). – Beroun: in nemore ad declivia occ.-merid. montis Doutnáč supra vic. Srbsko, 370 m s.m. (leg. Domin & Dostál 28. 6. 1939, PRC). – Central Bohemia, Bohemian Karst, Bubovice, NE slopes of Doutnáč hill, above the valley at an altitude of 350 m; the margin of an open forest and stony steppe, S slope orientation (leg. R. Businský 5. 6. 1977, ROZ 31764-31772). – Doutnáč hill (leg. M. Kovanda 3. 6. 1980, October 1980, 26. 5. 1982, PRA). – Srbsko, the summit area of Doutnáč hill, an oak forest, 6050b, 49°57'23.5"N, 14°09'09.5"E, 430 m a.s.l., ca 10 individuals (leg. ML 2. 8. 2007, CB 65274, HOLOTYPE; PRC 65274/a, ISOTYPE). Some specimens listed in Kovanda (1984) (i.e. in dumetis in clivo austr. collies Doutnáč prope pagum Srbsko, leg. M. Kovanda 1963 PR; in dumetis in summo collis Doutnáč prope pagum Srbsko, leg. M. Kovanda 1964 PR, 1965 PR) are probably lost. **6. Boubová hill:** Svatý Jan pod Skalou, the SW slope of Boubová hill, 400-410 m a.s.l. [not. M. Kovanda (Kovanda 1999)]. – Boubová hill, ca 0.3 km WSW of the summit, the margin of a forest road in an oak-hornbeam forest, 6050b, 49°57'11.9"N, 14°08'17.9"E, 400 m a.s.l., one ca 0.75 m high juvenile individual (not. 19. 6. 2009 J. Mottl, ML & PL). No specimen from this locality is available. **7. Velká hora hill:** Srbsko, W slopes of Velká hora hill, ca 200 m SW of the summit, in thermophilous scrub, 6050d, 49°56'58.6"N, 14°09'24.4"E, 420 m a.s.l., 3 juvenile individuals (leg. ML 4. 7. 2009, CB 71603). – Srbsko, ca 80 m SW of the summit of Velká hora hill, near the margin of the summit plateau, the margin of a forest gap, 6050b, 49°57'01.7"N, 14°09'28.2"E, 420 m a.s.l., one ca 6 m high individual (leg. ML 16. 7. 2009, CB 71601). – Srbsko, W slopes of Velká hora hill, ca 250 m W of the summit, in thermophilous oak forest, 6050b, 49°57'03.7"N, 14°09'18.6"E, 350 m a.s.l., one ca 1.5 m high juvenile individual (not. J. Mottl 2. 8. 2009, leg. ML 29. 10. 2009, CB 71604). A new locality. **8. Haknová hill:** Karlštejn, S slopes of Haknová hill, near the summit, a thermophilous oak forest, 6051c: 49°56'15.7"N, 14°11'55.7"E, 410 m a.s.l., 1 small tree (leg. PL & ML 10. 5. 2009, CB 71588), 49°56'15.8"N, 14°11'55.9"E, 410 m a.s.l., 1 small tree (leg. PL & ML 10. 5. 2009, CB 71589). – Karlštejn, ca 100 m ENE of the summit of the Haknová hill, 6051c, 49°56'17.9"N, 14°12'01.0"E, 420 m a.s.l., one overshadowed ca 4 m high individual (leg. PL & ML 10. 5. 2009, CB 71599). The first record for this locality is 1986: the summit area of Haknová hill near Karlštejn, 402 m a.s.l. (no specimen available) (Jankun & Kovanda 1988). **9. Plešivec hill:** Plešivec hill (leg. M. Kovanda 16. 10. 1985, PRA). – Karlštejn, ca 120 m SE of the summit of Plešivec hill, the margin of steppe, 6051c, 49°56'04.2"N, 14°11'24.5"E, 340 m a.s.l., one overshadowed ca 3 m high individual (leg. ML & PL 10. 5. 2009, CB 71598). **10. Mramor hill** (49°53'54.1"N, 14°07'43.3"E): Beroun: in nemore ad declivia collis Mramor prope pag. Měňany et Liteň, 450 m s.m., s. calcareo (leg. Domin & Dostál 2. 8. 1939, PRC). The specimens listed in Kovanda (1984) (i.e. in nemore in clivo septentr. collis Mramor prope pagum Liteň, leg. M. Kovanda 1980, 1981 PR) are probably lost and are the last records for this locality. A locality with unconfirmed occurrence.

Poorly localized specimens: Karlštejn (leg. M. Řezáč 2001, ROZ).

Herbarium specimens and records not confirmed

In 2005 (Vít 2006) and 2009, we repeatedly failed to confirm the two records cited for *S. eximia* by Jankun & Kovanda (1988) and Kovanda (1999) listed below (herbarium specimen not cited, see below). Considering the rather poor delimitation of these localities, we cannot rule out that the species was overlooked and is still present there. The distribution pattern of both species indicates that these records refer rather to *S. barrandienica* than *S. eximia*, but a field observation is needed to confirm this hypothesis. The specimen col-

lected by Hostim and mentioned below, which Kovanda (1984) referred to as *S. eximia*, we find impossible to identify with certainty. It consists of a sterile, probably epicormic shoot with lobed leaves with a greyish indumentum on the abaxial surface. Lobes are characteristic of hybrid *Sorbus* species, but *S. aria* s.l. can also have exceptionally lobed leaves, particularly on long sterile shoots. A greyish indumentum without any yellowish tinge is typical of *S. aria* s.l. A search carried out by us in the vicinity of the village in 2007 yielded many records of *S. aria* s.l., but no hybrid was recorded there.

Herbarium specimens and records:

Unconfirmed records: 1. Along the road from Hostim to Bubovice, 1 km from Bubovice, 350 m a.s.l. (Jankun & Kovanda 1988). **2.** The surroundings of the Králova studně spring (Kovanda 1999).
Uncertain determination: Böhmen, gehänge bei Hostín [= Hostim], Kalk (leg. Beck 21. 8. 1918, PRC).

Phenotypic variation and species-specific characters

Sorbus eximia and *S. barrandienica* populations are fertile. The plants produce fully developed seeds and are morphologically homogeneous both in vegetative and generative characters. There are no records based on our field observations of morphologically intermediate types between the species (they do not occur at the same localities). The taxa belong to the *S. latifolia* aggregate (parental combination *S. aria* s.l. × *S. torminalis*). The *S. aria* group is represented by *S. danubialis* and *Sorbus aria* s.l. in this region, and both of these taxa (along with *S. torminalis*) often occur sympatrically with the species studied. Plants intermediate between the hybrid species and their putative parents have not been observed. For morphological differences between *S. danubialis*, *S. aria* s.l. and the two hybrid species, see the key in Appendix 1. *Sorbus torminalis* is not included in the key because *S. eximia* and *S. barrandienica* are apparently closer to the *S. aria* group. The species studied differ from other Bohemian members of the *S. latifolia* agg. (*S. albensis*, *S. bohemica*, *S. gemella*, *S. rhodanthera*, *S. portae-bohemicae* and *S. milensis*) in having paler (orange to orange-red) fruits and shallowly lobed leaves. All Bohemian species except for *S. gemella* have darker (orange-red) fruits. *Sorbus gemella* differs in having rhomboidal and more deeply incised laminas. In *S. eximia* the leaves often have cochleariform lamina, which is a unique character for taxa of *Sorbus* occurring in the Czech Republic.

Chromosome variation and ploidy level of S. eximia

Sorbus eximia is cited as an example of a rare diploid apomictic species in several publications (Campbell & Dickinson 1990, Campbell et al. 1991, Nelson-Jones et al. 2002, Meyer et al. 2005, Dickinson et al. 2007, Rich et al. 2010). Our investigations have revealed that somatic cells of *S. eximia* and *S. barrandienica* have a triploid chromosome number (2n = 3x = 51). The diploid number previously reported for *S. eximia* (Jankun & Kovanda 1988) was not confirmed in the current study, not even for the populations cited by Kovanda (Koda hill near Srbsko; Jankun & Kovanda 1988). Screening the DNA ploidy levels of *S. eximia* and *S. barrandienica* using DAPI flow cytometry also did not detect any intraspecific variation. Observed sample/standard ratio for *S. eximia* was 0.60 (average CV of sample: 3.04 and standard: 1.66) and for *S. barrandienica* 0.60 (average CV of sample: 3.67 and standard: 2.35). Our recent study indicates the existence of only tetraploid cytotypes in the *S. aria* agg. in the Bohemian Karst (Lepší et al. in prep.), thus occurence

of diploid *S. eximia* is improbable. It is therefore concluded that both species studied are triploids and the existence of diploids or tetraploids as reported by Kovanda (1984) and Jankun & Kovanda (1988) must be regarded as dubious. Other hybrid species of the *S. latifolia* group in Bohemia (Jankun & Kovanda 1987, Lepší et al. 2008, 2009, P. Vít, unpubl.) also have the triploid number of chromosomes. The diploid chromosome number has thus so far only been reported with certainty for sexual species of *Sorbus*.

Genetic variation

Sorbus eximia and *S. barrandienica* showed minimal intra-specific genetic variation at seven nuclear microsatellite loci, indicating a monotopic origin (i.e., each species is a single evolutionary lineage). This phenomenon is common in several other agamospermous *Sorbus* taxa occurring in the Czech Republic (e.g., *S. albensis*, *S. portae-bohemicae*, *S. rhodanthera* and *S. milensis*; Lepší et al. 2008, 2009, P. Vít et al., unpubl.). While intra-specific variation was low, inter-specific differentiation is considerable as both of these species have distinct microsatellite patterns. Prevailing fragment length of each of the loci analysed and average gene diversity are presented in Table 2. The species differ from one another in six of the seven loci analysed. Average gene diversity of hybrid species is considerably (about tenfold) lower than that of species reproducing sexually (e.g. *S. torminalis* or *S. aria* s. str.; Vít 2006). This observation supports the independent status of each of the endemic *Sorbus* species as unique evolutionary units. The predominant, if not sole mode of reproduction of the taxa studied, which is inferred from the low morphological and genetic variation, is probably apomixis. These observations are consistent with the results for several other apomictic *Sorbus* taxa of hybrid origin (Liljefors 1953, Jankun & Kovanda 1986, 1987, 1988, Meyer et al. 2005).

Table 2. – Fragment length of each microsatellite loci (in bp) and average gene diversity over all loci (AGD) for each *Sorbus* species.

Taxon	N	Locus Mss1	Locus CH01H10	Locus Mss6	Locus CH02D11	Locus Ms14	Locus Mss5	Locus Ms6g	AGD	S.E.
S. eximia	10	172	78	254	120, 144, 178	128	112, 124	130	0.002405	0.003011
	2	172	78, 82	254	120, 144, 178	128	112, 124	130		
S. barrandienica	9	156	78	250	120, 144, 168	120	112, 124	126	0.008219	0.008045
	1	152	78	250	120, 144, 168	120	112, 124	126		

Morphometric analyses

Sorbus eximia and *S. barrandienica* were well separated in the canonical discriminant analysis (results not shown) from other Bohemian *Sorbus* species of the *S. latifolia* group (for details see Vít 2006, p. 55–61). In separate analysis of *S. eximia* and *S. barrandienica* (see Fig. 9), the species were also well separated. The incision between the 2nd and 3rd lobe of the leaf lamina and calyx length were the variables most tightly correlated with the first discriminant axis. When all of the characters measured are included, 62 (96.87%) of the 64 specimens of *S. eximia* and *S. barrandienica* tested were correctly classified in

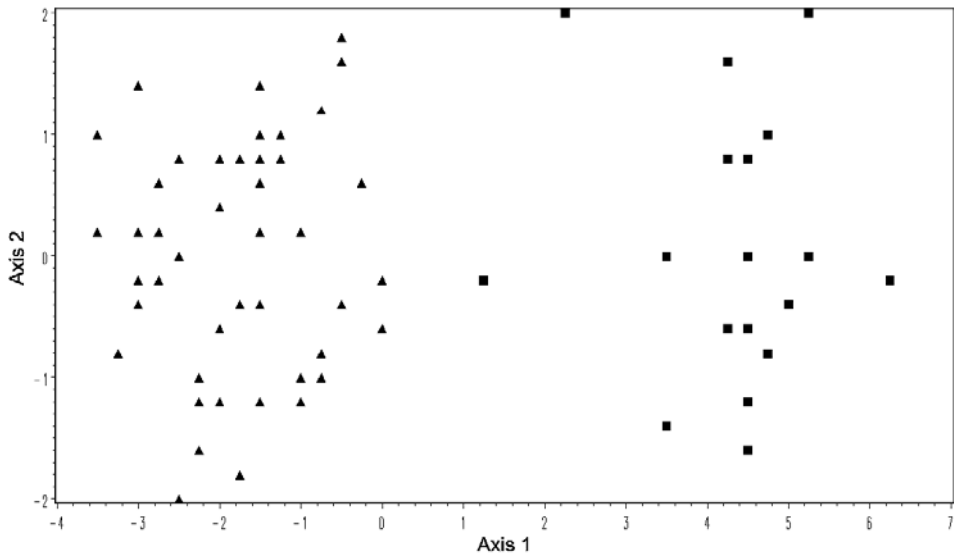

Fig. 9. – Canonical discriminant analysis of *Sorbus eximia* ▲ and *S. barrandienica* ■ using a morphometric data set of 17 characters.

a classificatory discriminant analysis (data not shown). The incorrectly classified samples may be a result of the phenotypic variation and/or problems of standardising sampling.

Elliptic Fourier analysis of leaf laminas

While descriptive morphometrics separated the two species on the basis of quantitative characters, an elliptic Fourier analysis allowed the separation of the two species by using the shape of the leaf lamina as a diagnostic character. Principal component analysis (PCA) performed on standardized Fourier coefficients revealed distinct differences between the species studied (Fig. 10). A morphological shape trend associated with the first principal component separates the two species based on the overall shape of the lamina. *S. eximia* has a broadly ovate to broadly elliptical leaf lamina, while that of *S. barrandienica* is more or less elliptical. Variation along the second (data not shown) and third axis demonstrated a tendency towards differentiation in the curve of the lamina base and apex. In *S. barrandienica* it tends to be rounded acute at the apex and cuneate at the base, while in *S. eximia* the apex is more or less broadly rounded acute to obtuse and the base rounded or broadly cuneate. The results of these analyses confirmed our field observations. No clear pattern was observed along the other PCA axes (data not shown). In total, seven PCA axes were found to significantly improve the discriminant power of the CVA analysis during forward selection in Canoco. A cross-validated discriminant analysis was performed on the principal component scores of these seven axes. The discriminant analysis resulted in an incorrect classification in eight of a total 194 cases (6 individuals of *S. eximia* were assigned to *S. barrandienica* and 2 individuals of *S. barrandienica* to *S. eximia*). These incorrectly classified samples were leaves untypically developed due to phenotypic varia-

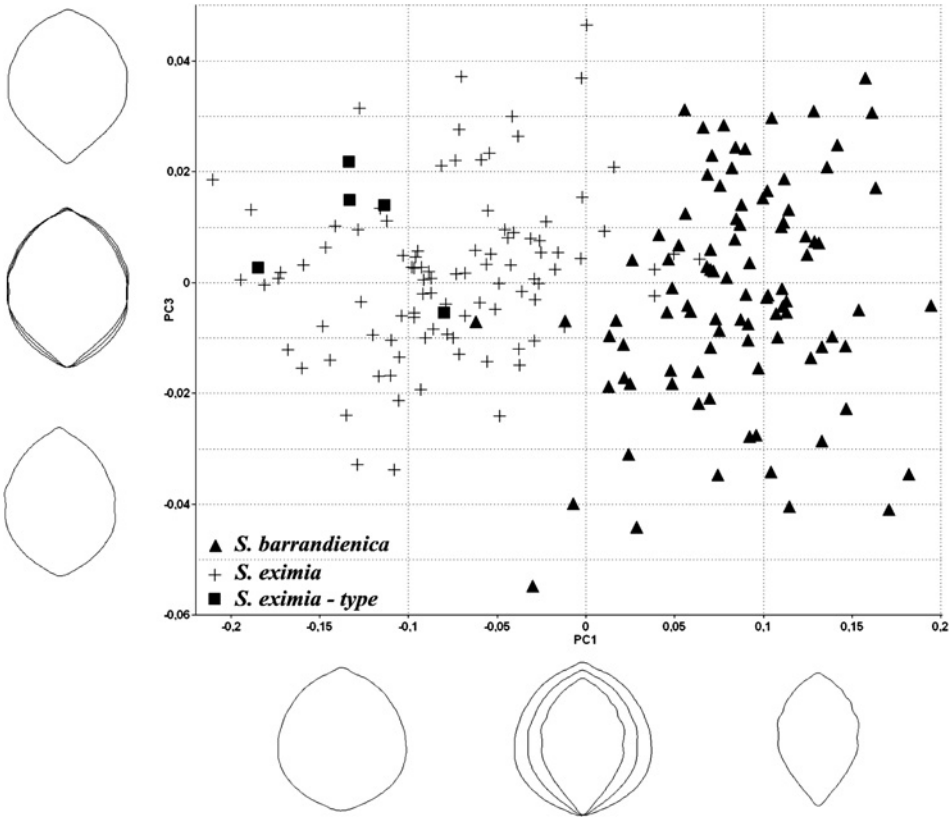

Fig. 10. – PCA of Fourier coefficients describing the total leaf lamina shape of *S. eximia* and *S. barrandienica*. The first and third ordination axes are displayed, which explain 81.8% and 2.5% of the overall variation, respectively. Reconstructed contours corresponding to the –2 and +2 SD positions on both axes are visualized along the particular axes. In the middle, these two contours are overlapped with the mean leaf shape (corresponding to the [0.0] point of the plot).

tion in this species (too overshadowed or exposed plants). All 5 leaves from the type specimen of *S. eximia* were determined as *S. eximia*. Neither the relative position nor the shape of the laminar lobes contributed to the discrimination of these two species, even when the shape of the lobes was more accurately described by using a greater (40) number of harmonics (data not shown).

Conservation status

All the specimens of the two taxa studied are found within the area of the Bohemian Karst protected landscape area and are (except the locality of *S. barrandienica* at Mramor hill) part of small-scale protected areas (in particular, Koda and Karlštejn national nature reserves, Kotýz national nature monument and Kobyla nature reserve). Despite this, the protection of the two endemic species is insufficient. The main threat stems from the

cessation of traditional forest management, which previously maintained open forests stands. The shady conditions that prevail in recent so-called tall forests are unfavourable for the long-term survival and regular reproduction of light-demanding *Sorbus* species. The general expansion of woods (especially of *Fraxinus excelsior*) into open (rocky and steppe) habitats represents another serious threat to these endemics.

Even before the taxonomic revision presented here, *Sorbus eximia* was regarded as a strongly endangered species (Holub & Procházka 2000). The new species *Sorbus barrandienica* should be included among the critically endangered plants of the Czech flora (C1; sensu Holub & Procházka 2000), as there are few individuals, frequent occurrence of old or dying trees and lack of juveniles at most localities. *Sorbus eximia* is considered strongly endangered (category C2) because there are considerably larger populations with lots of juveniles at its localities. Moreover, this species is able to spread into man-made non-relic biotopes, e.g. into abandoned quarries or pine plantations. Such habitats are now common in the Bohemian Karst. On the other hand, it is only recorded at three localities and the biggest one, Koda hill, is significantly affected by the spread of *Fraxinus excelsior*. Both endemic species should also be added to the list of species protected by law. According to the IUCN (2001), *S. eximia* and *S. barrandienica* rank among critically endangered species [status criteria B2b (iii) and B2b (iii,iv,v);C2a (i), respectively]. Particular attention should be paid to protecting these species in the future. Appropriate forest management (which would facilitate reproduction of these endemics) should be implemented at selected localities. Three localities are recommended: Koda hill for protection of *S. eximia* and Paní hora hill and/or Doutnáč hill for *S. barrandienica*. Supposed parental species occurring at the same localities as the endemics should also be included in the management plan, since they may generate new taxonomic diversity (by hybridization and introgression) in the future and thus play an important role in the ongoing evolutionary processes (cf. Ennos et al. 2005).

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Souhrn

V příspěvku je popsán nový apomiktický triploidní (2n = 3x = 51) druh jeřábu *Sorbus barrandienica* P. Vít, M. Lepší et P. Lepší (jeřáb barrandienský), náležející do skupiny *S. latifolia* agg. (rodičovská kombinace *S. aria* s.l. × *S. torminalis*). Byl rozlišen na základě taxonomické a chorologické revize jeřábu krasového (*Sorbus eximia*), hybridogenního druhu stejné rodičovské kombinace, který byl popsán v roce 1984 z Českého krasu (Kovanda 1984). Pomocí moderních biosystematických metod byly v rámci *S. eximia* rozlišeny dvě apomiktické linie, lišící se zřetelně morfologicky a geneticky. Průzkum ploidie pomocí průtokové cytometrie ukázal, že oba rozlišené taxony jsou triploidní. To je v rozporu s dřívějšími studiemi, kde byl u *S. eximia* zjištěn diploidní a tetraploidní stupeň

(Jankun & Kovanda 1988). Dokonce byla na diploidní úrovni pozorována i apomixie, jež je dodnes udávaná pouze v jednom nejistém případě u *S. subfusca*. Naše výsledky tyto závěry vyvracejí. Studium typového materiálu odhalilo, že jméno *S. eximia* se vztahuje k rostlinám na lokalitě v NPR Koda, populace udávané ze zbylých lokalit v Českém krasu náleží novému, zde popsanému druhu *S. barrandienica*. Terénním průzkumem bylo dodatečně zjištěno, že *S. eximia* roste na 4 lokalitách, z toho jedna vznikla výsadbou. Velikost populace je odhadována na 100 dospělých exemplářů a ca 200 juvenilních jedinců. *S. barrandienica* byl nalezen na 10 lokalitách, z toho dvě historické se nepodařilo potvrdit. Celá populace dnes čítá ca 50 exemplářů. Oba druhy nejčastěji rostou v teplomilných doubravách a dubohabřinách a v lesních lemech suchých trávníků. Vykazují malou genetickou a morfologickou variabilitu a jsou dobře diferencovaní od jeřábů vyskytujících se v Čechách. Nejvíce jsou ohroženy zánikem světlých lesů. *Sorbus barrandienica* navrhujeme zařadit do červeného seznamu taxonů ČR do kategorie kriticky ohrožený druh, *S. eximia* mezi silně ohrožené druhy (Holub & Procházka 2000). Pro přežití druhů je nutné na vybraných lokalitách zavést speciální management, k tomuto účelu doporučujeme lokality Doutnáč nebo Paní hora a Koda. K odlišení společně se vyskytujících jeřábů v Českém krasu poslouží následující klíč (čepel listů musí pocházet ze střední části fertilních brachyblastů):

1a Čepel listů mělce nebo pouze v horní třetině zastřihovaně dvojitě pilovitá (až mělce laločnatá), na rubu šedozelená, plody (korálově) červené **2**
1b Čepel alespoň některých listů laločnatá, na rubu nažloutle šedozelená, plody oranžové až oranžově červené **3**
2a Čepel listů široce eliptická až okrouhlá, mělce dvojitě pilovitá, s plochým okrajem, 6–12 cm dlouhá ..... ***S. aria* s.l.**
2b Čepel listů víceméně kosočtverečná až zaokrouhleně kosočtverečná, v horní třetině zastřihovaně dvojitě pilovitá (někdy až mělce laločnatá), se zvlněným okrajem, 4–10 cm dlouhá ..... ***S. danubialis***
3a Čepel listů víceméně eliptická, plochá, na bázi klínovitá vzácněji široce klínovitá, mělce laločnatá; zářez mezi druhým a třetím lalokem (0,40–) 0,45–0,60 (–0,75) cm dlouhý, kališní cípy (2,3–) 2,5–3,5 (–3,8) mm dlouhé, plody často delší než široké ..... ***S. barrandienica***
3b Čepel listu široce vejčitá až široce eliptická, často lžícovitě prohnutá, na bázi většinou zaokrouhlená nebo široce klínovitá, dvojitě pilovitá až pravidelně mělce laločnatá, zářez mezi druhým a třetím lalokem (0,25–) 0,40 (–0,55) cm dlouhý, kališní cípy (1,7–) 2,1–2,5 (–3,0) mm dlouhé, plody často stejně široké jako dlouhé nebo širší ..... ***S. eximia***

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Appendix 1. – Key for determining the species of the *Sorbus latifolia* agg. and *S. aria* s.l. occurring in the Český kras Karst.

1a Leaf lamina shallowly or coarsely double serrate (to shallowly lobed) distally, greyish-green beneath, fruits red 2

1b Leaf lamina (at least some) shallowly lobed, yellowish-greyish-green beneath, fruits orange to orange-red ... 3

2a Leaf lamina broadly elliptical to rounded, shallowly double serrate, with flat margin, 6–12 cm long .....***S. aria* s.l.**

2b Leaf lamina more or less rhomboidal to round rhomboidal, coarsely double serrate (to shallowly lobed) distally, with folded margin, 4–10 cm long .....***S. danubialis***

3a Leaf lamina more or less elliptical, flat, cuneate rarely broadly cuneate at base, shallowly lobed; incision between the second and the third lobe terminating the main veins (0.40–) 0.45–0.60 (–0.75) cm long, sepals (2.3–) 2.5–3.5 (–3.8) mm long, fruits often longer than wide .....***S. barrandienica***

3b Leaf lamina broadly ovate to broadly elliptical, often cochleariform, usually rounded or broadly cuneate at base, double serrate to regularly shallowly lobed, incision between the second and the third lobe terminating the main veins (0.25–) 0.40 (–0.55) cm long, sepals (1.7–) 2.1–2.5 (–3.0) mm long, fruits often as wide as or wider than long .....***S. eximia***