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Hypericum dubium Leers—New Data on Taxonomy and Biology

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Abstract New data on the taxonomy, karyology, reproductive biology and secondary metabolites of *Hypericum dubium* Leers (=*H. maculatum* subsp. *obtusiusculum* (Tourlet) Hayek) are given. The neotypification of the name *H. dubium* by plants from the *locus classicus* near the town of Herborn in Germany is presented. *Hypericum dubium* is characterized by stems with slightly conspicuous subsidiary lines, sepals with a finely denticulate or rarely entire apex, petals with pale and black, linear to striiform laminar glands. Karyological data confirm that the taxon is tetraploid ($2n=32$). Reproduction by facultative apomixis was discovered using a flow cytometric seed screen. In the spectrum of secondary metabolites, flavonoids, naphtodianthrones and phloroglucinol derivatives were found. The previously described taxon *H. carpaticum* Mártonfi is reclassified here to the level of nothospecies as *H. dubium* × *H. maculatum*. For the epitheton *balcanicum* a new combination *Hypericum* ×*carinthiacum* nothosubsp. *balcanicum* (N. Robson) Mártonfi is proposed. Finally, this paper provides a revised scheme of relationships among taxa of the *H. maculatum* group.

Keywords Distribution · Flow cytometry · Karyology · Morphology · Neotypification · Secondary metabolites

Introduction

The genus *Hypericum* L. (Clusiaceae, Guttiferae) comprises about 450 species of trees, shrubs and herbs that occur in almost all temperate zones of the world. The taxonomy of the genus is complicated; as currently circumscribed, it is subdivided into 30 sections (Robson 2003). According to the original monographer of the genus, *Hypericum dubium* Leers was delimited as a tetraploid taxon belonging to the section *Hypericum* (Robson 2001, 2002). However, Robson evaluated it only at subspecies level as *H. maculatum* subsp. *obtusiusculum* (Tourlet) Hayek (Robson 2002). Data presented in this paper suggest that Robson's *H. maculatum* subsp. *obtusiusculum* is distinct among *H. maculatum* taxa and is more appropriately defined as its own species. *Hypericum maculatum* Crantz (s. str.–i. e., without *Hypericum dubium*) is

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the most primitive species in the (mainly European) series *Hypericum* (characterized by stems persistently two to four lined) of *H.* sect. *Hypericum* subsect. *Hypericum*. It consists of two morphologically similar groups of populations, which are usually treated at subspecies level and which have overlapping distributions. *H. maculatum* subsp. *immaculatum* (Murb.) A. Fröhl. is confined to southern Romania, western and southern Bulgaria, northern Greece, F.Y.R.O.M. (the former Yugoslav Republic of Macedonia), southern Serbia, Bosnia and Herzegovina, and Montenegro (for a distribution map see Robson 2002: 70) and lacks black glands on the petals (the pale ones being linear and the leaves usually pale-gland-dotted). Its chromosome number is not known. *H. maculatum* subsp. *maculatum* is widespread in subalpine and alpine regions of southern Europe, and in hilly regions and lowlands further north (from Scotland (but not present in England), through northern Scandinavia, northern Russia to western Siberia in the north, and from Spain, through northern Italy, northern Greece, central Ukraine to southwestern Siberia in the south; introduced in British Columbia, Canada; for a distribution map see Robson 2002: 70).

Hypericum maculatum is probably the ancestral taxon that gave rise to some tetraploid *Hypericum* species, such as *Hypericum perforatum* L. ($2n=32$). Robson (2002) stated that this species is of allotetraploid origin, which arose by the putative hybridization between *Hypericum maculatum* subsp. *immaculatum* that was probably distributed also in Asia in the past and *Hypericum attenuatum* Choisy, a species native to eastern Siberia, the Far East, Mongolia, China and Korea. After hybridization the populations were likely stabilized by (facultative) apomixis (Noack 1939; Mártonfi et al. 1996; Matzk et al. 2001, 2003). Other work, however, has suggested that *H. perforatum* could be an autopolyploid (Brutovská et al. 2000).

Hypericum dubium (syn. *H. maculatum* subsp. *obtusiusculum*), the focus of this study, represents another taxon that is putatively derived from *H. maculatum*. It differs from *H. maculatum* as follows: stems with less conspicuous subsidiary lines, sometimes partly absent, inflorescence branches more widely ascending, at an angle of approximately 50° from the stem, sepals broadly to narrowly ovate, with apex finely eroded-denticulate or rarely entire, petals with laminar pale and black glands, linear to striiform and sometimes punctiform (Mártonfi et al. 1999; Robson 2002). Its putative distribution covers northwestern Europe and extends eastwards to western Germany and Bohemia (except for most of western Scotland), valleys of the Alps, Styria and western Hungary (Mártonfi et al. 1999, Robson 2002, for the distribution map see Robson 2002: 70). Robson (1958) stated that according to his observations of chromosome morphology and experiments using colchicine doubling that have led to tetraploid plants, *H. dubium* is an autotetraploid derived from *H. maculatum* subsp. *maculatum*. Later, Robson (2002) changed this opinion and suggested that on the basis of the presence of linear glands (sometimes pale) in the petals and the greater frequency of pale glands in the leaves, it is probable that *H. dubium* is derived from *H. maculatum* subsp. *immaculatum*. Similarly to *H. perforatum*, the apomictic origin of seeds was also recorded for *H. dubium* (Matzk et al. 2003).

Plants that are very similar to the species *H. dubium* were described from the territory of Slovakia as *H. carpaticum* Mártonfi (2001). These plants are pentaploid ($2n=40$) and differ from *H. dubium* by having narrower sepals, smaller pollen grains and seed weight. These plants are also facultatively apomictic (for details on morphology and biology see Mártonfi 2001). At the time of description it was

suggested that this species was most likely the result of a hybridization event between *H. maculatum* and *H. perforatum*, stabilized by inherited apomixis. Because of this presumed origin, Robson (2002: 107) included this taxon in the synonymy of hybrid taxa of the above parents (sub *H.* ×*desetangsii* nothosubsp. *carinthiacum* nothoforma *perforatiforme* (A. Fröhl.) N. Robson). However, new data presented later in this paper suggest an alternative scenario.

The group of taxa derived from *H. maculatum* is not sufficiently reproductively isolated and therefore they hybridize. Offspring derived from interspecific crosses within the *H. maculatum* group are morphologically intermediate relative to the parental taxa. The hybrid progeny derived from the same parental species can also display a different morphology, depending on its ploidy level. For example, triploids may arise as a result of fertilization of a reduced haploid (n=8) egg cell by unreduced pollen (n=16), or as a result of reduced parthenogenesis from hexaploid plants (the latter possibility is, however, very rare; Lihová et al. 2000). Pentaploids may arise by fertilization of an unreduced tetraploid (n=32) egg cell by reduced pollen (n=8), or by backcrosses (Mártonfi et al. 1996; Lihová et al. 2000; Matzk et al. 2001, 2003). Tetraploid species *H. dubium* and *H. perforatum* can also be affected by an introgressive hybridization (Crackles 1990; Robson 2002). This leads to the formation of transient types between the species. The outline of relationships in the group of *H. maculatum* including hybrids is shown in Fig. 1 (including new knowledge from this study).

In 1999 the author of this paper collected plants of the genus *Hypericum* in the region of the Veľká Fatra Mts. Among them were plants that could be classified only as *H. dubium*. This species had not been reported from the territory of Slovakia before. This led to more detailed investigation of the region, and to its discovery in close localities in the Choč Mts. The aims of the present study were (1) to report on *H. dubium* as a new species for the Flora of Slovakia, and to provide its detailed morphological description, data on reproductive biology, as well as secondary metabolites. The

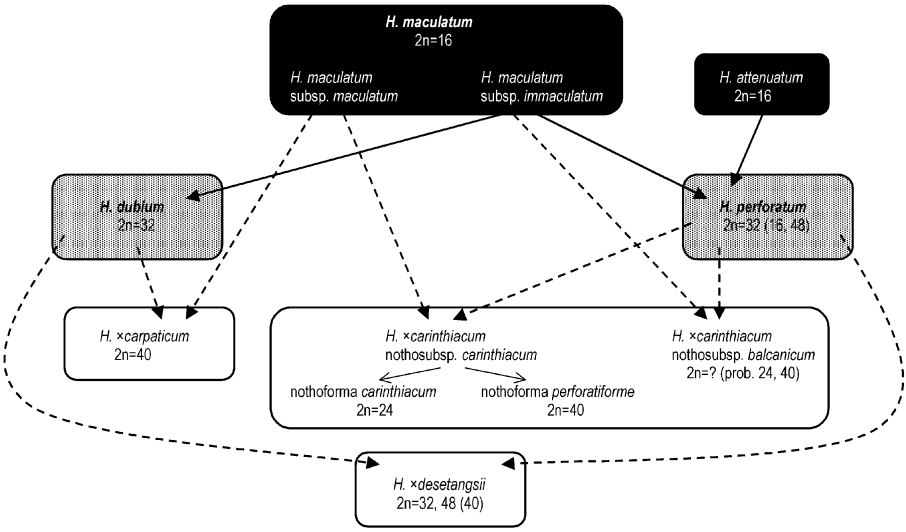

Fig. 1 Scheme of relationships in the *Hypericum maculatum* group. Relationships (*solid lines*) and hybrids (*dashed lines*). According to Robson (2002: 64), modified and completed

absence of type material of this species (cf. Stafleu and Cowan 1979; Robson 2002) led to a further aim: (2) typification of this name. The third aim was to present a modified taxonomic and nomenclatural concept of the studied group based on new findings.

Material and Methods

Plants

Material of *Hypericum dubium* was collected at three localities in Slovakia (see below, the section Distribution in Slovakia), and in Germany. After the discovery in Slovakia in 1999, two plants from locality nr. 1 and two plants from locality nr. 2 (see below) were cultivated on experimental plots in the Botanical Garden of P. J. Šafárik University in Košice, Slovak Republic. In addition, 10 plants were grown from seeds collected in 2002 from localities nr. 1 and 3 (five plants from each). Plants from both the natural localities and the experimental plots were used in karyological, flow cytometric and HPLC analyses. Voucher specimens are deposited in the KO herbarium, plants from Germany in BRA and in KO.

Karyology

Chromosome numbers were counted in root meristems of cultivated plants (localities nr. 1 and 2, two plants each) and from several germinated seeds collected from these plants. Root-tips were pre-treated using 0.002 M solution of 8-hydroxychinoline for 4 h at low temperature (4°C), and fixed for 2 h in a mixture of 97% ethanol and glacial acetic acid (3:1). Maceration was carried out by hydrolysis in 1 M HCl, at 60°C for 5 min. Root tip meristems were squashed in a drop of 45% acetic acid under a cellophane square (Murín 1960). The slides were stained in a 10% solution of Giemsa stock solution in Sörensen phosphate buffer.

Flow Cytometric Seed Screen

Flow cytometric analyses were carried out following Matzk et al. (2000). The analyses were done on mature dry seeds from two cultivated plants originating from locality nr. 1, and from two cultivated plants from locality nr. 2. To release nuclei from embryo and endosperm, 50 seeds per sample were chopped with a razor blade in a 2-ml buffer (0.107 g $MgCl_2 \cdot 6H_2O$, 0.5 g NaCl, 1.211 g Tris, 0.1 ml Triton X-100 in 200 ml aqueous solution, pH 7.0) supplemented with 1 mg/l DAPI (4′,6-diamino-2-phenylindole). The resulting nuclear suspension was filtered (42 μm) and stored on ice until measurement. The analyses were made on Partec Ploidy Analyser PA-II (Partec GmbH, Münster, Germany) at the Institute of Botany of the Czech Academy of Sciences in Průhonice, Czech Republic.

HPLC Estimate of Secondary Metabolites

Three fully opened flowers were taken from each of the 10 plants studied (cultivated individuals obtained from the seeds collected in localities nr. 1 and 3). Air-dried

flowers were extracted in methanol and immediately injected using the analytic sample injector–20 μl (Ecom, Prague). The modified gradient method was used for resolution of flavonoids, naphthodianthrones and acylphloroglucinols (Hölzl and Ostrowski 1987). HPLC (gradient pump LCP 3001, Ecom, Prague) system with Separon SGX C18 (7 μm, 3.3×150 mm) column (Tessek, Prague), and a variable UV/VIS detector LCD 2040 (Ecom, Prague) was used for sample analysis (wavelength 254 nm). Mobile phase: A: acetonitrile/water/H_3PO_4 (19:80:1) and B: acetonitrile/methanol/H_3PO_4 (59:40:1) (Merck, Darmstadt) were used as an eluent (flow rate=0.5 ml/min, gradient program=0 min–25% B, 5 min–30% B, 10 min–55% B, 15 min–100% B, 25 min–100% B); Integrator CSW (Ecom, Prague) was employed for data evaluation. The following standard compounds were used for comparative identification: hyperoside, isoquercitrin, quercitrin, quercetin and hypericin (Roth). Standard compounds 3,8″-biapigenin and hyperforin were isolated and purified by partition and by column chromatography. UV spectral analysis was carried out to confirm compound identification.

Results and Discussion

Neotypification of Hypericum dubium *Leers*

The taxonomy of the *Hypericum maculatum – H. dubium – H. perforatum* group is complicated, and characterized by sympatric occurrence of morphologically similar taxa. Of these taxa *H. perforatum*, *H. maculatum*, as well as *H. maculatum* subsp. *immaculatum* have been already typified (Robson 1968, 2002). In this context typification of the name *Hypericum dubium* Leers became inevitable.

Hypericum dubium Leers, Fl. Herborn.: 165, 1775 (for synonymy see Mártonfi et al. 1999, Robson 2002).

Ind. loc.: [Germany, Herborn] “H. ad Sepes am Sieghaus; am Homberg;”. *Neotypus (hoc loco designatus)*: Herborn, Homberg, 280 m a. s. l., 50°41′13″ N, 8°9′79″ E, 10 Jul 2006, *Friedrich Graffmann* (BRA; *isoneotypi* KO) (det. Pavol Mártonfi, 30 Aug 2006)–Fig. **2**.

Johann Daniel Leers (*1727 in Wunsiedel im Fichtelgebirge–†1774 in Herborn), in his “Flora Herbornensis” (Leers 1775) described the species *H. dubium* as a close relative of the species *H. quadrangulum* L. with the diagnosis “floribus trigynis, caule subqaudrato herbaceo, foliis ovatis, calycibus obtusissimis”. He considered the four-sided stem with less conspicuous subsidiary lines the main diagnostic character when it is compared to the four-sided stem of *H. quadrangulum*. He also pointed out that this taxon might be in fact a variety of *H. quadrangulum.* It should be remarked here that the name *H. quadrangulum* has been used in the sense of *H. tetrapterum* Fries for a long time and at the same time in the sense of *H. maculatum* Crantz. At present, *H. quadrangulum* is a rejected name (McNeill et al. 2006) based on the proposal by Robson (1990). *Hypericum dubium* was then used in parallel with the later name at subspecies level (*H. maculatum* subsp. *obtusiusculum* (Tourlet) Hayek Sched. Fl. Stiriac. 23–24: 27, 1912). The name *H. dubium* can be found in the following works: Smith (1796, 1800), Babington (1843), Bellynck (1855), Syme (1864), Schinz (1905), Buttler et al. (1996), Mártonfi et al. (1999), Kaplan and

Fig. 2 Neotypus of *Hypericum dubium* Leers (BRA)

Zelený (2002), Graffmann (2004). According to Stafleu and Cowan (1979) and Robson (2002), syntypes of *H. dubium* were probably included in Leers' herbarium, which was taken to Russia into the herbarium of G. F. Hoffmann. Leers' herbarium was allegedly destroyed in 1812 in the fire in Moscow (Stafleu and Cowan 1979: 811) or partially destroyed and partially lost (Robson 2002). According to Štěpánek (personal communication, 2005) a large part of Leers' herbarium may be included in the collection of Hoffmann, which is a herbarium collection of remarkable importance (Hoffmann's General Herbarium), deposited in Herbarium Universitatis Mosquensis (MW). This information led to an assumption that Leers' original material might be kept in Hoffmann's herbarium. The curator of the herbarium MW, Sergey Balandin (personal communication, 2005) confirmed that Hoffmann, unfortunately, usually replaced the original herbarium sheet labels provided by the collector with new ones that he produced by himself. Hoffmann gave some further information in his printed catalogue (Hoffmann 1825). I tried to find the possible type material of *H. dubium.* Hoffmann's catalogue (Hoffmann 1825: 320) and herbarium collection (Hoffmann's General Herbarium No. 6178) include a specimen identified as *H. dubium.* On the herbarium sheet label there is the following text: "*Hypericum dubium*. Helvet.". According to Balandin (personal communication, 2005) the text is probably Hoffmann's handwriting.

Hoffmann's catalogue (Hoffmann 1825) gives "Stockhorn. Helv." after the species name. This excludes the specimen as part of the original material. In addition, after the examination of the morphology of the herbarium specimen it is clear that this plant belongs to the species *H. maculatum*. After this investigation it was almost clear that the type material of *H. dubium* was really destroyed and neotypification is inevitable.

Leers' "Flora Herbornensis" was recalled after many years by compatriots from Herborn and in 2004 Friedrich Graffmann published "Neue Flora von Herborn und dem ehemaligen Dillkreis" (Graffmann 2004). In this work he reviewed Leers' collections in detail. When selecting the material for the neotype I aimed at the following: the plants should be from the same locality as *locus classicus* (unfortunately, one of the localities in protologue, Sieghaus, is built up, so only the second one, Homberg, was suitable for the collection of the neotype) and the material should correspond to Leers' description as much as possible. The characters in all plant organs correspond well with the original description, with sepals remaining the critical diagnostic feature. Leers (1775: 165) wrote: "Calyx pentaphyllus: Foliolis distinctis, ovatis, integerrimis, obtusissimis, flavo-viridibus, lineolis interruptis atris undique notatis." Robson (2002: 73) indicated that the sepals often had an apex that was finely eroded-denticulate, and entire sepals were found in Kikcudbrightshire, SW Scotland. In the plants from *locus classicus*, collected more than 200 years after the description of the species, denticules at the sepal apex are also present. However, they are not conspicuous and the sepals as a whole (without the use of stereomicroscope) give the impression of being entire. Therefore we suppose that Leers' original material could have had the denticules in the sepal apex; however, Leers did not pay attention to them. He compared them with other species with conspicuously dentate and glandular sepals. Therefore we consider the material from the *locus classicus* to be suitable for the selection of the neotype of the name *H. dubium*.

Hypericum dubium *Leers—New Data on Biology and Distribution*

In the next section I present a morphological description of the species, new karyological data, data on reproductive mode and secondary metabolites. The data are based on the material collected in Slovakia. A survey of its present occurrence in Slovakia is also given.

Description Perennial herb 0.4–1.0 m tall, erect or ascending from a creeping, rooting base, stems branched above for approximately 2/3 of their length. Stems four-lined, the subsidiary ones often less prominent. Leaves sessile, lamina 10–40× 8–20 mm, broadly to narrowly elliptic, paler beneath, apex rounded, margin plane, base rounded; with rather dense tertiary venation, laminar gland dots pale, sometimes also black, few, punctiform; intramarginal glands black, irregular in size. Inflorescence branches widely ascending, at an angle of 45–70° from the stem, pedicels 1.5–4 mm; bracts and bracteoles up to 5 mm long, narrowly triangular-ovate to narrowly elliptic, entire. Flowers 23–30 mm in diameter, stellate to reflexed, buds broadly ellipsoid, obtuse. Sepals 5, (4.2–)4.3–5.7(–5.8)×(2.1–)2.3–2.8(–2.9) mm, broadly to narrowly ovate (ratio length to width 1.6–2.5), with apex finely eroded-denticulate or rarely entire, erect to recurved in bud, recurved in fruit, veins 5(–7), not or slightly branched, laminar glands pale and sometimes also black, punctiform and sometimes striiform; intramarginal glands pale or occasionally black or often absent. Petals 5, golden yellow, 12.3–16.0×(4.6–)4.8–6.3 mm, sometimes distally unilaterally crenate with marginal black glands, laminar glands pale and black, linear to striiform and sometimes punctiform. Stamens 60–80, three-fascicled, 7.0–11.2 (–12.2) mm long, anther 0.8×0.9 mm long (thecae approximately 0.45 mm wide) with black gland. Ovary three-locular 2.0–4.2×1.5–2.5 mm, broadly ovoid to ovoid-ellipsoid; styles 3, free, 3–5 mm long. Capsule 5.5–10.0×3.8–5.5 mm, ovoid-ellipsoid to broadly ovoid, valves with longitudinal vittae, linear to striiform. Seeds dark brown, 0.8–1.2 mm, cylindric, testa finely linear-foveolate.

Karyology Tetraploid chromosome number $2n=32$ (Fig. **3**) was found in the material from the sampled Slovak populations. The chromosomes are very small (approximately 0.8–1.2 μm). Little data on chromosome numbers of *Hypericum dubium* have been published prior to this report (Table **1**). Robson (1957, 1958) stated that *Hypericum dubium* (under the name *Hypericum maculatum* subsp. *obtusiusculum*) is tetraploid with $2n=32$ without giving the collection site. On the contrary, Schwarz (1965) provided a chromosome count of $2n=16$ under the name *Hypericum erosum* (Schinz) Schwarz, however, this record is considered doubtful by Robson (1981, 2002); the material studied was probably confused with *Hypericum maculatum* Crantz. (Schwarz also mentioned the count $2n=32$ by Löve A. and Löve D. for *Hypericum dubium*, however, a bibliographic citation is missing; I did not succeed in identifying the source of this information). Robson and Adams (1968) published Robson's data from 1956: $2n=32$ from localities in England (Chailey, Sussex) and Scotland (Loch Tay, Pertshire; Drum, Aberdeenshire) and from the Botanical Garden in Munich as well. When the species was discovered in the Czech Republic (Mártonfi et al. 1999) a tetraploid chromosome number was confirmed from the locality in the Doupovské hory Mts., Zelený rybník pond near the village of Bražec,

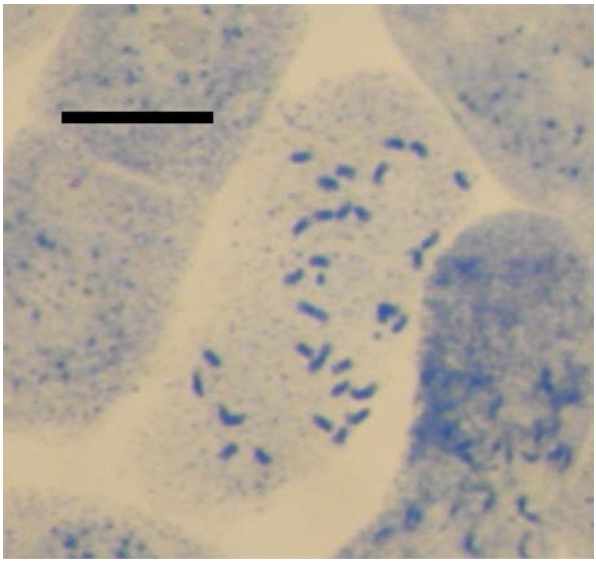

Fig. 3 Somatic metaphase plate of *Hypericum dubium* (2*n*=32). Scale bar=10 μm

E of Karlovy Vary, ca. 685 m a. s. l. (12 Jul 1996, *Mártonfi 2125*, KO). The most recent record 2*n*=32 is given by Matzk et al. (2003), without a note on the locality.

Reproduction Mode Analyses by FCSS (flow cytometric seed screen, Matzk et al. 2000, Fig. 4) showed that the plants of *H. dubium* originating from Slovakia are tetraploid, and two processes participate in their seed formation: (1) sexual reproduction, which leads to embryo with 2*n*=32 (2C peak of FCSS) formed from

Table 1 Chromosome counts in *Hypericum maculatum* and *Hypericum dubium*

Taxon, chromosome number	Original determination	Source
Hypericum maculatum		
2*n*=16	*H. maculatum*	Noack (1939), Robson (1957), Robson and Adams (1968), Reynaud (1975), Pogan and Rychlewski (1980), Arohonka (1982), Laane and Lie (1985), Dmitrieva (1986), Parfenov and Dmitrieva (1987), Pashuk (1987), Měsíček and Javůrková-Jarolímová (1992), Lövkvist and Hultgård (1999)
	H. erosum[a]	Schwarz (1965)
n=8	*H. maculatum*	Nielsen (1924), Winge (1925), Robson (1957), Laane (1969), Robson (1981)
Hypericum dubium		
2*n*=32	*H. maculatum* subsp. *obtusiusculum*	Robson (1957), Robson (1958), Robson and Adams (1968)
	H. dubium	Mártonfi et al. (1999), Mártonfi *hoc loco*

[a] *H. erosum* is a synonym for *H. dubium*, but probably the material collected by Schwarz was misidentified (for details see Robson 2002: 75).

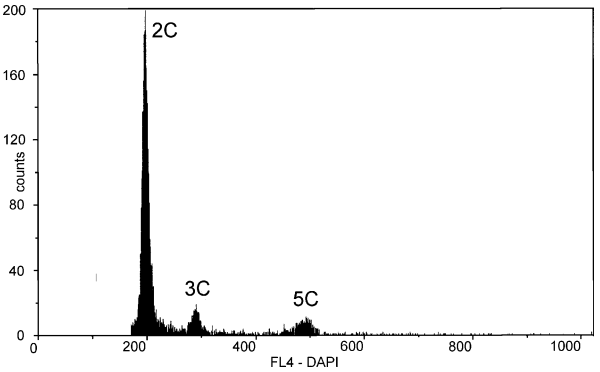

Fig. 4 Flow cytometric seed screen of *Hypericum dubium*. 2C peak represents both sexual and apomictic embryo, 3C peak represents endosperm from sexual reproduction, 5C peak represents endosperm from apomictic pseudogamous reproduction

reduced ovule and pollen (both $n=16$), and endosperm with 3C, and (2) apomictic pseudogamy reproduction, with unreduced ovule $2n=32$, embryo with $2n=32$ (2C peak of FCSS), and endosperm with 5C. Unreduced double fertilization is also possible and would be manifested by a 3C peak of embryo and a 5C peak of endosperm. Thus, the species *H. dubium* can be considered as a facultative apomict. Matzk et al. (2003) first discovered this, but the origin of the plants studied was not known (published under the name *H. maculatum* subsp. *obtusiusculum*). The results are similar in further *Hypericum* taxa (Matzk et al. 2001, 2003). It is not clear so far, whether the apomixis evolved independently in *H. dubium* or if it is a character obtained secondarily owing to recent introgression from *H. perforatum*.

Secondary Metabolites Apart from the flavonoids, biflavonoids, naphthodianthrones and phloroglucinol derivatives (present in the genus *Hypericum*), the secondary metabolites found in the material studied correspond to those found for *H. dubium* from other localities, i.e., in England (Mártonfi et al. 2006) and in the Czech Republic (Mártonfi et al. 1999). The following secondary metabolites are present (numbers in parentheses represent percentages of the compound in the sample of dry mass of fully open flowers, averages from 10 measurements): hyperoside+isoquercitrin (0.113%), quercitrin (0.108%), quercetin (0.023%), 3,8″-biapigenin (0.230%), pseudohypericin (0.477%), hypericin (0.128%). Rutin and hyperforin, typical for the majority of populations of *H. perforatum* and its hybrids, are not present (Mártonfi 2001; Mártonfi et al. 2001, 2006; for review see Kaul 2000; Hölzl and Petersen 2003).

Distribution in Slovakia In this paper *H. dubium* is presented as a new species for the Slovak flora. As stated by Mártonfi et al. (1999), the most proximate localities of the species are situated in Hungary (Bakony Mts., western Hungary) and the Czech Republic (Doupovské hory Mts., NW of the Czech Republic). In Slovakia the species has been found in localities in two orographic/phytogeographic regions in NW Slovakia: Chočské vrchy Mts. and Veľká Fatra Mts. Voucher specimens:

1. Slovakia, Chočské vrchy Mts., about 500 m east of the village of Valaská Dubová, small forest meadow, about 690 m a. s. l., 49°08′50″ N, 19°18′12″ E (6881d), (22 Jul 1999, *P. Mártonfi 2183–2186, 2190*; 11 Sept 2002, *P. Mártonfi 2568*).

2. Slovakia, Veľká Fatra Mts., in the margin of the village of Studničná, near forest, about 800 m a. s. l., 49°08′45″ N, 19°18′15″ E (6881d) (22 Jul 1999, *P. Mártonfi 2194, 2196, 2198–2200*).
3. Slovakia, Chočské vrchy Mts., the village of Valaská Dubová, near the village, pasture-land with *Pteridium aquilinum* L., about 690 m a. s. l., 49°08′37″ N, 19° 17′49″ E (6881d) (11 Sept 2002, *P. Mártonfi 2557*).

The species is also expected to be found in other regions in Slovakia and probably in other localities in the Czech Republic, too, which could lead to a more continuous distribution range. In addition, the species is supposed to occur also eastwards from the localities known to date.

Hypericum dubium is distributed mainly in western Europe. The closest occurrence of the species was reported from western Bohemia and western Hungary (Mártonfi et al. 1999). The new finding of the species in Slovakia extends its eastern distribution limit hundreds of kilometers eastwards.

Comments on H. ×carpaticum *Mártonfi*

H. ×carpaticum Mártonfi, Folia Geobot. 36: 374, 2001 (pro sp., in the sense of Art. 50.1. of ICBN; McNeill et al. 2006) was described as an apomictic taxon from the group *H. perforatum – H. maculatum*. It is a pentaploid ($2n=40$) taxon, which is morphologically very similar to *H. dubium*. The knowledge at the time of species description led to the hypothesis that the taxon is of a hybrid origin with the following parents: *H. perforatum* being a facultatively apomictic taxon, serving as a mother plant providing unreduced female gametes with 32 chromosomes, and *H. maculatum* as a pollen donor ($n=8$). It was suggested that the new taxon was stabilized and became sufficiently reproductively isolated from its parents due to inherited apomixis, and its phenotypic characteristics were shifted and stabilized (it differed both morphologically and by secondary metabolite content from the known hybrid plants of the above given parents) and spread into its own, even if relatively small, area. New data, however, revealed (see above) that *H. dubium*, a facultative apomict, occurs in relative proximity to the species *H. ×carpaticum*. Therefore, an alternative and much simpler explanation of the origin of *H. ×carpaticum* is that it is a hybrid betwen *H. dubium* as the mother plant (providing female gametes with 32 chromosomes), and *H. maculatum* as the father plant (providing pollen with 8 chromosomes). This corresponds to the morphological appearance of the plants of *H. ×carpaticum*, which are very similar to *H. dubium*, and, like both parent species, lack the secondary metabolites rutin and hyperforin (on the contrary to *H. perforatum* and its hybrids with *H. maculatum*, in which both rutin and hyperforin occur, for details see Mártonfi 2001; Mártonfi et al. 2006). A scheme of relationships among the taxa of the group *H. maculatum* is given in Fig. **1**, taking into account the here presented new findings.

Nomenclatural Notes

1. For the sake of correctness of the designation of the name *H. ×carpaticum* in accordance with Articles H.10.1., H.10.2. and H.3.1. of ICBN (McNeill et al.

2006) I give the following correction–reclassification of the name to the level of nothospecies:

Hypericum ×*carpaticum* Mártonfi, Folia Geobot. 36: 374, 2001 (pro sp.)
=*Hypericum dubium* Leers × *Hypericum maculatum* Crantz

2. New combination–Robson (2002) considered *H. dubium* a subspecies of the species *H. maculatum*; if both *H. dubium* and *H. maculatum* are treated at the species level, a new combination for the epitheton *balcanicum* is needed.

Hypericum ×*carinthiacum* nothosubsp. *balcanicum* (N. Robson) Mártonfi, comb. nova.

Bas.: *Hypericum* ×*desetangsii* nothosubsp. *balcanicum* N. Robson, Bull. Nat. Hist. Mus. Lond. (Bot.) 32: 107, 2002. (=*H. maculatum* Crantz subsp. *immaculatum* (Murb.) A. Fröhl. × *H. perforatum* L.)

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