

## ***Hypericum dubium* – a new species of the Czech flora**

***Hypericum dubium* – nový druh české flóry**

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Dedicated to František Procházka on the occasion of his 60<sup>th</sup> birthday

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A new species of the Czech flora, *Hypericum dubium* Leers [= *H. maculatum* subsp. *obtusiusculum* (Tourlet) Hayek] was found in the Dourovské hory Mts, in the northwest of the Czech Republic. Besides morphological description, karyological (2n = 32), chemical and chorological data (especially for the Czech Republic and Hungary) are given. The find means that the distribution limits of the species north of 50° of the northern latitude shift eastwards.

**Keywords:** *Hypericaceae*, *Hypericum dubium*, Czech Republic

### **Introduction**

In autumn 1995, J. Michálek and J. Hadinec directed attention of P. Mártonfi to their remarkable collections of plants from the genus *Hypericum* from the Dourovské hory Mts (Czech Republic). After studying them it was obvious that they belong to the species *Hypericum dubium* Leers [= *Hypericum maculatum* subsp. *obtusiusculum* (Tourlet) Hayek]. This finding started cultivation and a detailed study of this taxon from the territory of the Czech Republic. Karyological and chemical studies confirmed the correctness of original determination of the plants.

### **Material and methods**

#### *Plants*

The plants were collected on the localities given in the part on the species distribution. Several plants were taken to cultivation to the Botanical Garden of P. J. Šafárik University, Košice (Slovakia). Voucher specimens are deposited in the herbarium of Regional Museum in Sokolov, Czech Republic (OMS), herbarium of Department of Botany, Charles

University, Prague, Czech Republic (PRC) and Botanical Garden of P. J. Šafárik University in Košice, Slovakia (KO).

### Karyology

Chromosome numbers were counted in root meristems of cultivated plants. Root-tips were pre-treated by 8-hydroxychinoline, 0.002 M solution during 4 hours at low temperature (4 °C), fixed 2 hours in the mixture of 97 % ethanol and glacial acetic acid (3 : 1). Maceration was carried out by hydrolysis in HCl, 1 M solution at temperature 60 °C during 5 minutes. Root tip meristems were employed to prepare slides by squashing it in a drop of 45 % acetic acid under cellophane square. The slides were dyed in 10 % solution of Giemsa stock solution in Sörensen phosphate buffer.

### Palynology

Mature anthers were removed from herbarium specimens and prepared for LM and SEM using the slightly modified acetolysis procedure according to Erdtman (1960). For LM, pollen grains were mounted on slides in glycerine jelly. For SEM, pollen air dried using 95 % ethanol was mounted on a glass cover slip attached to an aluminium stub and then coated with gold, to a thickness of 300 Å, using a JEOL JFC 1100 sputter coater. Subsequent examination was with TESLA BS 340 scanning electron microscope.

### Extraction and HPLC estimation of secondary metabolites

Flower samples were taken from fully open flowers. Air-dried flowers were extracted in methanol and immediately injected by Rheodyne sample injector (20 µl). The gradient method was used for resolution of flavonoids, naphtodianthrones and acylphloroglucinols (Hölzl & Ostrowski 1987). Equipment: gradient pump (Ecom, Praha), variable UV-VIS detector (Hewlett-Packard, Palo Alto, model 1050), column (3×150 mm) SGX C18, 7µm (Tessek, Praha), integrator Apex (Ecom, Praha). HPLC purity solvents (Fluka) were used. The following standard compounds were used for comparative identification and quantitative determination: 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.

### Miscellanea

Abbreviations of herbaria are in accordance with Holmgren et al. (1990). OMS stands for Regional Muzeum, Sokolov, Czech Republic, and ZIRC for Regional Natural History Muzeum, Zirc, Hungary. In nomenclatural section, symbols for nomenclatural (=) and taxonomical (=) synonyms and for invalid names (–) are used. Abbreviations of authors are according to Brummitt & Powell (1992), abbreviations of journal titles according to Lawrence et al. (1968) and Bridson & Smith (1991).

## Results and discussion

### Nomenclature and taxonomy

The species *Hypericum dubium* was separated from other types of the genus *Hypericum* with 4-lined stem by Leers (1775, p. 165). The description and distribution of the species is in accordance with recent knowledge except for calyces ("Calyx pentaphyllus: Foliolis distinctis, ovatis, integerrimis, obtusissimis, ...") which suit this description (ovate and entire) only rarely. Even if the plant of the original type specimen had such calyces, it does not necessarily mean that this name cannot be associated with plants dealt in this work [Art. 7.2. of Code (Greuter et al. 1994): "...The nomenclatural type is not necessarily the most typical or representative element of taxon."]. Since according to Stafleu & Cowan (1979), herbarium and types of Johann Daniel Leers (1727–1774) are unknown and part of the herbarium was acquired by G. F. Hoffman, but destroyed in the Moscow fire of 1812, the selection of neotype will be probably necessary. Fröhlich (1911) included Leers' name in synonymy of the species *Hypericum maculatum* (subsp. *typicum* A. Fröhl., nom. inval. – Art. 24. 3.). Diagnostic characters were later revised and completed by chromosome numbers by Robson (1957, 1958, 1981, Robson & Adams 1968) who classified this taxon at the subspecies level [as *Hypericum maculatum* subsp. *obtusiusculum* (Tourlet) Hayek]. In this paper we accept the taxon on the species level.

*Hypericum dubium* Leers, Fl. Herborn.: 165, 1775.

Ind. loc.: Germany, "am Sieghaus; am Homberg" Typus: ignotus.

= *Hypericum obtusum* Moench, Meth. Pl. Hort. Bot. Agri Marburg.: 129, 1794; nom. illegit. superfl. (Art. 52.1.)

= *Hypericum leersii* C. C. Gmel., Fl. Bad. Alsat. 3: 253, 1808 & 5: 575, 1826; nom. illegit. superfl. (Art. 52.1.)

= *Hypericum desetangii* [var.]  $\beta$  *imperforatum* Bonnet, Bull. Soc. Bot. France 25: 277, 1878.

Ind. loc.: "Belgique, Champion (Bellynck!), Obourg (Martinis!), France: Alsace (Buchinger!), Nord (Cussac!), Calvados (Lenormand!), Eure-et-Loir!, Sarthe (Goupil!), Loire-Inférieure (Lloyd!); assez commun dans le rayon de la flore parisienne, Meudon, Versailles, Saint-Léger, forêt de Retz, etc.". Lectotypus (Robson, in prep.): Loire-Inférieure, Lloyd s. n., ANG (n.v.)

= *Hypericum acutum* var. *imperforatum* (Bonnet) Rouy et Foucaud Fl. France 3: 337, 1896.

= *Hypericum quadrangulum* subsp. *obtusiusculum* Tourlet, Bull. Soc. Bot. France 50: 307, 1903. Lectotypus (Robson, in prep.): the same element as for *Hypericum desetangii* [var.]  $\beta$  *imperforatum* Bonnet.

= *Hypericum quadrangulum* [var.] *imperforatum* (Bonnet) Tourlet, Bull. Soc. Bot. France 50: 308, 1903.

= *Hypericum quadrangulum* var. *epunctatum* Schinz, Vierteljahrsschr. Naturf. Ges. Zürich 49: 240, 241, 1905; nom. illegit. superfl. (Art 52. 1.)

= *Hypericum maculatum* var. *imperforatum* (Bonnet) A. Fröhl., Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Cl. 120 (1): 553, 1911.

= *Hypericum maculatum* subsp. *obtusiusculum* (Tourlet) Hayek, Sched. Fl. Stiriac. 23–24: 27, 1912.<sup>1</sup> (isonymum: A. Fröhl., Mitt. Naturwiss. Ver. Steiermark 51(1): 223, 1915.)

= *Hypericum quadrangulum* var. *occidentale* Franch., Fl. Loirdet-Cher: 98, 1885.

Ind. loc.: France, Loir-et-Cher, "R. Onzain, petit bois de Maré (Monin); environs de Mondoubleau (Legué); Baillou, bois de Roquelande (id.)". Lectotypus (Robson, in prep.): Onzain, petit bois de Maré, Monin s. n.

<sup>1</sup> Correct name for the taxon at the subspecies level into the *Hypericum maculatum* Crantz.

- = *Hypericum quadrangulum* var. *erosum* Schinz, Bull. Herb. Boissier, ser. 2, 3: 21–22, 1903.  
Ind. loc.: Switzerland, “Ct. Zürich: Hohe Rhone (Nägeli, Schinz), Fischenthal 740 m (Nägeli), Bärloch, am ober Rande des Frühtobels 1000 m (Nägeli), an den Rotengübeln 1030 m (Nägeli), Hirzegg 1050 bis 1080 m (Nägeli), Strahlegg 1040 m (Nägeli). Ct. Zug: Geissboden ob Zug, 950 m (Brunies und Schinz). Ct. Schwyz: ob Arth-Goldau (Brunies und Schinz).” Lectotypus (Robson, in prep.): Zug, Geissboden ob Zug, 950 m, Brunies & Schinz s. n., Z (n. v.).
- = *Hypericum quadrangulum* subsp. *erosum* (Schinz) Schinz, Vierteljahrsschr. Naturf. Ges. Zürich 49: 240, 1905.
- = *Hypericum maculatum* subsp. *erosum* (Schinz) Schinz & R. Keller, Fl. Suisse: 381, 1909.
- = *Hypericum erosum* (Schinz) O. Schwarz, Drudea 5 (1): 63, 1965.
- = *Hypericum quadrangulum* [var.]  $\beta$  *perforatum* Tourlet, Bull. Soc. Bot. France 50: 308, 1903.  
Ind. loc.: France, “Indre-et-Loire, ... des prairies du vallon de la Desmée, aux Hermites.” Typus: (Robson, in prep.).
- = *Hypericum quadrangulum* var. *punctatum* Schinz, Vierteljahrsschr. Naturf. Ges. Zürich 49: 240, 1905; nom. illegit. superfl. (Art 52.1.)
- = *Hypericum maculatum* var. *perforatum* (Tourlet) A. Fröhl., Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Cl. 120 (1): 553, 1911.
- = *Hypericum dubium* var. *perforatum* (Tourlet) Pugsley, J. Bot., London, 78: 35, 1940.
- = *Hypericum erosum* var. *perforatum* (Tourlet) O. Schwarz, Drudea 5 (1): 64, 1965.
- = *Hypericum maculatum* f. *nigrum* A. Fröhl., Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Cl. 120 (1): 554, 1911.  
Ind. loc.: sine. Typus: ignotus.
- = *Hypericum maculatum* f. *latisepalum* A. Fröhl., Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Cl. 120 (1): 554, 1911.  
Ind. loc.: sine. Typus: ignotus.
- = *Hypericum maculatum* f. *lucidum* A. Fröhl., Sitzungsber. Kaiserl. Akad. Wiss. Wien, Math.-Naturwiss. Cl. 120 (1): 554, 1911.  
Ind. loc.: sine. Typus: ignotus.
- = *Hypericum maculatum* subsp. *styriacum* A. Fröhl., Mitt. Naturwiss. Ver. Steiermark 51 (1): 222, 1915.  
Ind. loc.: Steiermark. Typus (Robson, in prep.): Austria, Styria, ad vicum Maria-Trost prope urbem Graz, c. 430 m, July 1911, Fröhlich in Hayek, Fl. Stir. Exsicc. 1198 (H). (n. v.)  
= *Hypericum erosum* subsp. *styriacum* (A. Fröhl.) O. Schwarz, Drudea 5 (1): 64, 1965.
- *Hypericum dubium* [var.] *a genuinum* Syme, Engl. Bot., ed. 3, 2: 151, 1864; nom. inval. (Art. 24.3.).

## Morphology

### Description<sup>2</sup>

Perennial herb 0.4–1.0 m tall, erect or ascending from creeping and rooting base, stems branched above for c. 2/3 of their length. Stems 4-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, making an angle of 45–70° with 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, vein 5 (–7), not or slightly branched, laminar glands pale and sometimes also black, punctiform and

<sup>2</sup> English version was prepared with the help of Prof. Robson's manuscript, numeric data are from Czech populations.

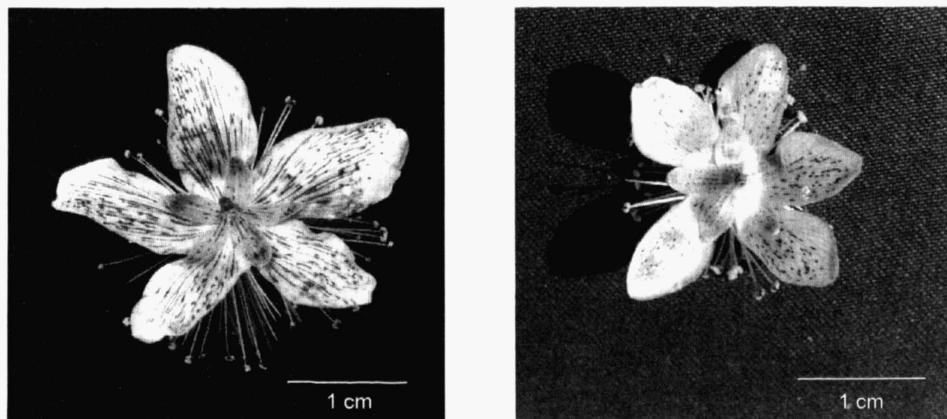


Fig. 1. – Sepals and petals of (a) *Hypericum dubium* Leers and (b) *Hypericum maculatum* Crantz.

sometimes striiform; intramarginal glands pale or occasionally black or often absent. Petals 5, golden yellow,  $12.3\text{--}16.0 \times (4.6\text{--})4.8\text{--}6.3$  mm, sometimes distally unilaterally crenate with marginal black glands, laminar glands pale and black, linear to striiform and sometimes punctiform (Fig. 1). Stamens 60–80, 3-fascicled, 7.0–11.2 (–12.2) mm long, anther  $0.8 \times 0.9$  mm (thecae ca. 0.45 mm wide) with black gland. Ovary 3-locular, 2.0–4.2  $\times 1.5\text{--}2.5$  mm, broadly ovoid to ovoid ellipsoid; styles 3, free, 3–5 mm long. Capsule 5.5–10.0  $\times 3.8\text{--}5.5$  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, weight of thousand seeds 0.0795g.

Table 1. – Comparison of main diagnostic characters among *Hypericum dubium* Leers, *H. maculatum* Crantz and hybrid *H. maculatum*  $\times$  *H. perforatum*. For chemical differences see Mártonfí et al. (1996).

Character	<i>Hypericum dubium</i>	<i>Hypericum maculatum</i>	<i>H. maculatum</i> $\times$ <i>H. perforatum</i>
Stems	4-lined, with subsidiary lines present or sometimes partly absent	4-lined, always with subsidiary lines	4-lined, with subsidiary lines partly or completely absent
Leaves	sometimes with pale glands, venation less densely reticulate	usually without pale glands, venation densely reticulate	with pale glands, venation less densely reticulate
Inflorescence	widely ascending, branches make an angle 45–70° with stem	rather strict, branches make an angle 25–45° with stem	widely ascending, branches make an angle 45–65° with stem
Sepals	$4.3\text{--}5.7 \times 2.3\text{--}2.8$ mm, apex finely eroded-denticulate, broadly to narrowly ovate (ratio length to width 1.6–2.5)	$3.1\text{--}4.5 \times 2.1\text{--}2.8$ mm, entire, broadly elliptic to broadly ovate (ratio length to width 1.3–1.9)	$6.0\text{--}7.5 \times 1.7\text{--}2.3$ , apex finely eroded-denticulate, narrowly oblong to lanceolate (ratio length to width 2.6–3.9)
Petals	$12.3\text{--}16.0 \times 4.8\text{--}6.3$ mm, entire to distally unilaterally crenate, laminar black glands mainly striiform, part of black glands punctiform	$9.0\text{--}11.0 \times 3.6\text{--}5.5$ mm, entire, laminar black glands mainly punctiform	asymmetric, $12.1\text{--}18.0 \times 4.6\text{--}6.1$ mm, usually distally unilaterally crenate, laminar black glands usually excentric, striiform to punctiform
Chromosome number	$2n = 32$	$2n = 16$	$2n = 40$ (rare 24)

Main diagnostic characters compared with *Hypericum maculatum* and hybrid *H. maculatum* × *H. perforatum* are given in Table 1. *Hypericum maculatum* (subsp. *maculatum*) is common in the Czech Republic, its hybrid with *Hypericum perforatum* is rare.

#### Karyology

Only a few data on chromosome numbers of *Hypericum dubium* have been published so far. Robson (1957, 1958) states that *Hypericum dubium* (under the name *Hypericum maculatum* subsp. *obtusiusculum*) is tetraploid with  $2n = 32$  without giving the place of collection. On the contrary Schwarz (1965) gives chromosome number  $2n = 16$  under the name *Hypericum erosum* (Schinz) Schwarz. However, the datum is considered doubtful by Robson (1981); this is probably the confusion of the material studied and *Hypericum maculatum* Crantz. (Schwarz also mentions the count  $2n = 32$  by Löve Á. & Löve D. for *Hypericum dubium*. However, bibliographic citation is missing; we did not succeed to find out the source of this information.) Robson & Adams (1968) published Robson's data from 1956:  $2n = 32$  from localities in England (Chailey, Sussex) and Scotland (Loch Tay, Perthshire; Drum, Aberdeenshire) and from the Botanical Garden in Munich as well. For the find from the Czech Republic (locality is given in the section on distribution, voucher Mártonfi 2125, KO) we corroborated tetraploid chromosome number  $2n = 32$  (Fig. 2).

#### Palynology

In accordance with classification of Clarke (1976), pollen grains of *Hypericum dubium* (Fig. 3) can be classified in *Hypericum perforatum*-type. Pollen of *Hypericum dubium* is small to medium size, subprolate to euprolate (shape classification follows Walker & Doyle 1975), polar axis: 18.8–26.1 µm, equatorial axis: 14.0–19.8 µm, P/E ratio: 1.27–1.4. The grains are 3-zonocolporate, ectoapertura is very long colpus, endoapertura is cruciform porus with meridional extensions which have slight costae. Micromorphological data (terms according to Vezey et al. 1992): Tectum is tectate (e. g. tectum coverage is more than 80 %), exine

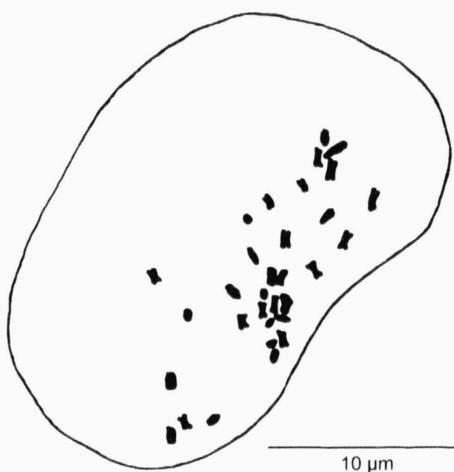


Fig. 2. – Drawing of somatic metaphase in *Hypericum dubium* Leers.

sculpture has perforations, average diameter of perforations is less than 1 µm, distance between edges of adjacent perforations is less than 1 µm and distance between edges of adjacent perforations is less than average diameter of perforations, thus exine is scrobiculate.

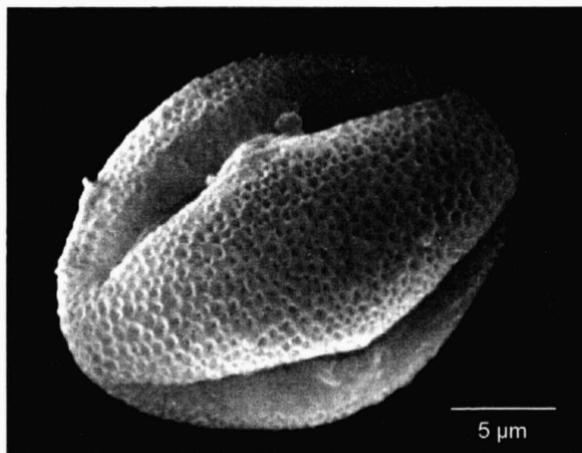


Fig. 3. – SEM micrograph of *Hypericum dubium* Leers pollen grain.

#### Secondary metabolites

The following secondary metabolites (Fig. 4) were found in methanol extract of flowers; the number in brackets represents per cent of compound in the sample of dry mass of fully open flower (average from 10 measurements): hyperoside + isoquercitrin (1.952 %), quercitrin (0.290 %), quercetin (0.816 %), 3,8"-biapigenin (0.230 %), pseudohypericin

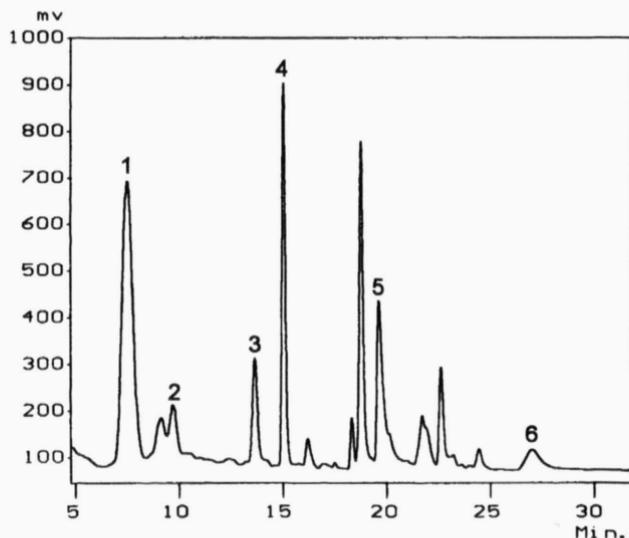


Fig. 4. – HPLC chromatogram of flower methanol extract of *Hypericum dubium* Leers. 1 – hyperoside + isoquercitrin, 2 – quercitrin, 3 – quercetin, 4 – 3,8"-biapigenin, 5 – pseudohypericin, 6 – hypericin.

(1.302 %), hypericin (0.309 %). In addition, further non-identified compounds were found in the samples and in one sample also hyperforin (1.272 %) was present.

### Distribution

#### General distribution

Up to this time, the species was reported from the northwestern Europe east to western Germany (except most of western Scotland), valleys of the Alps east to Styria (e. g. Scotland, England, Wales, Ireland, the Netherlands, Belgium, France, Germany, Switzerland, Austria – Robson, in prep.). The easternmost localities south of 49° of northern latitude are situated in West Hungary, Bakony Mts. Voucher specimens to the Hungarian localities: Montes Bakony. In silva inter Zirc and Borzavár, ca. 450 m s. m. 9 July 1934. *Polgár s. n.* (BP 261466, BP 261464, BP 261465). Montes Bakony, ad marginem silvae inter stationem ferroviae Porva-Csesznek ad pagum Borzavár, ca. 400 m s. m., 9 July 1934. *Polgár s. n.* (BP 261467). Comit. Veszprém. In fossis "Nádaser" pr. villam "Dobospuszta" iuxta pag. Sáska. 27 July 1954. *Szalay s. n.* (ZIRC). The easternmost occurrence from the area of 50°–60° parallel is in the Czech Republic.

#### Distribution in the Czech Republic

Zelený (1990) supposed that plants belonging to the taxon studied occurred at the territory of the Czech Republic. The first documented data are given only in this paper. They concern northwestern Bohemia, where the species *Hypericum dubium* occurs in the region of the Doušovské hory Mts (see the list of localities below and Fig. 5). Earlier collections of this species from the territory of the Czech Republic have not been known.

Note 1. The occurrence of the hybrid *Hypericum maculatum* × *Hypericum perforatum* from the locality close to the place of occurrence of *Hypericum dubium* (only 7 km by air line) was noted in 1938 (Fröhlich 1960). Since we did not succeed to find original voucher in herbaria and the photograph does not show details of the plant found, the find can be neither confirmed, nor disproved, nor supposed to be the species *Hypericum dubium*.

Note 2. Schwarz (1965) reported *Hypericum erosum* (Schinz) O. Schwarz from Bohemian Elbe sandstones and from southern Bohemia, however, at the same time he gives  $2n = 16$  for *Hypericum erosum*. He did not mention voucher material and we know no occurrence of *Hypericum dubium* on the above territory. At the same time Schwarz (1965) classified *Hypericum dubium* Leers in the synonymy of *Hypericum fallax* Grimm (= *Hypericum maculatum* Crantz). Obviously these data do not concern the species *Hypericum dubium* at all.

Note 3. Fröhner (1971) also reported *Hypericum erosum* (Schinz) O. Schwarz from the Krkonoše Mts, Pec pod Sněžkou. *H. dubium* is unlikely to occur at this altitude in the Czech Republic and it can be a non-typical specimen of *H. maculatum* or misidentification with the hybrid *H. maculatum* × *H. perforatum*.

#### List of localities<sup>3</sup>

1. Doušovské hory Mts. Former village of Tunkov. Ledviny region – meadows and grassy clearings on SW foot of Lesná hill (812 m a. s. l.), above little road from former village of Tunkov to former village of Žďár, 680–700 m a. s. l.; 2. 8. 1995, J. Hadinec et J. Michálek s. n.; OMS 580/95, 580A/95, 580B/95, 580C/95, PRC.

<sup>3</sup> In the list there are several villages marked as "former" because they are situated in places belonging nowadays to a military area. Thus they were abandoned.

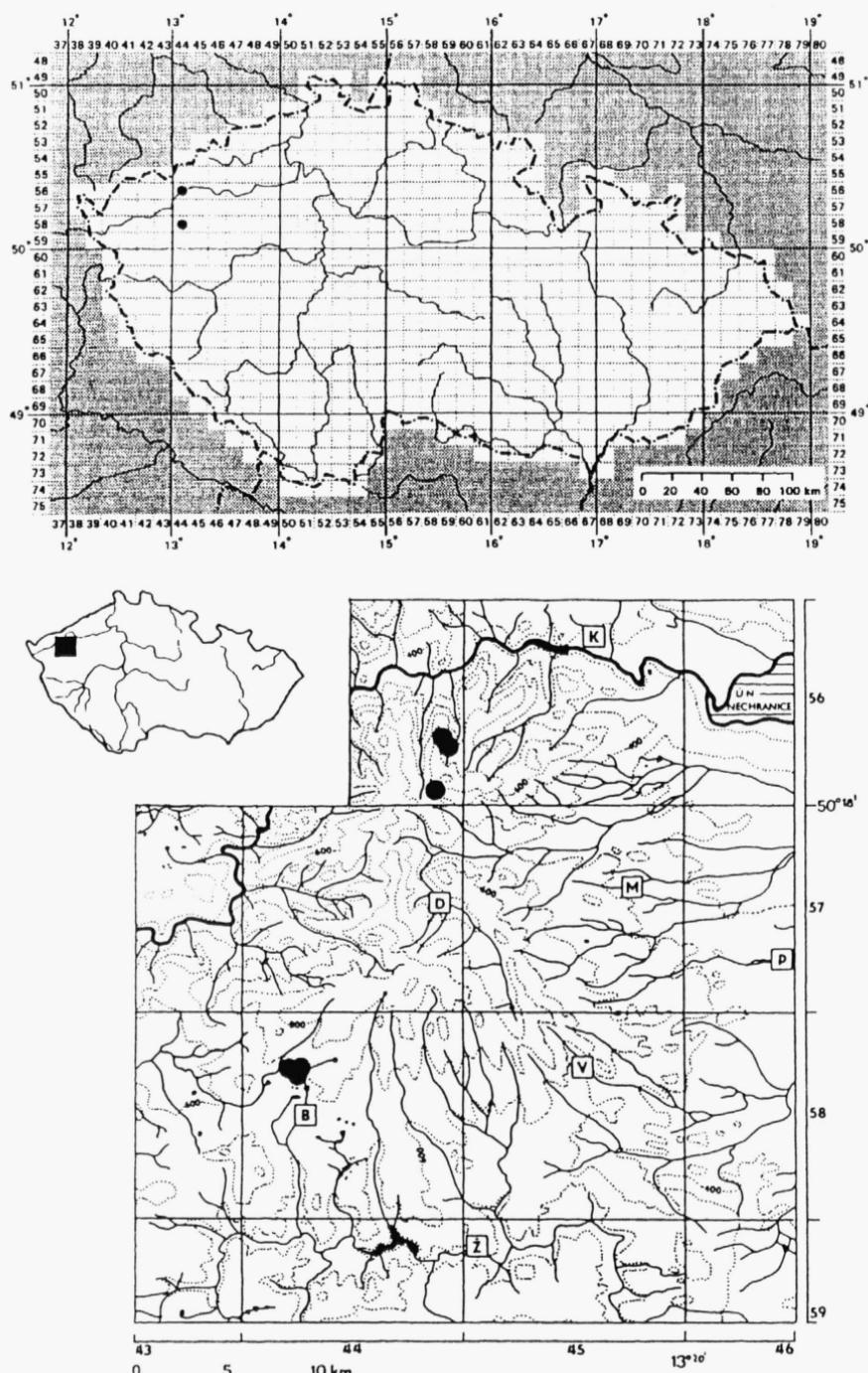


Fig. 5. – Distribution of *Hypericum dubium* in the Czech Republic. Map of the Czech Republic (top) and detailed map of the Doušovské hory Mts (bottom) are shown. Villages: B – Bochov, D – Doušov, K – Klášterec nad Ohří, M – Mašťov, P – Podbořany, V – Valeč, Ž – Žlutice.

2. Dourovské hory Mts. Havraň hill (736 m a. s. l.). NE foot of basalt heap, forest and shrub border, 680–700 m a. s. l.; 3. 8. 1995, J. Hadinec et J. Michálek s. n.; OMS 610/95.
3. Dourovské hory Mts. Former village of Martinov, former village of Humnice. Meadows with shrubs on S-SE foot of Havraň (736 m a. s. l.), W from the road between former villages, 660–675 m a. s. l.; 3. 8. 1995, J. Hadinec et J. Michálek s. n.; OMS 613/95.
4. Dourovské hory Mts. Village of Bražec – former village of Horní Tašovice. Zelený rybník pond 1 km NE from village of Horní Tašovice – overgrown and wet sand-pit below dike, 685 m a. s. l.; 4. 8. 1995; J. Hadinec et J. Michálek s. n.; OMS 625/95, PRC.
5. Dourovské hory Mts. Village of Bražec. Wet meadow in pond system called "Hliňáky", 600–700 m SW from Bražec (along bank of discharged pond), 695 m a. s. l.; 4. 8. 1995; J. Hadinec et J. Michálek s. n.; OMS 655/95.
6. Dourovské hory Mts. Village of Bražec. Mown wet meadows, 350m W from the village, 700 m a. s. l. – insularly near the meadow drive; 23. 7. 1996; J. Michálek s. n.; OMS 375/96.
7. Dourovské hory Mts. Zelený rybník pond near the village of Bražec, E from Karlovy Vary, ca. 685 m a. s. l. 12. 7. 1996. Mártonfi 2125 (KO), (karyologically tested  $2n = 32$ ).

### *Introgession*

In England, an introgression between *H. dubium* and *H. perforatum* was described (e. g. Crackles 1990). With regard to certain contact of these plants in the Czech Republic, hybridization and introgression cannot be excluded also in this territory. However, such plants have not been found so far. They can be morphologically determined on the basis of different degree of character intermediarity and the evidence of their presence can be given by a detailed morphometric study of the populations.

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### **Súhrn**

Nový druh pre kvetu Českej republiky, *Hypericum dubium* Leers (=*H. maculatum* subsp. *obtusiusculum*) bol zistený z oblasti Dourovských hôr. Práca poskytuje okrem morfologických a chorologických (obr. 5) údajov aj dátu karyologické (z územia Českej republiky bol pre druh zistený tetraploidný počet chromozómov  $2n = 32$  (obr. 2), čo súhlasí s udávanými počtami inde v Európe), ďalej dátu palynologické (obr. 3) a chemickú charakteristiku na základe identifikovaných sekundárnych metabolítov pomocou kvapalinovej chromatografie (obr. 4). Na území Českej republiky je teda nutné rozlišovať bežný taxón *H. maculatum*, zriedkavý taxón *H. dubium* (Dourovské hory, ale možno aj inde, zatiaľ prehliadaný) a zriedkavý pentaploidný, prípadne triploidný hybrid *H. maculatum* × *H. perforatum*. Je možné, že *H. dubium* hybridizuje s *H. perforatum* a môže vytvárať introgresanty, zatiaľ však neboli z územia potvrdené. Tri vyššie uvedené taxóny možno rozlíšiť na základe týchto znakov:

- (a) stonky vždy so 4 lištami, takmer rovnocennými, listy bez priesvitných žliazok, žilnatina husto sieťovitá, vetvy súkvetia zvierajú so stonkou uhol 25–45°, kalíšne lístky 3,1–4,5 × 2,1–2,8 mm, celistvookrajové, eliptické až vajcovité, korunné lupienky 9,0–11,0 × 3,6–5,5 mm, celistvookrajové, tmavé žliazky na korunných lupienkoch prevažne vo forme bodiek a krátkych čiarok,  $2n = 16$  ..... *Hypericum maculatum*  
 (b) stonky so 4 lištami, dve sú menej výrazné, prípadne čiastočne chýbajú, listy niekedy s priesvitnými žliazками, žilnatina menej husto sieťovitá, vetvy súkvetia zvierajú so stonku uhol 45–70°, kalíšne lístky 4,3–5,7 × 2,3–2,8 mm, na vrchole jemne zúbkaté, úzko až široko vajcovité, korunné lupienky 12,3–16,0 × 4,8–6,3 mm, ce-

listvookrajové, prípadne na jednej strane so zubom, či niekoľkými zúbkami, tmavé žliazky na korunných lupienkoch prevažne vo forme dlhých a krátkych čiarok,  $2n = 32$  ..... *Hypericum dubium*  
 (c) stonky so 4 lištami, dve sú menej výrazné, prípadne čiastočne alebo úplne chýbajú, listy s priesvitnými žliazkami, žilnatina menej husto siet'ovitá, vetyvy súkvetia zvierajú so stonku uhol 45–65°, kališne lístky 6,0–7,5 × 1,7–2,3 mm, na vrchole jemne zúbkaté, úzko vajcovité až kopijovité, korunné lupienky asymetrické, 12,1–18,0 × 4,6–6,1 mm, zvyčajne na jednej strane s niekoľkými zúbkami, tmavé žliazky na korunných lupienkoch často hustejšie na strane lupienka oproti zúbkovaniu, v tvare čiarok, na zúbkoch lupienkov bodkovité,  $2n = 40$  (ojedinele  $2n = 24$ ) ..... *Hypericum maculatum* × *Hypericum perforatum*

## References

- Bridson G. D. R. & Smith B. (1991): Botanico-Periodicum-Huntianum-Supplementum, B-P-H/S. – Hunt Institute for Botanical Documentation, Pittsburgh.
- Brummitt R. K. & Powell C. E. (eds.) (1992): Authors of plant names. – Royal Botanic Gardens, Kew.
- Clarke G. C. S. (1976): The northwest European pollen flora. 7. *Guttiferae*. – Rev. Palaeobot. Palynol., Amsterdam, 21: 125–142.
- Crackles F. E. (1990): *Hypericum × desetangii* Lamotte nm. *desetangii* in Yorkshire, with special reference to its spread along railways. – Watsonia, Arbroath & London, 18: 63–67.
- Erdtman G. (1960): The acetolysis method. A revised description. – Svensk Bot. Tidskr., Stockholm, 54: 561–564.
- Fröhlich A. (1911): Der Formenkreis der Arten *Hypericum perforatum* L., *H. maculatum* Cr. und *H. acutum* Mnch. nebst deren Zwischenformen innerhalb des Gebietes von Europa. – Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturwiss. Cl., Wien, 120: 505–599.
- Fröhlich A. (1960): Kříženci třezalek (*Hypericum*) v ČSR. – Preslia, Praha, 32: 97–99.
- Fröhner S. (1971): Kritische Pflanzen und floristische Neuheiten aus dem Riesengebirge und Isergebirge. – Opera Corcont, Vrchlabí, 7–8: 65–76.
- Greuter W., Barrie F. R., Burdet H. M., Chaloner W. G., Demoulin V., Hawksworth D. L., Jorgensen P. M., Nicolson D. H., Silva P. C., Trehane P. & McNeill J. (eds.) (1994): International code of botanical nomenclature adopted by the Fifteenth International Botanical Congress, Yokohama, September 1993. – Regnum Veg., 131, Utrecht.
- Holmgren N. H., Holmgren P. K. & Barnett L. C. (1990): Index herbariorum. 8. ed. Part 1: The herbaria of the world. – New York Botanical Garden, New York.
- Hölzl J. & Ostrowski H. (1987): Johanniskraut (*Hypericum perforatum* L.) HPLC-Analyse der wichtigen Inhaltsstoffe und deren Variabilität in einer Population. – Deutsche Apotheker-Zeitung, Berlin, 127: 1227–1230.
- Lawrence G. H. M. et al. (1968): Botanico-Periodicum-Huntianum, B-P-H. – Hunt Botanical Library, Pittsburgh.
- Leers J. D. (1775): Flora Herbornensis. – Berolini.
- Mártonfi P., Repčák M. & Mihoková L. (1996): *Hypericum maculatum* Crantz subsp. *maculatum* × *H. perforatum* L. (*Hypericaceae*): Corroboration of natural hybridization by secondary metabolite analysis. – Folia Geobot. Phytotax., Praha, 31: 245–250.
- Robson N. K. B. (1957): 112/12. *Hypericum maculatum* Crantz. – Bot. Soc. Brit. Isles Proc., Arbroath, 2: 237–238.
- Robson N. K. B. (1958): *Hypericum maculatum* in Britain and Europe. – Bot. Soc. Brit. Isles Proc., Arbroath, 3: 99–100.
- Robson N. K. B. (1981): Studies in the genus *Hypericum* L. (*Guttiferae*) 2. Characters of the genus. – Bull. Brit. Mus. Nat. Hist., ser. bot., London, 8: 55–226.
- Robson N. K. B. & Adams P. (1968): Chromosome numbers in *Hypericum* and related genera. – Brittonia, New York, 20: 95–106.
- Robson N. K. B.: Studies in the genus *Hypericum* L. (*Guttiferae*). Sect. 9. *Hypericum*. – Bull. Brit. Mus. Nat. Hist., London ser. bot. (in preparation).
- Schwarz O. (1965): Die kritischen *Hypericum*-Arten der mitteleuropäischen Flora. – Drudea, Jena 5: 59–66.
- Stafleu F. A. & Cowan R. S. (1979): Taxonomic literature. Volume II: H-Le. – Bohn, Scheltema & Holkema, Utrecht & Dr. W. Junk Publishers, The Hague.
- Vezey E. L., Shah V. P. & Skvarla J. J. (1992). A numerical approach to pollen sculpture terminology. – Pl. Syst. Evol., Wien, 181: 245–254.

- Walker J. W. & Doyle J. A. (1975): The bases of angiosperm phylogeny: Palynology. – Ann. Missouri Bot. Gard., St. Louis, 62: 644–723.
- Zelený V. (1990): 58. *Hypericaceae* Juss. – třezalkovité. – In: Hejný S. & Slavík B. (eds.), Květena České republiky 2: 376–389, Academia, Praha.

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