Thaiszia - J. Bot., Košice, 34: 001–010, 2024
https://doi.org/10.33542/TJB2024-1-01

THAISZIA
JOURNAL OF BOTANY

Papaver dubium in relic habitats in the Veľká Fatra Mountains

Dana Bernátová[1], Ladislava Paštová[2], Ján Kliment[3] & František Krahulec[2*]

[1] Francúzskych partizánov 27, 038 61 Vrútky, Slovakia
[2] Institute of Botany, Academy of Sciences of the Czech Republic, CZ 252 43 Průhonice; Czech Republic; ladislava.pastova@gmail.com; frantisek.krahulec@ibot.cas.cz (corresponding author)
[3] Textorisovej 3, 036 01 Martin, Slovakia; jan.kliment54@gmail.com

Bernátová D., Paštová L., Kliment J. & Krahulec F. (2024): *Papaver dubium* in relic habitats in the Veľká Fatra Mountains. – Thaiszia – J. Bot. 34: 001–010.

Abstract: The occurrence of *Papaver dubium* L. under overhangs in the Veľká Fatra Mts., Slovakia, is evaluated with respect to its morphology, chromosome number and habitat conditions. We consider its occurrence under overhangs in high altitudes as a relic from the end of Last Glacial or early Holocene.

Keywords: relic occurrence, overhangs, chromosome number, *Papaver dubium*, morphology.

Introduction

The Veľká Fatra Mts. is a mountain range situated in the south-western part of the Western Carpathians in Slovakia. It is the most south and west situated range reaching the altitudes above the timberline. This mountain range is characterized by the diversity of underlying rocks: granites in the northern part and calcareous marls and nappe outliers in the southern part. The southern part has broad and flat ridges on marls, and deep valleys, gorges, and rock walls in the parts with nappe outliers. Characteristic features of the southern part of the Veľká Fatra Mts. are avalanche paths, which are common, having the biggest area in the Slovak Carpathians (Kňazovický 1967). Another common feature typical in the region of the nappe outliers is rock overhangs. Their history, especially with their mollusc fauna, was summarized by Ložek (2007). The area is characterized by the common occurrence

of alpine species at lower altitudes and thermophilus species at higher altitudes. This is the case for both plants and animals (Ložek 2007).

During the research of the flora of this region, Bernátová (1986) discovered that these overhangs have a unique flora forming distinct plant communities. Later, even a new endemic subspecies of *Papaver tatricum* (A. Nyár.) Ehrend. has been described (*Papaver tatricum* subsp. *fatrae-magnae* Bernátová) and several species with their only occurrence in the Western Carpathians were found, such as: *Arabis nova* Vill., *Chenopodium foliosum* (Moench) Asch., *Sisymbrium austriacum* Jacq. subsp. *austriacum*. These species are probably relics from the end of the last Glacial or early Holocene. A lower probability is that they are later introduced by birds as distribution agents, because overhangs are used by various animals as a place of shelter.

The plant communities of this specific habitat are unique, and a new plant community alliance has been described: *Erysimo witmannii-Hackelion deflexae* Bernátová 1986. Subsequent studies led to the description of several more communities (Bernátová 1991), some of which were accepted during vegetation synthesis for the whole of Slovakia (Jarolímek et al. 1997).

One of the species discovered under the overhangs is *Papaver dubium*. It belongs to a group of closely related species (or subspecies), with few characters to differentiate between them. In addition to the morphological characters, the colour of fresh and dry latex and the chromosome number is also used to differentiate the taxa. The survey of particular taxa of the *P. dubium* agg. occurring in Slovakia and neighbouring countries is given in Table 1.

Taxonomic concept varies between species and subspecies, several of them changed during the time. Survey of particular taxa of *P. dubium* agg. and synonymy as they were treated in species lists and floras of Slovakia and neighbouring countries (see also Tab 1.):

P. dubium L. s.str. [CZ, PL, UA]
    P. dubium subsp. *dubium* [A, HU, SK]
P. dubium subsp. *confine* (Jordan) Hörandl [A, SK]
    P. confine Jordan [CZ]
P. dubium subsp. *stevenianum* (Mikheev) Kubát & Šípošová [SK]
    P. stevenianum Mikheev [UA]
P. dubium subsp. *austromoravicum* (Kubát) Hörandl [A, HU, SK]
P. dubium subsp. *albiflorum* (Boiss.) Dostál [HU]
P. maculosum Schur.
    P. albiflorum (Boiss.) Pacz. [UA]
    P. dubium subsp. *albiflorum* (Boiss.) Dostál
P. maculosum subsp. *maculosum* [CZ]
    P. albiflorum subsp. *albiflorum*
P. maculosum subsp. *austromoravicum* (Kubát) Kubát [CZ]
    P. albiflorum subsp. *austromoravicum* Kubát
    P. dubium subsp. *austromoravicum* (Kubát) Hörandl
P. lecoqii Lamotte [CZ]

P. maeoticum Klokov [UA]
P. tumidulum Klokov [UA]

Because of small morphological differentiation and differences in descriptions (width of leaf segments, hairs on the stem), and differences in ecology of *P. dubium* and closely related taxa, we decided to study the variation and chromosome numbers of plants from the Veľká Fatra Mts. in detail to be more certain with the identification of these plants. With regard to the rather strange habitat, we also describe the ecological conditions there.

Methods

Localities of analysed plants

Papaver dubium has been found under overhangs at two localities situated in different valleys:

1. Blatnica village, Dedošová dolina valley, overhangs on south oriented slopes, ca 700 m SW of Plavá Mt., 48°56'32.46"N, 19°1'9.76"E, alt. 950 m, slope 40°. Relevé published by Bernátová (1991: tab. 4, rel. no. 1). The size of overhang is: width 35 m, height ca 20 m, and depth 6.5 m. In the text it is named Salaš.

2. Blatnica village, Blatnická dolina valley, overhangs under Slnečné skaly, on south oriented slopes of Ostrá Mt., 48°54'37.8"N, 18°57'30.3"E, alt. 750 m, slope 0°; two relevés published by Bernátová (1991: tab. 4, rel. nos. 2, 3). The species has not been found there in the last few years.

Morphology (including latex colour)

Plants collected in the field were compared with those cultivated from seeds in the experimental garden of the Institute of Botany Czech Academy of Sciences in Průhonice, Czech Republic. Seeds for this purpose were collected in the field and from plants cultivated at Blatnica village on the foothills of the Veľká Fatra Mts. Seeds were stored for several seasons at room temperature and then in the refrigerator for two months. After this treatment they germinated. Latex colour was observed in fresh plants (fresh latex) as well as on dried ones (dry latex). The cultivated plants were used for the study of the chromosomes; root tips of plants cultivated from both seeds collected in the field as well as from seeds collected in the garden were used.

Temperature measurements

Air temperature at the soil surface was measured by means of minimum and maximum thermometer in 1992 at the Salaš locality. In that time, more detailed devices were unavailable and because of difficulties in accessibility (locality is dangerous), thus visits were impossible.

Cytology

Slide preparation of mitotic chromosomes: Root tips were taken from potted plants and treated with a saturated aqueous solution of p-dichlorbenzene for 2 hours at room temperature. Then, they were fixed in fresh ethanol-acetic acid fixative (3:1, v/v) and stored in a freezer until use. The fixed root tips were washed in distilled

water (twice) and citrate buffer (0.01 M citric acid and 0.01 M sodium citrate buffer, pH 4.8) for 5 min each. Thereafter, the root tips were incubated in pectolytic enzyme mixture [0.3% (w/v) cellulase, 0.3% (w/v) cytohelicase (Sigma, St. Louis, MO, USA) and 0.3% (w/v) pectolyase Y-23 (Duchefa Biochemie, Haarlem, NL) in citric buffer] for 3 hours at 37 °C. After digestion, the enzyme mixture was replaced by distilled water. The slides were prepared using the smear method (Pijnacker & Ferwerda 1984) following Ross et al. (1996) with several modifications (Paštová et al. 2019). The digested root tip was carefully transferred onto a microscope slide in a drop of water. A cell suspension was produced with needles and 20 µl of ice-cold 75% acetic acid was added to the suspension. The slide was placed on a hot plate (49 °C) and stirred with a needle to spread the cells for 3.5 min. Finally, 150 µl of ice-cold ethanol-acetic acid fixative (3:1, v/v) was added twice, the slides were then washed with ethanol and air-dried. Chromosomes were determined from one plant cultivated from seeds collected in the field and one plant from seeds collected at Blatnica.

Results

Chromosome numbers

Chromosome number 2n=42 was found several times (Fig. 1). Observed chromosomes were metacentric or sub-metacentric, with small differences of their size.

Morphology

Capsule shape is considered as an important character for distinguishing individual species (or subspecies of this group of species; *P. dubium* agg.). Capsules (n=61) collected from cultivated plants have the following length and width and their ratio (Tab. 2).

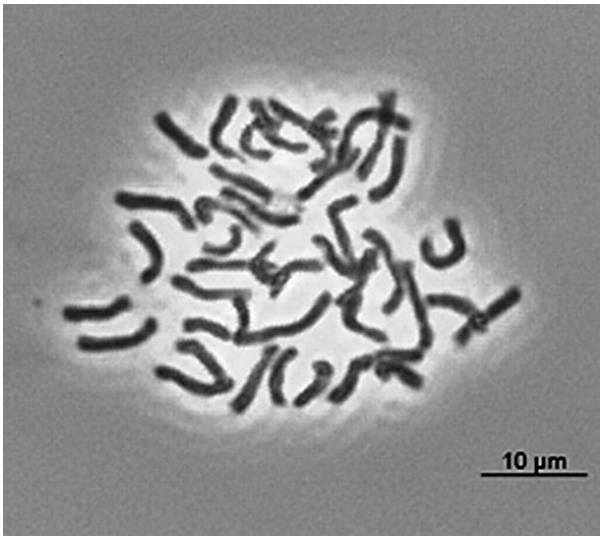

Fig. 1 Chromosomes of *Papaver dubium* cultivated from seeds collected at Salaš overhang.

Tab. 1 Treatment of particular taxa of the *Papaver dubium* agg. in Slovakia and neighbouring countries.

Slovakia [SK]	Slovakia [SK]	Czechia [CZ]	Austria [AU]	Poland [PL]	Hungary [HU]	Ukraine [UA]
P. d. subsp. *dubium*	*P. d.* subsp. *dubium*	*P. dubium*	*P. d.* subsp. *dubium*	*P. dubium*	-	*P. dubium*
P. d. subsp. *confine*	*P. d.* subsp. *confine*	*P. confine* *P. lecoqii*	*P. d.* subsp. *confine*	-	*P. d.* subsp. *confine*	-
P. d. subsp. *austromoravicum*	*P. d.* subsp. *austromoravicum* *P. d.* subsp. *stevenianum*	*P. maculosum* subsp. *austromoravicum* *P. maculosum subsp. maculosum*	*P. d.* subsp. *austromoravicum*	-	*P. d.* subsp. *albiflorum*	*P. albiflorum* *P. stevenianum* *P. maeoticum* *P. tumidulum*
Marhold & Hindák (1998)	Šípošová et al. (2002)	Kubát (2019)	Hörandl (1994), Fischer et al. (2008)	Mirek et al. (2020)	Gergely (2009)	Mosyakin & Fedoronchuk (1999)

Tab. 2 Capsules of the *Papaver dubium* from the Salaš locality: length, width, and their ratio. Capsules were collected from ten cultivated plants.

Length (mm)	s.d.	Width (mm)	s.d.	Length/width ratio	s.d.
13.5	3.2	5.0	1.1	2.7	0.6

Fig. 2 Herbarium specimen of the *Papaver dubium* plant collected at Salaš overhang.

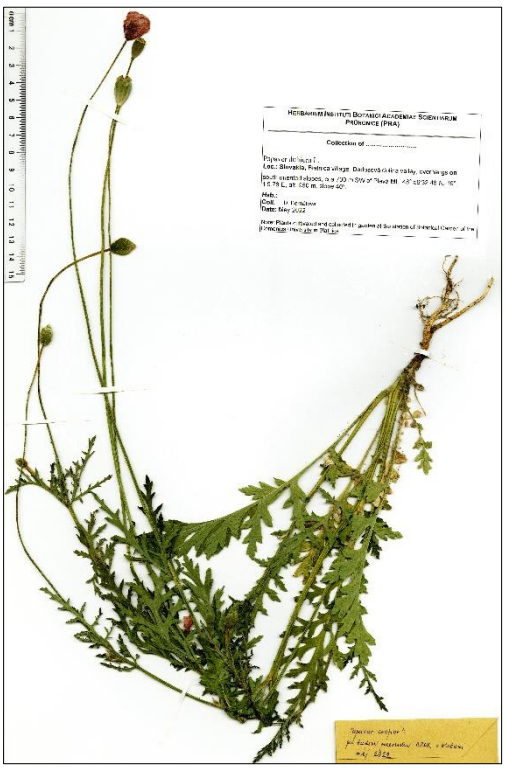

Fig. 3 Herbarium specimen of *Papaver dubium* plant cultivated in Blatnica.

Capsule length/capsule width ratio range covering 80% of values was 2.2-3.3.

Flower buds: of rhomboid shape, broadest in the lower half.

Leaf lobes: broader (above 4 mm) for several plants collected in the field, than at plants from culture (under 2.5 mm), the difference can be observed at Fig. 2 and Fig. 3.

Hairs on lower part of flower stalk: plants from the field and plants in cultivation have only appressed hairs, except one plant, which had patent hairs (Fig. 4).

Fresh latex was white, becoming red after several minutes and almost black during a day.

Habitat conditions

Populations of *Papaver* occur in very specific conditions: they grow on rather steep slopes (to 40°) under overhangs with a south facing aspect, in the massif of Salaš Mt., at an altitude of ca 940 m, and at the Slnečné skaly at an altitude 750 m. The soil is rich in calcium carbonate and clay, rather dry. Vegetation is sparse, with bare, and rich in coarse gravel on the soil surface. In wintertime, these overhangs are fully exposed to sun with almost no snow cover; this results in great amplitudes between day and night. In summertime, it is partially shaded by stands of beech forests. The plant community *Poo nemoralis-Hackelietum deflexae* variant with *Papaver dubium* has been described (Bernátová 1991).

Air temperature at the soil surface was measured in 1992: in spring from 5. 3. to 20. 5. it falls to -7 °C, and the range then was 5–41 °C between 20. 5. and 7. 10.; and in winter to -13.8 °C, soil surface was without snow cover.

Results

Identity of plants from the Veľká Fatra Mts.

Plants from the Veľká Fatra Mts. were originally determined as *Papaver dubium* (Bernátová 1991). Chromosome number 2n=42 fully agrees with those known from Slovakia (Kubát 1980), Austria (Hörandl 1994) and Czechia (Kubát 1988); other taxa (*P. confine*, *P. maculosum*, *P. lecoqii*) of this group have 2n=28. Comparison of morphological characters showed some differences with the details given in the literature. The shape of capsules, especially length to diameter ratio, corresponds to values given e.g. by Kubát (2019) or Šípošová et al. (2002). The value 2.7 is directly between values 2.2 and 3.3 (Tab. 1). The shape of flower buds corresponds to the figure given by Hörandl (1994: 421).

We found greater variation for the shape of leaf lobes and hairiness on the basal part of the flower stalks than is given in the literature. Leaf lobes are more variable in plants from the field (one with broader leaf lobes, Fig. 2 and figure 2 in Hörandl 1994), in cultivation all plants had finely divided leaves cf. Fig. 3 and Fig. 4. Flower stalks had mostly strongly appressed hairs in most of plants from the field and culture; Hörandl (1994) described them as “abstehende Behaarung” (= patent hairs). Only one plant from the field had such hairs, for that reason this character seems as not very exclusive.

Latex colour is considered as important character in most of present determination keys. However, due to changes in the colour of the latex during the drying process, the colour changes should be observed after a period of 24 hrs.

The combination of characters together with chromosome number has confirmed the original identification as *P. dubium* s. str.

Because variation is rather high both of plants in field as well as in culture, it seems there are more genotypes in field.

Habitat conditions

Overhangs are a typical habitat of the Veľká Fatra Mts., especially of its south-western part. Their vegetation is influenced by their orientation. Those open to the north, northwest and northeast are cold and have relic species such as *Cortusa matthioli* L. and *Pinguicula alpina* L. in relatively low altitudes, and newly described endemic, *Papaver tatricum* subsp. *fatrae-magnae* has evolved there.

A different species composition occurs in overhangs with a south, southeast, and southwest orientation. Several species have a unique occurrence in the Western Carpathians: *Arabis nova*, *Chenopodium foliosum*, *Sisymbrium austriacum* subsp. *austriacum*, and another relic, *Hackelia deflexa* (Wahlenb.) Opiz. occurs there.

Papaver dubium is confined to this latter habitat. Its environmental conditions are very specific: immature soil rich in calcium, low snow cover during the winter, evidently with high temperature amplitudes during the winter days and nights, due to the southeast to southwest exposition with no shading. The present ecological conditions resemble conditions, which are supposed to be at the end of the Last Glacial in neighbouring Turčianska kotlina basin. It is probable that the occurrence of *P. dubium* under overhangs in the Veľká Fatra Mts. is related to its distribution in the late glacial period or early Holocene. Another possibility is its later introduction by birds, which use overhangs as natural shelters. But the occurrence of several other relic species together with the presence of variation in characters at the locality supports its relic occurrence.

In Slovakia, this species occurs mainly in ruderal habitats in the Pannonian region. The occurrence of *P. dubium* in the Veľká Fatra Mts. is different from the other localities. The highest known localities are those in the Veľká Fatra Mts. (Šípošová et al. 2002). In Czechia, distribution is similar to that in Slovakia, with most of the localities in warmer regions (Kubát 1988). It has a different distribution in Austria, where it is distributed in higher altitudes (Hörandl 1994), especially in the Alps. Recent Exkursionsflora (Müller et al. 2021) characterizes *P. dubium* as “kalk meidend” (= avoiding calcium) in Germany. In Czechia and Slovakia, the most important factor seems to be habitat disturbance, in Czechia the soils are characterized as mildly acid and mildly basic (Kubát 1988).

Age of biota under overhangs

The flora of overhangs seems to be connected with the plant communities of bare ground at the end of the glacial period. The occurrence of species having the only one locality in the Western Carpathians supports our view. At the end of the Last

Fig. 4 Herbarium specimen of the *Papaver dubium* plant cultivated from seeds at experimental garden at Průhonice. Plant on the left has patent hairs on the stalk.

Glacial, the neighbouring Turčianska kotlina basin was covered with communities associated with open ground. This is based mainly on the studies by Ložek (2007), who described conditions in many localities in the Turčianska kotlina basin as well as overhangs in the Veľká Fatra Mts. The synopsis of his results is given in his paper (Ložek 2007). His opinions influenced our view on the relic nature of occurrence of the whole plant communities connected with this habitat, which were described as a special alliance *Erysimo witmannii-Hackelion deflexae*. This uniqueness is also reflected in the fact, that they remained unclassified into higher units in the present system of vegetation classification of Slovakia (Jarolímek et al. 1997). In synthesis of European vegetation (Mucina et al. 2016), it is classified in its own order *Hackelio deflexae-Blitetalia foliosi* Mucina 2016 belonging to *Sisymbrietea* class.

Acknowledgement

We thank to Jiří Machač for the scanning of herbarium specimens, and to Miroslav Chmelař for care of the plants in the garden and regular collection of ripe capsules. Michael Wilcox (England) is acknowledged for the language correction.

References

Bernátová D. (1986): *Erysimo witmannii-Hackelion deflexae* all. nov. vo Veľkej Fatre. – Severočeskou Přír. 19: 55–59.

Bernátová D. (1991): Rastlinné spoločenstvá pod skalnými previsni vo Veľkej Fatre. – Preslia 63: 21–46.

Fischer M. A., Adler W. & Oswald K. (eds) (2008): Exkursionsflora für Österreich, Liechtenstein und Südtirol. Ed. 3. – Land Oberösterreich, Biologiezentrum der Oberösterreichischen Landesmuseen, Linz.

Gergely K. (2009): Uj Magyar füveszkönyv. Magyarország hajtásos növenyei. – Aggteleki Nemzeti Park Igazgatózág.

Hörandl E. (1994): Systematik and Verbreitung von *Papaver dubium* L. s. l. in Österreich. – Linz. Biol. Beitr. 26/1: 407–435.

Jarolímek I., Zaliberová M., Mucina L. & Mochnacký S. (1997): Rastlinné spoločenstvá Slovenska. 2. Synantropná vegetácia. – Veda, Bratislava.

Kňazovický L. (1967): Lavíny. – Vydav. Slov. Akad. Vied, Bratislava.

Kubát K. (1980): *Papaver dubium* agg. v Československu, pp. 185–188. – In: Hindák F. (ed.): Zborník referátov z III. zjazdu Slovenskej botanickej spoločnosti pri SAV. Zvolen, 30. 6. – 5. 7. 1980. – SBS pri SAV, Bratislava, VŠLD, Zvolen.

Kubát K. (1988): 34. Papaveraceae Juss. – makovité. – In: Hejný S. & Slavík B. (eds.): Květena České socialistické republiky 1: 482–494.

Kubát K. (2019): 66. Papaveraceae – makovité. – In: Kaplan Z., Danihelka J., Chrtek J. jun., Kirschner J., Kubát K., Štech M. & Štěpánek J. (eds.): Klíč ke květeně České republiky. Ed. 2. – Academia, Praha.

Ložek V. (2007): Zrcadlo minulosti. Česká a slovenská krajina v kvartéru. – Dokořán, Praha. (Chapt. 6 devoted to the Veľká Fatra Mts.).

Marhold K. & Hindák F. (1998): Zoznam nižších a vyšších rastlín Slovenska. [Checklist of non-vascular and vascular plants of Slovakia.] – Veda, Bratislava.

Mirek Z., Piękoś-Mirkowa H., Zając A. & Zając M. (2020): Flowering plants and pteridophytes of Poland. A checklist. 3rd Edition. – W. Szafer Institute of Botany, Kraków.

Mosyakin S. L. & Fedoronchuk M. M. (1999): Vascular plants of Ukraine. A nomenclatural checklist. – National Academy of Sciences of Ukraine, M. G. Kholodny Institute of Botany, Kyïv, xxiii + 345 pp.

Mucina L., Bultmann H., Dierßen K., Theurillat J.-P., Raus T., Čarni A., Šumberová K., Willner W., Dengler J., Gavilán García R., Chytrý M., Hájek M., Di Pietro R., Iakushenko D., Pallas J., Daniels F. J. A., Bergmeier E., Santos Guerra A., Ermakov N., Valachovič M., Schaminée J. H. J., Lysenko T., Didukh Y. P., Pignatti S., Rodwell J. S., Capelo J., Weber H. E., Solomeshch A., Dimopoulos P., Aguiar C., Hennekens S. M. & Tichý L. (2016): Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. – Appl. Veg. Sci. 19 (suppl. 1): 3–264. doi.org/10.1111/avsc.12257

Müller F., Ritz Ch. M., Welk E. & Wesche K. (eds) (2021): Rothmaler Exkursionsflora von Deutschland. Gefäßpflanzen: Grundband. Ed. 22. – Springer Spektrum.

Paštová L., Belyayev A. & Mahelka V. (2019): Molecular cytogenetic characterisation of *Elytrigia* ×*mucronata*, a natural hybrid of *E. intermedia* and *E. repens* (Triticeae, Poaceae). – BMC Plant Biol. 19: 230. doi.org/10.1186/s12870-019-1806-y

Pijnacker L. P. & Ferwerda M. A. (1984): Giemsa C-banding of potato chromosomes. – Can. J. Genet. Cytol. 26/4:415–419.

Ross K. J., Fransz P. & Jones G. H. (1996): A light microscopic atlas of meiosis in *Arabidopsis thaliana*. – Chrom. Res. 4/7: 507–516.

Šípošová H., Bernátová D. & Kubát K. (2002): 1. *Papaver*. L. Mak. pp. 25–60. – In: Goliašová K. & Šípošová H. (eds.): Flóra Slovenska V/4. – Veda, Bratislava.

Received: October 23th 2023
Revised: January 3rd 2024
Accepted: February 5th 2024