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Petasites kablikianus (Asteraceae) in the Slovak Carpathians: distribution, chromosome number and genome size

Matej Dudáš[1]*, Jana Fabianová[1], Lenka Mártonfiová[2] and Pavol Mártonfi[1]

1 Institute of Biology & Ecology, Faculty of Science, P. J. Šafárik University, Mánesova 23, SK-041 54, Košice, Slovakia

2 Botanical Garden, P. J. Šafárik University, Mánesova 23, SK-043 52 Košice, Slovakia

ABSTRACT: The paper treats the distribution and ecological characteristics of the butterbur species *Petasites kablikianus* in the Slovak Carpathians (Central Europe). Study of herbarium specimens, review of published sources and results of field research show its wide distribution in northern and central Slovakia, mainly in wet biotopes such as gravel riverbeds and rocky banks of mountain streams. The majority of populations occur from the submontane to montane level (mainly at elevations of 600-1200 m a.s.l.) with the altitudinal maximum at ca. 1850 m a.s.l. Chromosome number analysis showed $2n = 60$. The nuclear DNA content measured in leaves was $2C = 5.96 \pm 0.17$ pg, or 5828 Mbp. Characters used for determination are also discussed. A distribution map is attached.

Keywords: *Petasites kablikianus*, nuclear DNA content, karyology, chorology, Slovakia

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INTRODUCTION

The number of species of the genus *Petasites* Mill. (Asteroideae, Asteraceae) or butterbur reaches over 28. They are distributed throughout the Northern Hemisphere in Europe, northern Africa, North America and northern Asia, but not in Greenland or Iceland. In Europe, 12 species are native. In Slovakia, the genus is represented by three species – *Petasites albus*, *P. hybridus, P. kablikianus* and their hybrids (Toman 1972; Dostál & Červenka 1992; Meusel & Jäger 1992; Štech 2004).

Petasites kablikianus Tausch ex Berchtold Lotos 1: 120, 1851 [syn. *Tussilago kablikiana* Opiz Sezn. Rostl. Květ. Čes. 73, 1852, nom. inval. – *Petasites albus* β var. *glabratus* Maly Enum. Pl. Phan. Austriac.: 108, 1848. – *P. glabratus* (Maly) Borb. Term. Közl. 34: 124, 1895] is a species of Carpathian origin with a Sudeten-Carpathian-Dinaric range (Toman & Sýkora 1968; Hendrych 1987). It grows singly or in groups in wet habitats, in sandy to gravel soils, on cold and wet limestone, often under adverse conditions. The species prefers banks of mountain streams, silts of rivers and streams, wet ditches, springs, wet screes and edges of mountain forests along streams from the submontane to subalpine belt in the Czech Republic (Krkonoše Mts.), northern and central Slovakia and western Ukraine (Carpathian Mts.) and southern Poland (with frequent distribution in hillyareas along the Polish-Slovak border) (Toman & Procházka 1979; Dostál & Červenka 1992; Kliment 1999; Zając & Zając 2001; Štech 2004; Chopik & Fedoronchuk 2015). In the northern parts of mountains of the Balkan Peninsula (Fig. 1), it ranges from Croatia and Bosnia and Herzegovina (from Mt. Velebit to Mt. Zelengora) through northern Montenegro (Mt. Durmitor), northern Albania (Vermeshë Mts.) and northwest Macedonia (Šar Mts. and Mt. Jablanica) to northwest Serbia and western Romania, extending (sparsely) as far as Bulgaria (Mt. Pirin and the Central Rhodopi Mts.). The southernmost limit of its range is in northeast Greece (Rhodopi Mts.) (Nyárády 1964; Štech 2004; Assyov & Petrova 2006; Niketić & Zlatković 2006; Tan *et al*. 2010; Milanović & Stupar 2017; Teofilovski 2018).

*correspondence: dudas.mato@gmail.com

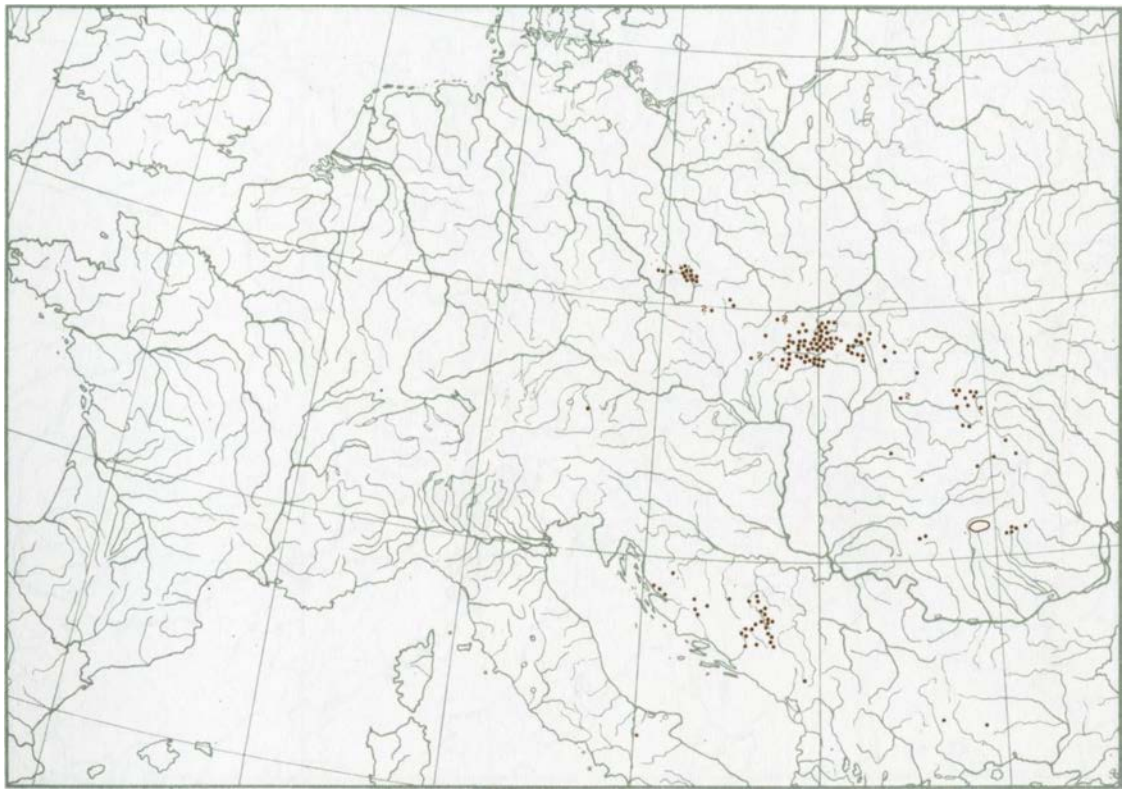

Fig. 1. Distribution of *Petasites kablikianus* in Europe (according to Meusel & Jäger 1992).

The aims of this study were to (1) map the distribution of *P. kablikianus* in Slovakia, (2) briefly describe the communities where it grows, (3) determine its chromosome number and (4) measure its genome size.

MATERIALS AND METHODS

Collection of distribution data. The species' occurrence in Slovakia was mapped on the basis of herbarium specimens deposited in public herbarium collections in Central Europe [BRA, BRNM, HUM, KO, MJG, MPS, NI, PR, PRC, SAV, SLO, TM, ZV; acronyms according to Thiers (2018) and small local collections according to Vozárová & Sutorý (2001)]; field study realised during 2017-2018; available published sources (drawn from the database of locations of higher plants maintained by the Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Bratislava, Slovakia); and almanacs compiled by teachers of floristic courses offered by Slovak and Czech botanical societies in Slovakia (Mártonfi 1992; Benčaťová & Ujházy 1998; Mráz & Mrázová 2003).

The map was designed in the ArcGis program, version 9.2. The list of localities was edited according to Goliašová & Michalková (2016). For the herbarium specimens, the collector, year of collection and herbarium acronym are given. The references for records published in sources not listed in the References section are given in an abridged form including the page of a particular *P. kablikianus* record. For unpublished field records, the year is given, followed by the name(s) of their author(s). The records are arranged following the phytogeographical division of Slovakia (Futák 1980) and assigned to quadrants of the CEBA grid template (Niklfeld 1971; Jasičová & Zahradníková 1976).

Karyology. For the karyological studies, plants of *P. kablikianus* were collected at two localities: (1) Branisko Mts., Lipovce, left bank of the stream Lačnovský Potok, below the holiday chalets, collected by J. Fabianová, 26. 5. 2017; and (2) Malá Fatra Mts., Vrátna Valley, north of the Vrátna Chalet, along the yellow tourist path, collected by M. Dudáš, 1. 7. 2017. The plants were potted, put in a shady place and cultivated on premises of the Botanical Garden in Košice.

Root-tip meristems from the potted plants were used for chromosome number counts. The root tips were pretreated in a 0.002 M aqueous solution of 8-hydroxyquinoline for 16 h at 4°C (overnight), then fixed in a mixture of 96% ethanol and 98% acetic acid in a ratio 3:1 for at least 1 h. The meristems were washed with distilled water, after which they macerated in 1 M HCl in a water bath at 60°C for 3 min and washed again with distilled water for 10 min. Slides were made using the cellophane square method (Murín 1960). The slides were stained with a 7% solution of Giemsa stain, (modified solution)

from Fluka Analytical in Sörensen phosphate buffer, dried and observed in a drop of immersion oil. A Leica DM 2500 microscope equipped with an HDCE-X5 camera and ScopeImage 9.0 software was used for observation of chromosomes.

Flow cytometry and analysis of genome size. Plants from three locations were used for analysis of genome size: (1) Branisko Mts., Lipovce, (described above), (2) Bukovské Vrchy Mts., Uličské Krivé, roadside between the villages of Zboj and Uličské Krivé, alongside the stream Zbojský Potok, scollected by J. Fabianová, 11. 6. 2017 and (3) Malá Fatra Mts., Vrátna Valley (described above).

The plants were cultivated in the same way as for chromosome counts (see Karyology). Three plants of *P. kablikianus* from each locality were used for analysis of genome size. Approximately 0.5 cm^2 of young leaves of a *P. kablikianus* sample and the employed standard were chopped with a new razor blade in a Petri dish for about 30 sec in 1 ml of ice-cold GPB buffer [200 ml H_2O, 20 mM 4-morphinepropan sulphonate, 30 mM sodium citrate, 0.5 mM spermin, 4 HCl, 30 mM KCl, 80 mM KCl, 20 mM NaCl, and 0.5% (v/v) Triton X-100 (pH 7.0) (Loureiro *et al.* 2007)], to which 3% polyvinylpyrrolidone was added. *Solanum lycopersicum* cv. Stupicke, with a determined genome size of 2C = 1.96 pg DNA (Doležel *et al.* 1992), was used as the standard. The suspension was filtered through nylon mesh (42 µm) and stored at 7°C. A fluorochrome solution (50 µl of propidium iodide as DNA intercalator, 50 µl of RNase and 5 µl of β-mercaptoethanol) was then added. After 10 min of incubation at 7°C, the samples were measured using a CyFlow ML flow cytometer (Partec GmbH, Münster, Germany) in the Laboratory of Flow Cytometry (Institute of Biological and Ecological Sciences, P. J. Šafárik University, Košice). A green laser beam (532 nm/150 mW) was used as the exciting source. Each sample was measured thrice on different days (Lysák *et al.* 1999). The minimal flow rate was 1300 and the values of CV were up to 5.5%. The measured data were analysed using the FloMax 270 program, and genome size was measured according to Doležel & Bartoš (2005).

RESULTS AND DISCUSSION

Distribution in Slovakia. *Petasites kablikianus* is a species that occurs relatively frequently in the flora of the submontane to subalpine level in northern and central Slovakia. Its distribution in the Slovak Carpathians is illustrated by the distribution map (Fig. 2) and list of localities (see Appendix). The occurrence of this species was documented from 22 phytogeographic districts and subdistricts. The centre of its distribution is located in the central Carpathians (*Eucarpaticum*) and area of the Eastern-Beskydy flora (*Beschidicum orientale*). Most of the records are located in districts and subdistricts of the Slovenský Raj Mts., the Krivánska Malá Fatra Mts.,

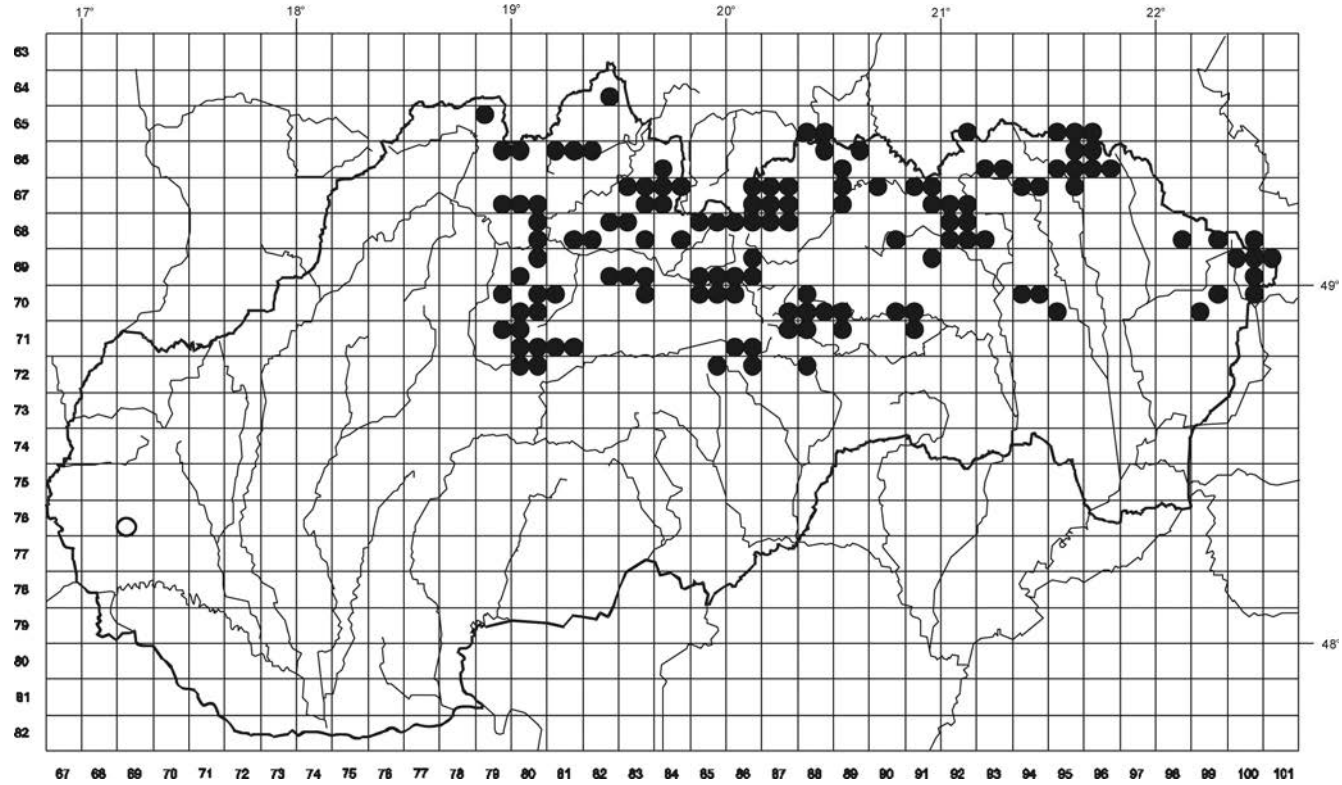

Fig. 2. Distribution of *P. kablikianus* in Slovakia (black dots), white dot – questionable occurrence.

the Veľká Fatra Mts., the Chočské vrchy Mts., the Nízke Tatry Mts., the Tatry Mts., the Pieniny Mts., the Západné Beskydy Mts., the Spišské Vrchy Mts., the Šarišská Vrchovina hill area, the Čergov Mts., the Nízke Beskydy hill area, the Slanské Vrchy Mts. and the Bukovské Vrchy Mts. Sporadically, the species was recorded in the Malé Karpaty Mts., the Kremnické Vrchy Mts., Muránska Planina Mts., the Slovenské Rudohorie Mts., the Vihorlat Mts., the Liptovská Kotlina Basin and the Spišské Kotliny Basins.

Petasites kablikianus grows in tall hygrophilous herb fringe communities of hilly areas and the montane to (sub)alpine belts. Kliment & Jarolímek (2002) relegated these plant communities to the class *Mulgedio-Aconitetea*, order *Petasito-Chaerophylletalia*, alliance *Petasition officinalis* Sillinger 1933 and associations *Agropyro caninae-Petasitetum kablikiani* Pawłowski et Walas 1949 em. Kliment et Jarolímek 2002 and *Petasitetum officinalis-glabrati*. Regionally, the species is a member of mountain river bed communities of the class *Betulo-Adenostyletea* Br.-Bl. et Tx. 1943, whereas the upper altitudinal limit of its distribution reaches 1180–1200 m (Kopecký 1971). It prefers rocky banks of mountain streams and gravel benches in moutain river beds, in lower regions extending to spring areas and wet roadsides, dwelling mainly at an altitude of (500-) 600–1200 (-1400) m a.s.l. The lowest altitude detected was 260 m a.s.l. in the Ondavská Vrchovina hill area near the village of Beloveža on a left-hand tributary of the Topľa River in the association *Alnetumincanae* (Dostál 1982). The occurrence from the Malé Karpaty Mts. at 250 m a.s.l. is not correct (probably the result of a misplaced herbarium label). The highest occurrence of the species (between 1520 and 1850 m a.s.l.) was reported by Hadač *et al.* (1948) on the slopes of Mt. Hrubý Štít in the Temnosmrečinská Dolina Valley in the Vysoké Tatry Mts. (see Appendix). *Petasites kablikianus* also colonises travertine heaps with loose vegetation (Toman & Krahulec 1990).

It does not grow in the area of the Pannonian flora (*Pannonicum*). The report from the Slovenský Kras Karst, near the village of Zádiel at the source of the stream Blatný Potok (Valachovič & Janovicová 1999) is uncertain and needs to be confirmed (Virók *et al*. 2016).

The first published distribution of the species from the area of former Czechoslovakia was given by Toman & Starý (1966) and Toman & Sýkora (1968). The distribution showed a distinct geographical disjunction between the localities in the Hrubý Jeseník Mts. in Moravia and the localities in the Malá Fatra Mts. and the Západné Beskydy Mts. in Slovakia. This disjunction was minimised by new findings in the Slovak Beskydy Mts. in northern Slovakia (Toman & Procházka 1979). Later, Kopecký (1983) published a report with new localities in the Slovak Beskydy Mts., the Oravská Magura Mts. and the Roháče Mts., thereby extending the known distribution of the species in northern Slovakia. A detailed characterisation of the species' distribution along selected segments of the Biela Orava River was given by Kopecký (1970). In the valley of the Biela Orava River, *P. kablikianus* is a very frequent species in gravel beds and on rocky banks from the upper part of the valley along the whole river with its tributaries up to its mouth in the Oravská Priehrada Reservoir. *Petasites kablikianus* is also common in the area of left-hand tributaries of the Biela Orava River, mainly the Mutnianka and Polhoranka Brooks, which drain the massif of Mt. Babia Hora. Kopecký (1971) also published a detailed distribution of *P. kablikianus* in valleys of the streams Studený Potok and Roháčsky Potok, which drain the Západné Tatry Mts. and the Roháče Mts. Further data pertaining to the Západné Tatry Mts. (the valley Tomanova Dolina) were published by Unar *et al*. (1985). From the high region of the Oravská Magura and Západné Tatry Mts., *P. kablikianus* descends into the valleys Dolina Chochołowska and Dolina Kościeliska on the Polish side of the mountains. In Poland, the distribution of the species is concentrated in mountainous and hilly areas in southern parts of the country (Zając & Zając 2001). Other records of *P. kablikianus* from mountains in central Slovakia (the Chočské Vrchy, Nízke Tatry and Veľká Fatra Mts.) together with an identification key were published by Toman & Krahulec (1990). Kliment (2008) noted frequent occurrence of the species in the whole area of the Veľká Fatra Mts., while Bernátová & Kubát (1980) gave detailed information about the species' distribution in the valleys Blatnická Dolina and Gaderská Dolina. Widespread occurrence was also noted along streams in the Tatry Mts. (Hadač & Šmarda 1960; Dostál 1982) and in the Slovenský Raj Mts. (Leskovjanská 2014). While the species grows very frequently in the Slovenský Raj Mts., in the adjacent Muránska Planina Mts. it was found for the first time in 2002 (Bernátová *et al*. 2002) and only four populations are known to exist there at the present time (see Appendix).

In eastern Slovakia, *P. kablikianus* forms large and vital populations, which are continuously connected to those in northern Slovakia and southern Poland (Dostál 1982; Zając & Zając 2001). The first large locality is the gravel riverbed and banks of the Ladomírka River with its tributaries in the Ondavská Vrchovina Hills (Dostál 1983). The second large one was first reported by Dostál (1982) from the Čergov Mts. in the valley of the Ľutinka Brook and its tributaries. Later Dostál (1989) stated that the populations in the Čergov Mts. were the largest in eastern Slovakia. We confirmed both of these locations during our field observations in 2017 and 2018.

In general, the majority of populations in the area of central and northern Slovakia are under territorial protection as national parks or protected landscape areas. The status of the populations seems to be stable, but they can be locally disrupted by logging activity.

Table 1. Genome size in plants of *P. kablikianus* collected at three localities in the Slovak Carpathian Mts.

Locality	*Genome size (min)*	*Genome size (max)*	*Genome size (average)*	*Standard deviation (SD)*
Lipovce	5.70	6.23	5.90	0.14
Vrátna	5.60	6.22	5.92	0.20
Uličské Krivé	5.98	6.24	6.09	0.08

Petasites kablikianus is considered to be a pioneer species with the ability to colonise initial habitats (Uziębło 2011). As an apophyte with high abundance, it also occurs in human-affected communities in such habitats as forest narrow-gauge track embankments in the valley of the Biela Orava River (Kopecký 1983): around bridges, in ditches along forest roads and on road embankments in the valley of the stream Studený Potok (Kopecký 1983); on the banks of the Ladomírka River (Dostál 1983); and along tourist paths in the Tatry Mts. and the Slovenský Raj Mts., where it grows frequently. Dostál (1982) noted spreading of the species connected with the building of forest roads in northeastern Slovakia.

Chromosome number. From the territory of Slovakia, Uhríková & Májovský (1980) mentioned a diploid number of chromosomes in somatic cells of $2n = 60$ for plants from the Medová Baba locality (Bukovské Vrchy Mts.). We confirmed the same chromosome number for plants from the Lipovce (Branisko Mts.) and Vrátna Valley (Malá Fatra Mts.) localities. As for other countries, the same chromosome number was reported for plants from Poland (Pogan *et al.* 1990), the Czech Republic (Měsíček 1992) and Bulgaria (Kuzmanov 1986).

In Slovakia, a diploid chromosome number $2n = 60$ was also reported for the species *Petasites hybridus* and *P. albus* (Májovský 1974; Hindáková & Schwarzová 1977; Fabianová 2018).

Genome size. The genome size of *P. kablikianus* (Table 1) measured from leaves was 2C = 5.96 ± 0.17 pg, which represents 5828 Mbp (1 pg DNA = 978 Mbp; Doležel *et al.* 2003). For other *Petasites* species in Slovakia, genome sizes were measured by Fabianová (2018) and found to be 5.76 ± 0.15 pg (5639 Mbp) for *P. hybridus* and 5.37 ± 0.20 pg (5606 Mbp) for *P. albus*.

Determination of the species. After the completion of blossoming, *P. kablikianus* is easily overlooked and can be confused with *P. hybridus*. Early in spring, excursions by botanists are not very frequent and the species is overlooked. On summer excursions, because of the large leaves, the species has not been collected for determination. The main characters on leaves unfailingly ensure correct determination. The most conspicuous of these is

Fig. 3. *Petasites kablikianus*: leaf shape. Locality: Slovenský Raj Mts., Prielom Hornádu, between the Lesnica and Čingov sites (photo by M. Dudáš, 2018).

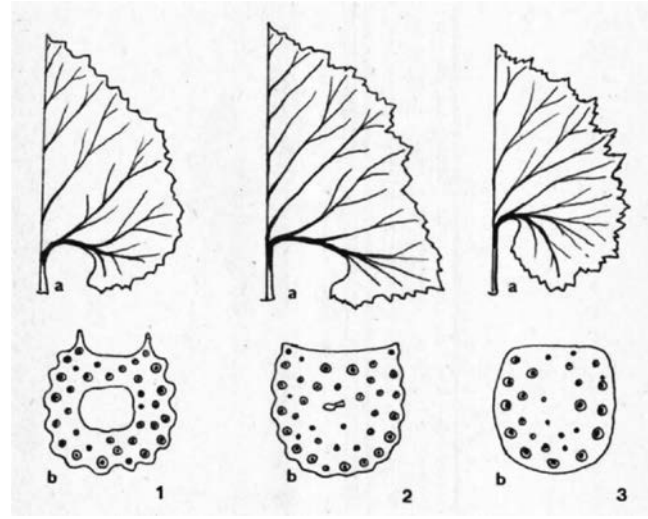

Fig. 4. Leaf shape (a) and cross section of leaf stipe (b) in plants of *Petasites hybridus* (1), *P. kablikianus* (2) and *P. albus* (3) according to Toman & Krahulec (1990).

the orbicular to triangular-cordate shape of the leaves (Fig. 3), which have convergent basal lobes with the margin regularly to irregularly toothed and 3-5 lateral veins bordering the sinus. The stipe of leaves (over half of their length) is somewhat wrinkled, without a central cavity in cross-section and having a wide channel on the top side with sharp margins (Fig. 4), whereas the stipe of *P. hybridus* leaves is markedly ribbed with a central cavity, a deep narrow channel and a wing-like appearance on the margins (Dingwall 1976; Toman & Krahulec 1990).

Crossing between *Petasites* species is frequent on sites where more than one occur and the populations grow close to each other. Hybrids have intermediate characters with a wide range of variability. Live plants are necessary for determination, herbarium specimens being for the most part problematical (Štech 2004). *Petasites* × *intercedens* Matouschek (*P. hybridus* × *P. kablikianus*) and *P.* × *celakovskyi* Matouschek (*P. albus* × *P. kablikianus*) have been recorded in the Slovak Carpathians (Dupľáková 2002).

CONCLUSION

The distribution of *P. kablikianus* in Slovakia is concentrated in the northern and northeastern parts of the country. In northern Slovakia, it is not definitely documented and would seem to be more abundant than presented in our list of localities and on the attached map. Future field observations are sure to result in new findings of populations in northern and central parts of the Slovak Carpathians. Our measurements showed larger nuclear genome size in *P. kablikianus* compared to the other two *Petasites* species, *P. albus* and *P. hybridus*, all three sharing the same ploidy level of $2n = 2x = 60$.

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Botanica SERBICA

REZIME

Distribucija, broj hromozoma i veličina genoma vrste *Petasites kablikianus* (Asteraceae) u slovačkim Karpatima

Matej Dudáš, Jana Fabianová, Lenka Mártonfiová i Pavol Mártonfi

Rad se odnosi na distrtibuciju i ekološke katakteristike vrste *Petasites kablikianus* u slovačkim Karpatima (centralna Evropa). Pregled herbarskog materijala, literature, kao i terenska istraživanja pokazuju njegovu široku distribuciju u severnom i centralnom delu Slovačke, uglavnom na vlažnijim staništima, kao što su šljunkovite obale reka i kamenite obale planinskih potoka. Većina populacija se javlja u submontano - montanoj zoni (uglavnom 600-1200 m), sa maksimumom nadmorske visine od 1850 m. Analize broja hromozoma pokazuju da je $2n = 60$. Sadržaj nuklearne DNK meren u listovima je 2C = 5.96 ± 0.17 pg, ili 5828 Mbp. Determinacioni karakteri su, takođe, diskutovani. Prikazana je i mapa distribucije.

Ključne reči: *Petasites kablikianus*, sadržaj nuklearne DNK, kariologija, horologija, Slovačka