

POTENTILLA VERNA REDISCOVERED IN HUNGARY: DIAGNOSTIC FEATURES, PLOIDY AND ECOLOGY

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Potentilla verna, an Atlantic – Central European species, had not been reported from Hungary since the 1890s (earlier records were known from Kőszeg Mts in W Hungary). The species was re-found altogether on 4 localities 2015 onwards in NE Hungary, both in Gömör-Torna Karst and Cserehát regions; these sites represent the one of the south-easternmost occurrences of the recent range. The north-east Hungarian populations occur in the *Sedo acris-Festucetum valesiacae* association of the Festuco-Brometea class. *P. verna* behaves here unambiguously as a calciphilous species. The populations were cytologically uniform, the same ploidy level for all 64 analysed individuals was proven. Determined chromosome counts of $2n = \sim 40\text{--}42$ and genome size of $1.75 \text{ pg} / 2C (\pm 0.009 \text{ SD})$ correspond to hexaploids of *P. verna*. According to macromorphological measurements, most of the quantitative values are in the lower range compared to European Floras. We suppose that this deviation is due to the extremely dry environmental conditions in Hungarian habitats. Since *P. verna* is a less-known species and previous descriptions often follow old keys only, we prepared (and provide here) a new identification key based on recent measurements.

Key words: distribution, extinction, genome size, hexaploid, morphology, rocky grasslands

INTRODUCTION

Potentilla L. subgenus *Potentilla* (Rosaceae, Rosoideae) with about 50 species occurring in Europe is a taxonomically intricate, complicated group, with several nomenclatural confusions, and lacks in the understanding of evolutionary processes. Main reasons behind these issues are repeated hybridization and apomixis connected with polyploidy and reticulate patterns (Kurtto *et al.* 2004, Wall *et al.* 1968). *P. verna* L. (sect. *Aureae* (Rydb.) Juz.) comprises a morphologically extremely variable polyploid (tetraploid to dodecaploid) complex. Some authors (i.e. Smith 1971) consider it as a collection of polyploid apomicts of various, hybrid origin. Recently, it is often treated as a derivate of *P. pusilla* Host (syn.: *P. puberula* Krašan) (Soják 2010). Notwithstanding these confusions, this taxon is morphologically well characterized in the sect. *Aureae*

by having elongate internodes (stolons), and exclusively simple hairs on the underside of the leaves, therefore it is represented in several European floras with a relatively uniform description, mainly under the names *P. neumanniana* Rchb., *P. tabernaemontani* Asch. or *P. verna* L. Its nomenclature had been discussed for a long time (for details see Kurtto *et al.* 2004, Soják 2009), here we follow the concept of Soják (2009) that proposed the use of the name *P. verna* L., with a conserved type, for this taxon. This has also been accepted by the Nomenclature Committee for Vascular Plants (Brummitt 2011), hopefully leading to a stable nomenclatural and taxonomic solution.

Potentilla verna is an Atlantic – central European species that occurs from the lowlands to the submontane regions (Fig. 1). The species is not rare in the Alps, and in the Eastern and Western Carpathians, the taxonomic position of the stands in the northern Balkans (incl. Southern Carpathians) is not yet fully clear (Fischer *et al.* 2008, Goliašová 1992, Kurtto *et al.* 2004). Despite its relatively wide distribution in the neighbouring countries, and several records close to the recent borders, it has been reported and collected in Hungary at a single site near Kőszeg (western Hungary) in 1892 (Király 1996), and it is considered as extinct in the country (Király 2007).

Here we report on new occurrences of the species in Hungary in the north-eastern part of the country, with the analysis of historical data (incl. review of erroneous records), the investigation of the morphological features, chromosome counts and ploidy of the newly found populations, the circumscription of the ecological conditions of the sites, and the nature conservation aspects of the (re)discovery.

MATERIAL AND METHODS

Field studies and plant material

The field study was carried out between 2018 and 2022. For each locality, the geo-coordinates and altitude were determined using a MobilMapper 50 GPS handheld device in WGS84 projection. Distribution maps were compiled using ArcGIS software. The material of the following herbaria was used for comparative morphological studies: BP, DE, SAMU (for herbarium acronyms see Thiers 2024+). Recently collected voucher specimens were deposited in DE. Ecological indicator values not corresponding to the Ellenberg's system were converted following Király (2006).

Morphological investigations were based on the assessment of 10 specimens in each of four populations. In the assessment we included 19 morphological features (17 of them were quantitative). The taxonomic treatment of vascular plants is in accordance with Király (2009), those of the phytosociological units with Borhidi (2003).

Chromosome counts

Materials for chromosome counts were sampled from three plants collected in Galvács and Meszes, which are potted in personal garden of the first author. Chromosome counts were determined in mitotic metaphases of root-tip cells of potted plants. The root tips were pre-treated in a 0.002 M aqueous solution of 8-hydroxyquinoline for 16 h at 4 °C (overnight), then fixed in the mixture of 96% ethanol and 98% acetic acid in the ratio 3:1 for at least one hour. After fixation, the meristems were washed in distilled water, macerated in 1-N HCl at 60 °C for 6 min and washed again in distilled water for 10 min. Cellophane square method (Murín 1960) was used to prepare root tip squashes on glass slide. Prepared squashes were stained in 7% Giemsa stock solution in Sørensen phosphate buffer for ~30 min, washed in distilled water, and dried. Slides were inspected in a drop of immersion oil using a Leica DM 2500 microscope equipped with a DFC 290 HD camera under $\times 1,000$ magnification and the Leica application suite software (ver. 3.5.0).

Flow cytometry

Plants investigated for ploidy level analysis were collected from all four populations (64 individuals in total). Further four additional specimens were used to estimate the genome size. We conducted a two-step flow cytometry (FCM) procedure (Doležel and Göhde 1995) according to Loureiro *et al.* (2007) with some modifications. Nuclei were isolated from leaves. Equally sized pieces of leaves of $n = 1\text{--}5$ individuals (so-called “bulked samples” when $n \geq 2$) were chopped (homogenized) in 1 ml of general-purpose buffer (Loureiro *et al.* 2007) using razor blade. For genome size estimation, equally sized parts of leaves from *P. verna* and proportionally sized leaf piece of reference standard (RS) *Solanum pseudocapsicum* L. with $2C = 2.62$ pg (Temsch *et al.* 2010; see also Kolarčík *et al.* 2020) were prepared always separately for each of investigated plants (altogether four individuals). After filtering through 42 μm nylon filter, the samples were incubated with RNase (50 $\mu\text{g mL}^{-1}$), PI (50 $\mu\text{g mL}^{-1}$), and β -mercaptoethanol (2 $\mu\text{L mL}^{-1}$) for at least 15 min before measurements. We used the flow cytometer Partec CyFlow ML (Partec GmbH, Münster, Germany) equipped with a 532 nm green laser beam and FloMax software (ver. 2.70, Partec GmbH, Münster, Germany).

FCM results were displayed as FCM histograms, and these allows for clear identification of G_0/G_1 peaks of both *Potentilla verna* and RS (G_0 and G_1 refers to nuclei of specific phases of cell cycle). More than 5,000 nuclei were analysed in one record with at least 1,300 nuclei for both, *P. verna* and RS. The coefficient of variation for *P. verna* and RS G_0/G_1 peaks was below 5%. DNA content ($2C$ val-

ues) for FCM samples was calculated from positions of the G_0/G_1 peak means according to the formula (Doležel and Bartoš 2005): DNA content of DA (pg, 2C value) = DNA content of RS (pg, 2C value) $\times [(G_0/G_1 \text{ peak mean of DA})/(G_0/G_1 \text{ peak mean of RS})]$. For genome size estimation, each investigated plant was repeatedly measured four times in four different days (between-day fluctuation was always below 2%) and values were averaged per individual.

RESULTS AND DISCUSSION

Historical records

Records under the name "*Potentilla verna*" were reported in numerous older sources from central and northern Hungary (Borbás 1875, Bontz 1896, Zoltán 1904, Prodán 1909, Kitaibel in Gombocz 1945), however, these events are considered a consequence of the controversial use of the name. These records are not supported by vouchers, consequently they had not been accepted by the standard Hungarian Floras (Jávorka 1924–25, Soó and Jávorka 1951, Soó 1966, Simon 2000, Király 2009), and in most cases, later corrections showed that they referred to *P. incana* G. Gaertn., B. Mey. et Scherb. (*P. arenaria* auct.).

The only region of Hungary with unambiguous evidence of presence of the species is the Kőszeg Mountains (foothill of the Eastern Alps, western Hungary), although some early records are erroneous in this area as well (i.e. Freh 1876, corrected by Borbás 1887). In the wider region of Kőszeg (without details on the locality) the species was reported by Freh (1883), Borbás (1887), and Waisbecker (1882, 1891a). Noteworthy that all these records are pre-WW 1, and significant part of the surroundings of Kőszeg became thereafter part of Burgenland, Austria. The only certain locality lying in recent-day Hungary is the village of Cák, where the plant was repeatedly collected in the 1890s, but published only by Waisbecker (1891a, b). The species has not been found later in the area, despite several efforts 1990 onwards (Király 1996). The record of Csapody (1980) from "Írottkö" (highest peak of the Kőszeg Mts) is quite dubious due to the lack of vouchers, and the several other species misidentifications in his paper.

Historical vouchers of *P. verna* from Hungary (Fig. 1): Kőszeg Mts region: "In lapidicius Cák" (stone pit near Cák), 1890 and 1891, W. Piers (SAMU); "Czáki kőfejtő" (stone pit near Cák), 2 V 1892, A. Waisbecker (SAMU).

(After revisions, Király 1996 reported three other vouchers from BP, collected by the above authors between 1890 and 1893 near Cák, however, these specimens have not been re-found during the recent revision in BP, neither in the material of other *Potentilla* taxa).

Recent records

During recent field research, *Potentilla verna* was found in 4 new localities in north-eastern Hungary. According to Goliašová (1992), the species is scattered in the neighbouring south-eastern Slovakia, both in hilly and mountainous regions, therefore the new stands are supposedly in contact with the adjacent populations and can be considered as formerly overseen native occurrences (although some of them are situated in a secondary habitat).

The former iron ore mine in Rudabánya is supposed to be a secondary habitat for the species. Until the late 1980s, the habitat of the population was the area of an operating mine, part of which was recultivated in the early 1990s and planted with Scots pine, furthermore, succession had begun. The

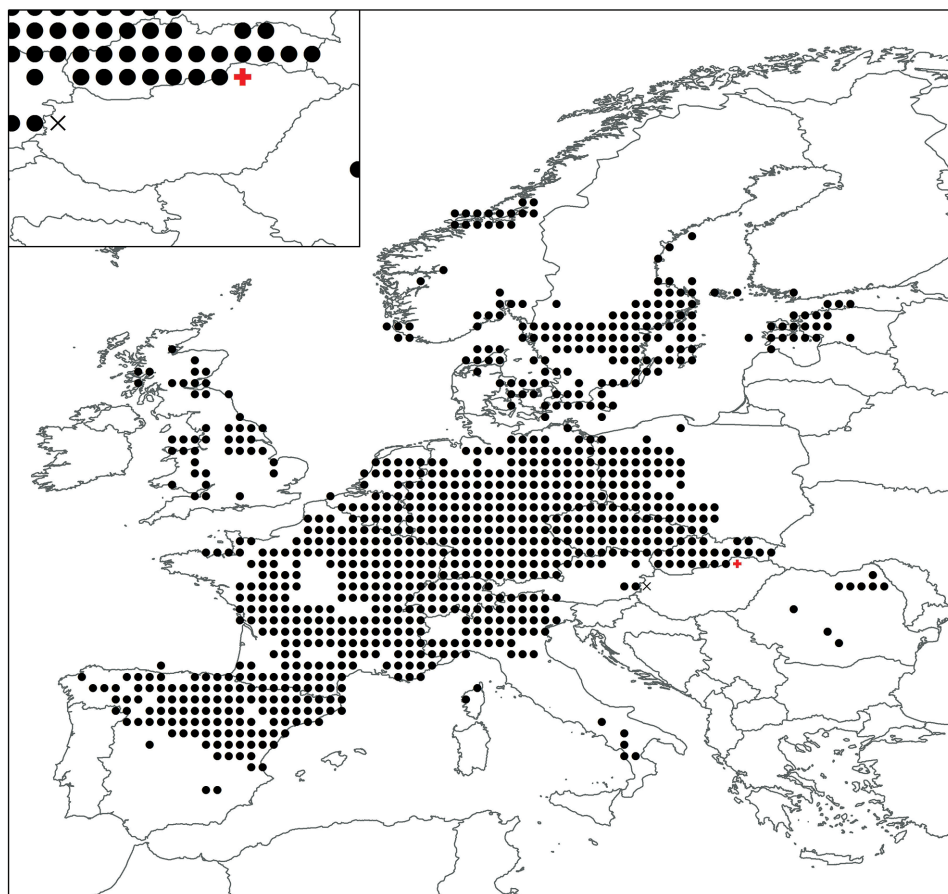


Fig. 1. Distribution of *Potentilla verna* in Europe. (After Kurtto *et al.* (2004), only category 'native', converted in GARS grid; • = original occurrences, + = new occurrence, x = extinct)

habitat on the Vár-hegy in Meszes is also considered to be a secondary one, as it is located on the edge of a planted pine forest right next to a paved road. We suppose that the species used to occur in natural rocky grassland in the surrounding hills (a considerable part of the rocky grasslands of Vár-hegy has been destroyed by former mining activities). The other two localities (Galvács: Boroszló-Hegy, Meszes: Cseres) represent natural rocky grasslands having similar environmental conditions like regularly occupied habitats of the species in Europe (Fig. 3).

In Hungary, based on current observations, the natural populations occur in the *Sedo acris-Festucetum valesiacae* association of the Festuco-Brometea class. For comparison, we note *Potentilla verna* in Slovakia is also a Festuco-Brometea, and additionally, a Sedo-Scleranthetea species (Goliašová 1992), similarly to the records from the Czech Republic (Soják 1995). In the latter

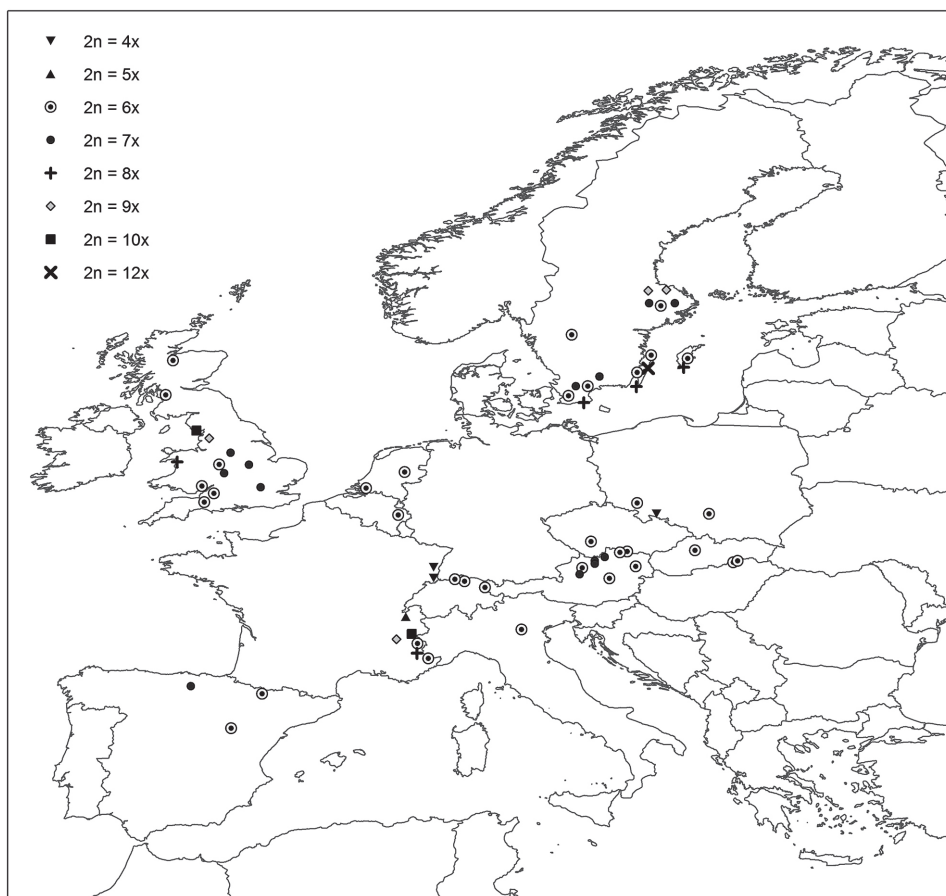


Fig. 2. Ploidy levels in populations of *Potentilla verna* (after Dobeš 1999, complemented)

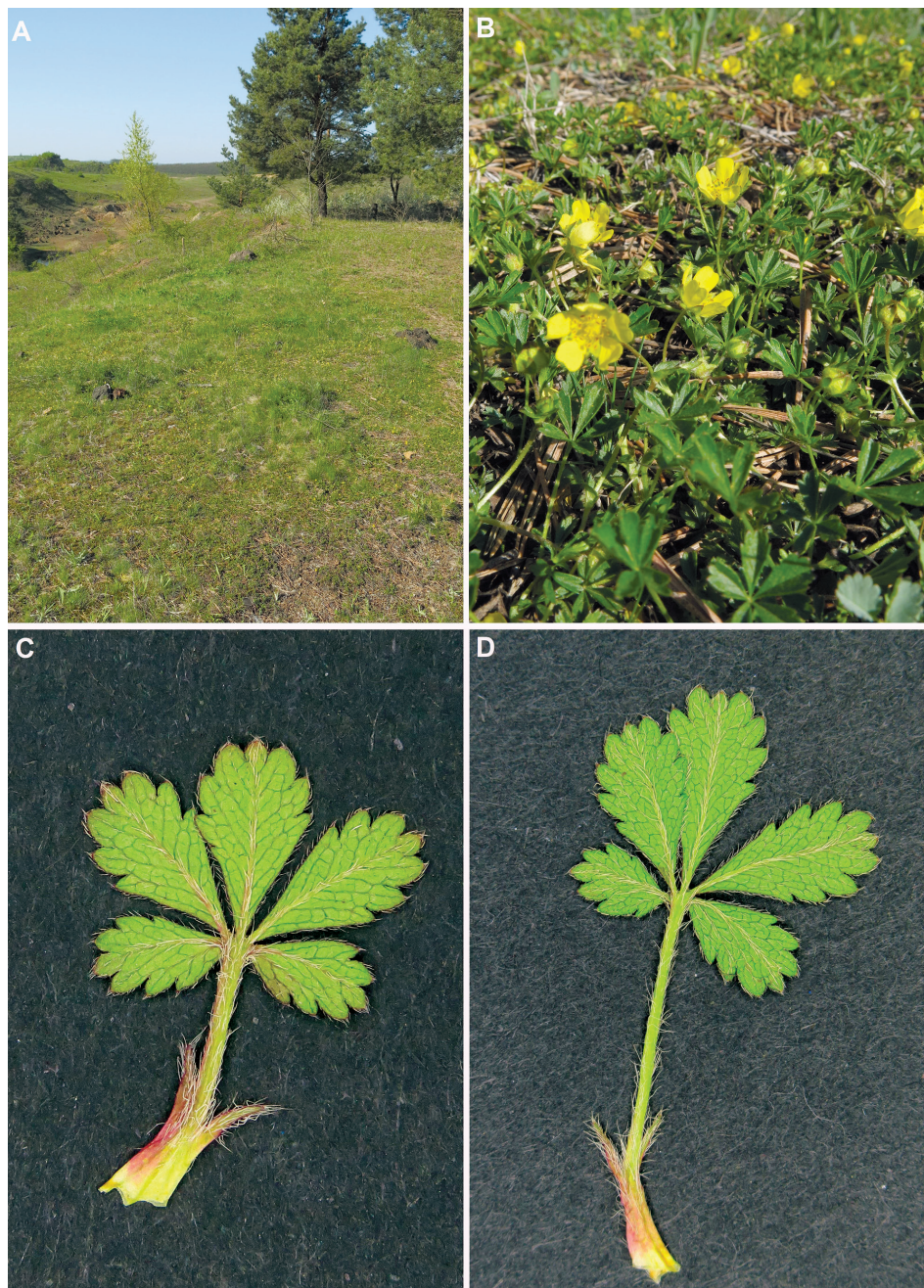


Fig. 3. Habitat of *Potentilla verna* (secondary grassland in the iron ore mine near Rudabánya) (A); Flowering specimens of *Potentilla verna* (Rudabánya) (B); Typical leaves of *Potentilla verna* (lower side) (Rudabánya) (C–D) (photo: V. Virók)

country, the species was reported also from dry and acidic associations, as Nardo-Callunetea and Hyperico perforari-Scleranthion perennis. In Germany, it also occurs in dry pine forests of Erico-Pinetea class, and in the associations of the Festuco-Brometea class (Rothmaler 1994).

List of recent localities of *Potentilla verna* in north-eastern Hungary (Fig. 1): – Gömör-Torna Karst region: Rudabánya 1: Vasércbánya (7689.2), N 48.3805°, E 20.6287°, 256–270 m a.s.l., on Gutenstein Limestone Formation, *Sedo acris-Festucetum valesiacae* (Virók V., 9 X 2015, DE-Soo-41127). – Cserehát region: Galvács 1: Boroszló-hegy (7590.4), N 48.4290°, E 20.7795°, 241–245 m a.s.l., Szendrő Phyllite Formation, *Sedo acris-Festucetum valesiacae* (Virók V., 22 IV 2022, DE-Soo-48364); Meszes 1: Cseres (7590.4), N 48.4412°, E 20.8093°, 220–230 m a.s.l., Szendrei Phyllite Formation, *Sedo acris-Festucetum valesiacae* (Virók V., 12 IV 2022, DE-Soo-48364, DE-Soo-48465); Meszes 2: Vár-hegy (7590.4), N 48.4412°, E 20.8093°, 192 m a.s.l., Rakacaszend and Rakaca Marble Formation, secondary dry grasslands (Virók V., 6 V 2021, DE-Soo-48367).

Both the recently unconfirmed western, and the newly found north-east Hungarian localities of *Potentilla verna* are situated on the south-eastern edge of the European range, representing low-lying outposts of the distribution area in the closed oak forest belt.

Morphology and identification key

The species represents a morphologically highly variable polyploid complex ($2n = 28, 35, 42, 49, 56, 63, 70, 84$), hybridizing with numerous other *Potentilla* species. Several local populations have been described as infraspecific taxa, or even on rank of species (Kurtto *et al.* 2004). The different ploidy levels in the distribution range of the species are shown on Figure 2. Infraspecific taxa are primarily distinguished by the shape and articulation of the leaf blades (Soják 1995). Soják (2010) considered that *P. verna* has evolved from the hybrid *P. × puberula* (*P. heptaphylla* L. × *P. incana* G. Gaertn., B. Mey. et Scherb.) by the gradual loss of stellate and partially reduced stellate hairs.

The results of morphological measurements in the newly found north Hungarian populations are shown in Table 1. Most of the quantitative values are in the lower range compared to other European Floras. Exceptions are the diameter of flowers and the length of sepals, which are in the range of the mean values. We suppose that this small deviation is due to the extremely dry environmental conditions in Hungarian habitats.

Since *Potentilla verna* is a less known species in Hungary and previous descriptions easily follow old keys, we prepared (complementing the keys of Soják 1995 and Király 2009) a new identification key based on recent morphological measurements for *Potentilla* species having digitate leaves with (3–)5–7(–9) leaflets, not solitary flowers and 5 yellow petals.

Table 1
Morphological features of 4 north-east Hungarian populations of *Potentilla verna*, compared to three major European references (see also Fig. 3)

Feature	Present study	Wall <i>et al.</i> (1968)	Soják (1995)	Ertter <i>et al.</i> (2014)
Habit	mat forming	mat forming	mat forming	mat forming
Creeping branches	present		present	–
Rooting at the nodes	yes	yes	–	sometimes
Hairs on lower side of leaves	simple	simple	simple	simple
Length of hairs on the stem and leaves (mm)	(0.4–)1.5–2.1(–2.5)*	–	0.7–2(–3)	0.5–2.5
Nr. of leaflets	(3–)5	5–7	5–7	5–7
Length of leaflets (mm)	(7.5–)9.8–13.8(–21.2)	8–40	10–20	(8–)10–30
Width of leaflets (mm)	(3.7–)4.5–6.4(–8.5)	4–15	5–10	4–15
Nr. of teeth on leaflets per side	2–3(–4)	–	–	2–4(–5)
Stipules of basal leaves	linear	linear	–	acuminate to acute
Petiole length (mm)	(6.4–)8.5–18.8(–56.3)	–	–	10–60
Position of hairs on petiole	ascending to appressed	–	–	ascending to appressed
Length of petiolule (mm)	0–1	–	–	0–2
Length of pedicel (mm)	(8.9–)11.5–15.3(–22.4)	–	–	10–30(–40)
Diameter of flowers (mm)	(11.2–)13.6–16.1(–17.6)	–	(7–)12–20	–
Length of sepals (mm)	(3.5–)4.3–4.9(–5.5)	–	–	3–5
Length of petals (mm)	(3.8–)5.0–6.3(–7.4)	6–10	5–8	5–8(–10)
Width of petals (mm)	(2.4–)2.9–4.1(–5.2)	–	–	4–6
Length of epicalyx-segments (mm)	(1.8–)2.4–3.3(–3.8)	–	–	3.5–5
Width of epicalyx-segments (mm)	(0.8–)0.9–1.3(–1.5)	–	–	1–1.5
Length of filament (mm)	(0.8–)1.3–1.8(–2.0)	–	–	0.8–2
Length of anthers (mm)	(0.3–)0.5–0.6(–0.7)	–	0.5–1	0.5–0.8
Length of fruits (mm)	(1.1–)1.3–1.4(–1.6)	–	1.5–1.8	1.5–2

*(min) lower quartile – upper quartile (max)

- 1a Base of the styles thickened; stem ascending, erect (if prostrate, the stem is not rooting at nodes), 15–50 cm long
P. collina agg., *P. argentea* agg., *P. recta* agg., *P. inclinata*
- 1b Base of the styles not thickened; stem prostrate, ascending, 5–20 cm long 2
- 2a Mat forming plant with creeping branches, usually rooting at nodes; branches partly covered with stipules; leaflets (3–)5(–7) 3
- 2b Compact plant without creeping longer branches; the base of the branches is densely covered with stipules; leaflets (5–)7 4
- 3a Abaxial side of leaflets with simple, straight hairs only; hairs on petioles appressed or subappressed, straight, rigid; stipules of basal leaves linear ($2n = 28, 35, 42, 49, 56, 63, 70, 84$) *P. verna* L.
- 3b Abaxial side of leaflets densely covered with stellate hairs overlapping or touching each other ($2n = 14, 28, 35$)
P. incana G. Gaertn., B. Mey. et Scherb. (*P. arenaria* auct.)
- 4a All petioles with patent hairs; surface of sepals hairy; epicalyx-segments linear-lanceolate or oblong ($2n = 14, 21, 28, 30, 35$) *P. heptaphylla* L.
- 4b All petioles with appressed or erecto-patent hairs; surface of sepals glabrous or hairy along the edge; epicalyx-segments narrow-linear ($2n = 42$) *P. patula* Waldst. et Kit.

Note 1. Morphotypes of *P. heptaphylla* with 5 leaflets are also known, they can easily be mixed up with *P. verna*. However, *P. heptaphylla* does not form large mats and it does not have creeping/rooting longer branches. The base of the branches is densely covered with stipules. It has only patent hairs on the petioles that are thinner, not rigid. Hairs of *P. verna* are usually translucent, hairs of *P. heptaphylla* are whitish.

Note 2. Hybrids are excluded from the key.

Chromosome counts, ploidy level and genome size

Small chromosomes were frequently overlapped, which allows just approximate determination of chromosome counts. Number of chromosomes was determined to $2n = \sim 40\text{--}42$ (Fig. 4). FCM records were clear, both *Potentilla verna* and RS G_0/G_1 peaks were separated, and they were of required quality [low CV, symmetrical peaks (Fig. 4), and between day fluctuations among repeated measurements]. No variation in ploidy level was observed among investigated plants (64 individuals / four populations in total). The genome size (2C value) measured per individual of *P. verna* plants was 1.75 pg (Galvács1),

1.74 (Meszes1), 1.76 (Meszes2), and 1.75 pg (Rudabánya1) (Fig. 4), with the mean value of 1.75 pg (± 0.009 SD). Maximal genome size difference between samples was 1.14%.

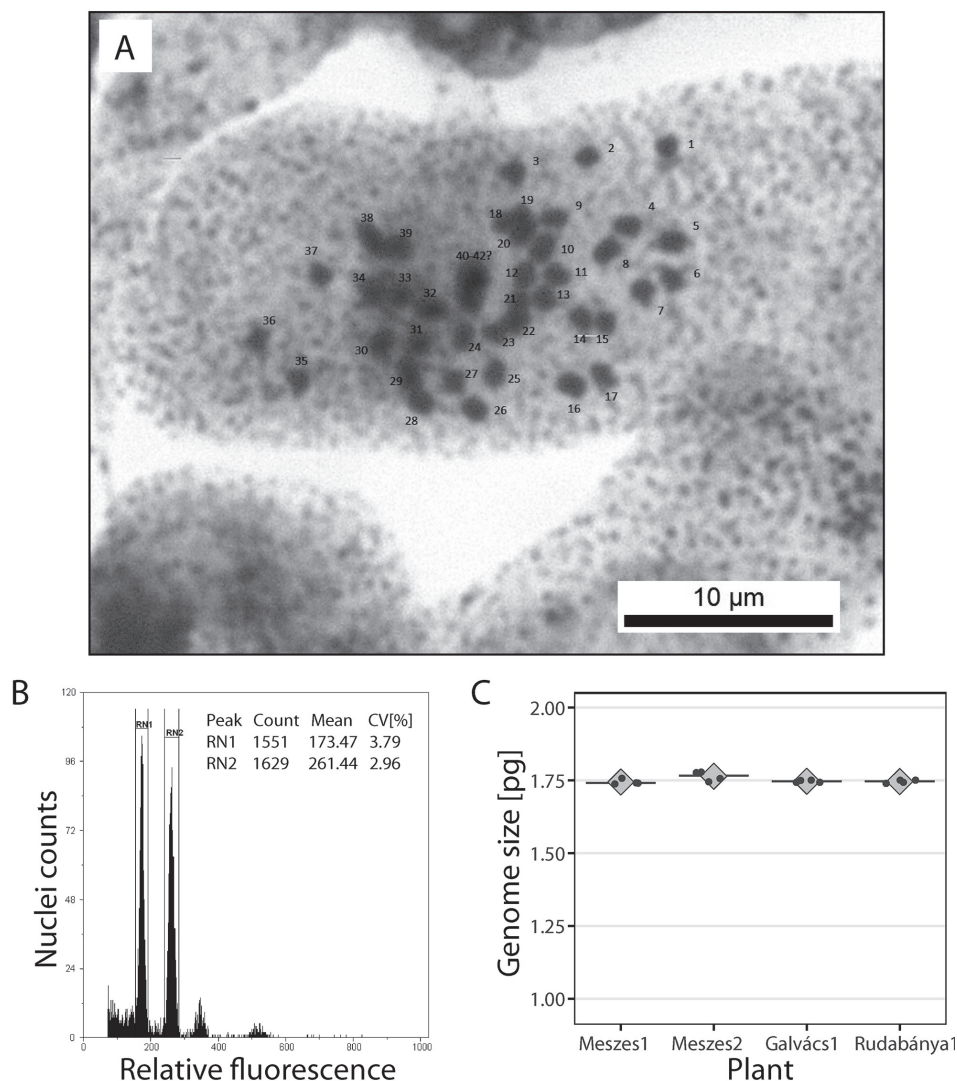


Fig. 4. Cytological analysis of northeastern Hungarian stands of *Potentilla verna*. A) Representative mitotic chromosomes in $2n = \sim 40-42$; B) Flow cytometry record, RN1 region – nuclei of *P. verna* in G_0/G_1 phase of cell cycle, RN2 region – nuclei of reference standard *Solanum pseudocapsicum* ($2.62 \text{ pg} / 2C$) in G_0/G_1 phase of cell cycle; C) Genome size variation among four *P. verna* individuals, dots – four repeats per plant, diamonds – mean value per plant, horizontal line – median value per plant

Populations of *P. verna* in north-eastern Hungary are cytologically uniform, we have confirmed the same ploidy level for all 64 analysed individuals. Determined chromosome counts of $2n = \sim 40\text{--}42$ and genome size of $1.75 \text{ pg} / 2C (\pm 0.009 \text{ SD})$ correspond to hexaploids of *P. verna* (Dobeš 1999, Šmarda *et al.* 2019).

Ecology

Potentilla verna grows in its entire range on dry, nutrition-poor soils in relatively warm, sunny sites (Ellenberg *et al.* 2001, Jurko 1990, Landolt 1977). The only indicator with considerable variation in the various sources is the soil reaction. In Switzerland and Slovakia, it prefers neutral or acidic soils (Jurko 1990, Landolt 1977), that is also in accordance with some reports on its preference to Nardo-Callunetea or Erico-Pinetea Soják communities (Rothmaler 1994, Soják 1995). On the other hand, it was shown by Ellenberg *et al.* (2001) in wider central European perspective as a rather calciphilous species. In Hungary, Borhidi (1995) classified it as a plant of slightly acidic soils, however, that was (connected with the historical west Hungarian records) only an assumption since the plant has not been found in the country 100 years ago. Based on recent field studies, in north-eastern Hungary *P. verna* clearly behaves as a calciphilous species.

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