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Genome size and leaf morphology variations of *Vaccinium uliginosum* in the Central Western Carpathians

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ABSTRACT:
A total of 67 individuals belonging to the species *Vaccinium uliginosum sensu lato* were sampled from diverse locations in the Central Western Carpathian Mountains in order to analyse their ploidy, genome size and leaf morphology using flow cytometry, chromosome counting and various morphometric methods including field measurements, graphical analysis of scanned leaves and stomatal measurements. The high elevations of Low and High Tatra mountain ranges above 1600 m a.s.l. serve as glacial refugia for the diploid lineage; between and around them on the forelands, the tetraploid lineage occurs. Contrary to the established practice in Slovak botany, it was not possible to distinguish between the diploid and tetraploid individuals based on leaf morphology alone, even though the lineages show a higher degree of niche separation than in the Alps. The variation in the leaf shape appears to occur randomly and the variation in leaf and plant size is influenced by both cytotype and elevation. We demonstrated a case of a diploid individual at an unusually low elevation, as well as an elevational range (approx. 1600–1650 m a.s.l.) where the two cytotypes overlap. This study comprehensively maps the occurrence, morphology and cytotype of *V. uliginosum* in Slovakia for the first time.

Keywords: flow cytometry, leaf morphology, morphometrics, ploidy, stomata, *Vaccinium uliginosum*

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INTRODUCTION

Bog bilberry, *Vaccinium uliginosum* L. *sensu lato,* is a circumboreal and circumpolar species of bilberry which also occurs in many southern alpine areas and often plays a dominant role in several alpine ecosystems, ranging from bogs and mires, shrublands, forests, and dry uplands to arctic and alpine tundra, heaths, and ridges. Some 30 taxa at various taxonomic levels have been described in this morphologically variable, diploid–polyploid complex. However, it is now usually recognised as a single species where di-, tri-, tetra- and hexaploid specimens have been documented by various authors (Alsos *et al.* 2005).

In the most extensive molecular study of diversity in *V. uliginosum s.l.*, Alsos *et al.* (2005) characterised three distinct lineages:

- The Beringian lineage, which occurs in Beringia, Alaska and the west coast of North America, mainly tetraploid with notable triploid and hexaploid populations;

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- The Amphi-Atlantic lineage, which covers most of Europe, southern Greenland and the south-eastern reaches of the North American range, almost exclusively tetraploid;
- The Arctic-Alpine lineage, which covers the Arctic regions and, notably, glacial refugia populations in the high mountains of Southern Europe and the Caucasus, almost exclusively diploid (Alsos *et al.* 2005; Eidesen *et al.* 2007).

Where these lineages meet, populations with different ploidies may coexist even at the same location. Such a situation has been described in great detail in the Alps, where the Amphi-Atlantic lineage in the northern part meets the Arctic-Alpine lineage in the southern part. Diploids and tetraploids show some degree of niche separation in this region, which is mostly due to the tetraploids occurring exclusively at low altitudes, while above 1500 m a.s.l. both cytotypes co-occur. Accordingly, tetraploids occur in a broader range of plant communities than diploids (Regele *et al.* 2017). Three separate locations have been identified where diploids and tetraploids grow side by side and are virtually indistinguishable in terms of basic morphometry, such as plant height and leaf shape parameters (width, length, circularity, area, and leaf outline shape). The single statistically differing parameter appeared to be the shape of the flowers (Silbernagl & Schönswetter 2019).

The reason for the isolation of the Arctic-Alpine lineage in the southern mountains above 1500 m a.s.l. has been hypothesised as follows: the south European populations survived the last glaciation in several smaller refugia and following the retreat of the glaciers, their expansion northwards was blocked by the rapidly expanding tetraploid Amphi-Atlantic lineage (Eidesen *et al.* 2007).

The Carpathian Mountain range provides multiple high altitude refugia for the diploid *V. uliginosum*. One of them is located in the High Tatra and Low Tatra mountains in the Central Western Carpathians. This particular refugium serves as a meeting point between lineages similar to that in the Alps, since northwards in the peat bog ecosystems in Poland the populations of *V. uliginosum* are tetraploid, whereas in high-elevation stands in the High Tatras, the populations are diploid (Combik & Mirek 2015).

In Slovakia, *V. uliginosum* is listed as an endangered and protected species, since centuries of agriculture, pasturing and the draining of peat bogs have destroyed the once much more widely distributed species. For example, in the 1950s a significant population still existed in the peat bogs along the river Rudava in the Záhorie lowland, which was subsequently destroyed by peat mining (Stanová & Kosorínová 2000). The current distribution of the species in Slovakia is provided in the www.biomonitoring.sk database, where field mapping records can be viewed separately for *V. uliginosum* and *V. gaultherioides*. This is in accordance with Flora of Slovakia, vol. III (Bertová & Futák 1982), which also distinguishes between *V. uliginosum sensu stricto* (tetraploid) and *V. gaultherioides* (diploid). In 1982, the first karyological analysis of *V. uliginosum s.l.* in Slovakia was performed, confirming the presence of the species in the Western Carpathians in both diploid and tetraploid karyotypes. Two samples were analysed, one diploid and one tetraploid, both from the high elevation stands in the High Tatra mountains. The authors themselves concluded that these results did not align with the established distribution pattern of the two karyotypes and suggested further research (Májovský & Uhríková 1982). The study conducted by Combik & Mirek (2015) focused on several species of Polish flora, including *V. uliginosum s.l.* from the Central Western Carpathians. Their study also indicated the presence of two different cytotypes within the same region but included data for only three samples.

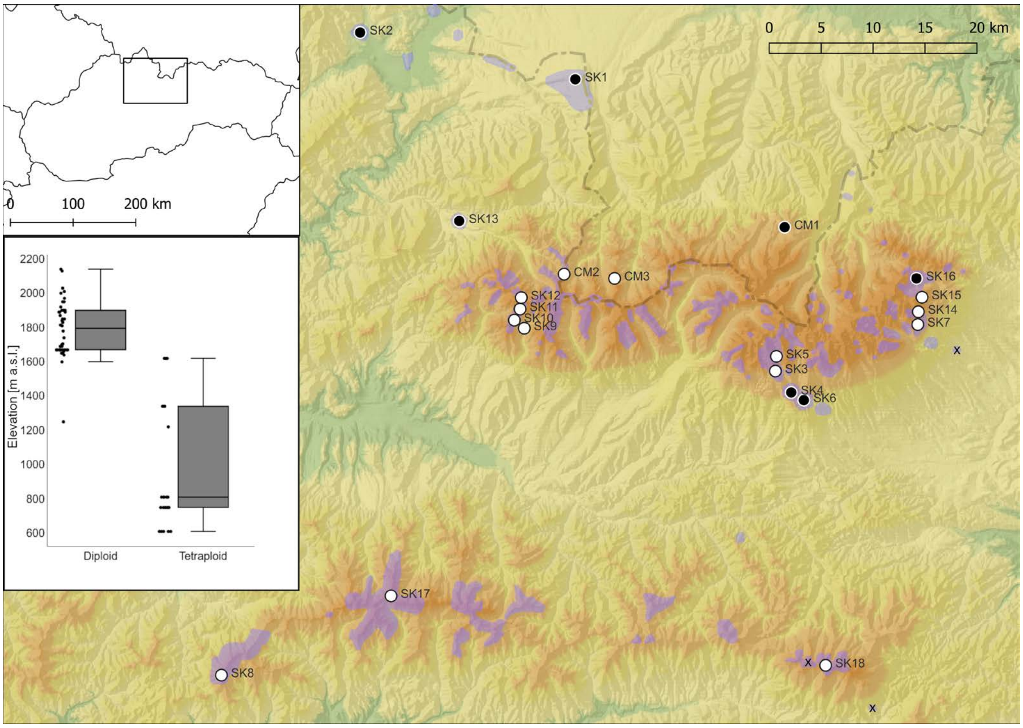

Fig. 1. Populations sampled and the ploidy level of the individuals studied in the Central Western Carpathians. Population IDs correspond to Table 1 (white – diploid; black – tetraploid). CM1 – CM3 indicate populations sampled in Combik & Mirek (2015). Purple areas indicate the range of distribution of *Vaccinium uliginosum s.l.* according to the public data in the www.biomonitoring.sk database. Areas marked with an x indicate a location where sampling was attempted, but no individuals were found. The upper insert indicates the position of the sampling area on the border between Slovakia and Poland in Europe. The lower insert indicates the elevational distribution of the studied individuals.

The aim of this study is:

- to determine the extent of diploid and tetraploid populations of *Vaccinium uliginosum* L. *sensu lato* in the Central Western Carpathians,
- to map the macro- and microscopic morphological features of the specimens, focusing on the leaves,
- to determine whether it is possible to distinguish between the diploid and tetraploid cytotypes based on the recorded morphological features, and
- to review the available floristic and taxonomic literature from this region and compare it with the presented results.

Similar to the studies carried out by Regele *et al.* (2017) and Silbernagl & Schönswetter (2019), various leaf shape parameters are utilised. To expand on previous research, the size and density of the leaf stomata are also included in the morphometry.

MATERIALS AND METHODS

Sampling. A total of 67 individual plants from 18 locations were collected by Z.V. in the summers of 2020, 2022, 2023 and 2024, as listed in Table 1 and visualised on a map in Fig. 1. Sample locations were selected based on the available data of the species distribution provided in the www.biomonitoring.sk database and Stanová (2020). When possible, north-south oriented tran-

Table 1. Sampled populations of *Vaccinium uliginosum s.l.* from northern Slovakia. Value of n indicates the number of specimens sampled at the location. RGS – relative genome size ± standard deviation. * indicates the chromosome number confirmed by chromosome counting.

ID	Sampling location	Predominant plant community	Latitude N	Longitude E	Elevation [m a.s.l.]	n	RGS ± SD	Ploidy	WUP Herbarium code
SK1	Rudné bog	*Sphagnion medii, Pino-Ledion*	49.38954	19.78173	750	5	0.661 ± 0.009 n = 15	4*	BBE_001-005
SK2	Klin bog	*Sphagnion medii*	49.42940	19.49964	610	5	0.665 ± 0.005 n = 15	4*	BBE_006-010
SK3	near Solisko	*Loiseleurio-Vaccinion*	49.14000	20.04418	1670	5	0.313 ± 0.008 n = 15	2*	BBE_011-015
SK4	Štrbské pleso bog	*Sphagnion medii*	49.12167	20.06490	1340	3	0.652 ± 0.009 n = 9	4	BBE_016-018
SK5	near Skok waterfall	*Loiseleurio-Vaccinion*	49.15263	20.04547	1690–1815	5	0.308 ± 0.014 n = 15	2*	BBE_019-023
SK6	Uhlištiatka bog	*Sphagnion medii*	49.11508	20.08139	1220	1	0.656 ± 0.010 n = 3	4	BBE_024
SK7	Škaredý žľab	*Pinion mugo*	49.17991	20.23087	1650	1	0.309 ± 0.001 n = 3	2	BBE_025
SK8	Prašivá	*Loiseleurio-Vaccinion*	48.87865	19.31779	1670	5	0.326 ± 0.011 n = 15	2	BBE_143- 147
SK9	near Žiarska chata	*Vaccinion myrtilli*	49.17673	19.71485	1250	1	0.303 ± 0.003 n = 3	2	BBE_106
SK10	Jalovec pass	*Loiseleurio-Vaccinion*	49.18362	19.70180	1650–1858	5	0.309 ± 0.007 n = 15	2	BBE_107-111
SK11	near Príslop	*Loiseleurio-Vaccinion*	49.19315	19.70930	1950–2142	5	0.309 ± 0.004 n = 15	2	BBE_112-116
SK12	Spálená valley	*Loiseleurio-Vaccinion*	49.20294	19.71096	1600–1950	5	0.314 ± 0.006 n = 15	2	BBE_117-121
SK13	Medzi bormi bog	*Sphagnion medii*	49.26851	19.62959	810	5	0.657 ± 0.007 n = 15	4	BBE_122-126
SK14	Skalnaté pleso	*Loiseleurio-Vaccinion*	49.19081	20.23152	1830–1900	2	0.322 ± 0.011 n = 6	2	BBE_127-128
SK15	near Svišťovka	*Loiseleurio-Vaccinion*	49.20327	20.23635	1950–2142	4	0.314 ± 0.006 n = 12	2	BBE_129-132
SK16	Trojrohé pleso bog	*Sphagnion medii*	49.21944	20.22914	1620	4	0.668 ± 0.018 n = 12	4	BBE_133-136
SK17	near Poľana	*Vaccinion myrtilli*	48.94689	19.54019	1840–1890	5	0.316 ± 0.008 n = 15	2	BBE_137-141
SK18	Stredná hoľa	*Vaccinion myrtilli*	48.88711	20.11005	1850	1	0.315 ± 0.006 n = 3	2	BBE_142

sects through the distribution area were selected (see Regele *et al.* 2017). Up to 5 individuals were investigated per sampling location, depending on availability. Due to the clonal growth of *V. uliginosum*, a distance of at least 10 m was maintained between individuals. From each individual, an approximately 10 cm long branch was sampled, kept fresh on a moist paper towel at 8°C for up to a week and stored in the -20°C freezer after returning to the facility. If berries were present, they were also included in the sample. Habitat data were taken from www.biomonitoring.sk, Šuvada (2023) and Stanová (2020) and verified in the field. More detailed descriptions of the sample habitats are provided in Supplementary Table 1.

Following analysis, material from all the sampled individuals was preserved as herbarium specimens in the WUP herbarium (see Table 1).

Flow cytometry. Flow cytometry (FCM) of 4',6-diamidino-2-phenylindole (DAPI) stained nuclei was used to estimate the relative genome size (RGS) of the frozen leaf samples. The internal standard used to determine DNA content was *Petroselinum crispum* (2C = 4.46 pg; Temsch *et al.* 2021). Green leaf tissue was processed using a method adapted from Suda *et al.* (2006). Briefly, leaves (approx. 1 cm^2 from both the sample and standard) were chopped in 1.3 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20) using a new razor blade. The crude suspension was filtered through a 40 µm nylon filter and centrifuged at 200 g for 4 min. The pellet was resuspended in 100 µl of fresh Otto I buffer and the samples were left for 30 min at room temperature. The staining solution consisted of 1 ml of Otto II buffer (0.4 M Na_2HPO_4. 12 H_2O) supplemented with DAPI (at a final concentration of 4 µg/ml) and β-mercaptoethanol (2 µg/ml). After 10 min of incubation at room temperature, the samples were filtered again and the fluorescence intensity was measured using a MACSQuant® Analyzer 10 Flow Cytometer (Miltenyi Biotec). The gain of the V1 fluorescence channel was adjusted so that the G_0/G_1 peak of the internal standard plant was located at 800 on a 1000 linear scale axis. For *V. uliginosum*, the tetraploid G_0/G_1 peak was then expected at approximately 500 and the diploid G_0/G_1 peak at 250. The flow rate was set to 50 µL/min and the relative fluorescence intensity of 3,000–7,000 particles was recorded to obtain at least 2,000 nuclei signals (Suda & Trávníček 2006).

FlowJo v10.10.0 software was used to evaluate the histograms, which were manually gated (the gating strategy is provided in Supplementary Fig. 1). The reliability of the measurements was assessed by calculating the coefficients of variation (CV) for the G_0/G_1 peaks of both the analysed sample and the reference standard. Histograms with CVs lower than 5% and a peak height of at least 50 particles were accepted. Due to tissue degradation during the transfer of the samples from locations SK3, SK8, SK9, SK10 and SK11, here CVs lower than 10% were also accepted (Suda & Trávníček 2006). Analyses were performed in triplicate for each sampled plant.

RGS was calculated as the ratio of the relative fluorescence of the sample to that of the standard. (Temsch *et al.* 2021).

Root tip microscopy and chromosome counting. To confirm the chromosome numbers in the *V. uliginosum* specimens, selected individuals (those bearing ripe fruits at the time of collection) were subjected to chromosome counting using fuchsin-stained root tip squashes. The seeds were extracted from the ripe berries, cold-stratified at 5°C for a month, incubated in of 500 ppm gibberellic acid solution for 24 hours at lab temperature, disinfected in 3% H_2O_2 for 15 minutes and then germinated on sterilised filter paper in a Petri dish, moistened with deionised water with the pH adjusted to 4.5 with 0.1 N sulphuric acid. The Petri dishes were incubated at laboratory temperature under normal light conditions.

The germinated seeds were analysed as soon as the radicles reached 5 mm in length. The radicles were pre-treated by incubation in a 0.4% colchicine solution overnight at 5°C to maximise the number of cells in metaphase. Afterwards, they were fixed in a mixture of glacial acetic acid/concentrated ethanol (1:3) at lab temperature for an hour, washed in distilled water and hydrolysed in 1 N HCl at 60°C for 10 minutes. The root tips were removed from the radicle and placed on a glass slide, where they were stained with basic fuchsin solution (Tanaka 1961). The excess dye was washed off with 30% acetic acid and the root tips were squashed under a cover slip. The slides were observed on a Nikon Eclipse E600 microscope under 600× magnification, both in normal light and with a FITC filter.

Morphometrics. Plant height, leaf size, flower size and the number of flowers and berries per node have all previously been suggested for discriminating between *V. uliginosum s.s.* (tetraploid) and *V. gaultherioides* (diploid) (Bertová & Futák 1982; Jansen *et al.* 2000; Fischer *et al.* 2008; Silbernagl & Schönswetter 2019). Since not all the individuals bore flowers or fruits at the time of collection, we focused on vegetative characters. Plant height was recorded in the field. Five mature leaves from the middle part of different branches of each collected specimen were glued onto paper and scanned at a 600 dpi resolution. Size measurements were taken using Lamina (Bylesjö *et al.* 2008) and the following characters were analysed: leaf length, leaf width, leaf perimeter, leaf area and leaf circularity, i.e. $4\pi(\text{area}/\text{perimeter}^2)$ – where a value of 1.0 indicates a perfect circle.

To characterise leaf shape, we employed LeafAnalyser (Weight *et al.* 2007) to assess variations in the leaf shape by placing 48 evenly distributed landmarks along the leaf edges. The leaf margin thresholds and leaf tips were checked and adjusted manually. The major trends in the whole dataset were summarised using the principal component analysis (PCA) tool provided by the software.

Stomata measurements were carried out microscopically using the nail polish cuticle imprint method (Lattier *et al.* 2019). Three healthy leaves per plant individual were selected and for each leaf, an entire half along the midrib on the abaxial leaf surface was treated with a thin coat of clear, nitro-cellulose fingernail polish. Once the nail polish had dried, strips of clear packing tape were applied to the coated area. The tape strips were subsequently removed carefully with forceps and the resulting cuticle peels containing relief impressions of the stomata were mounted on microscope slides. The slides were observed using a Leica ICC50 W microscope under 200× magnification. The length of 10 randomly selected stomata including guard cells was measured using LAS V4.13 software. For stomatal density, one image per leaf with the dimensions of 600 x 450 μm was captured and the number of stomata in each image counted.

Statistical analysis. The data were assembled and evaluated in MS Excel. The pairwise correlations between the measured parameters were tested using Spearman's rank correlation and the t-test. The significance of the differences between the diploids and the tetraploids was calculated using a one-way ANOVA on ranks (Kruskal-Wallis test). PCA was conducted using the LeafAnalyser tool (Weight *et al.* 2007) and SRplot (Tang *et al.* 2023).

RESULTS

Ploidy of the *Vaccinium uliginosum* samples. The ploidy of all the samples was determined by flow cytometry (Table 1) and confirmed by direct chromosome counting (Fig. 2). FCM analyses yielded high-resolution histograms

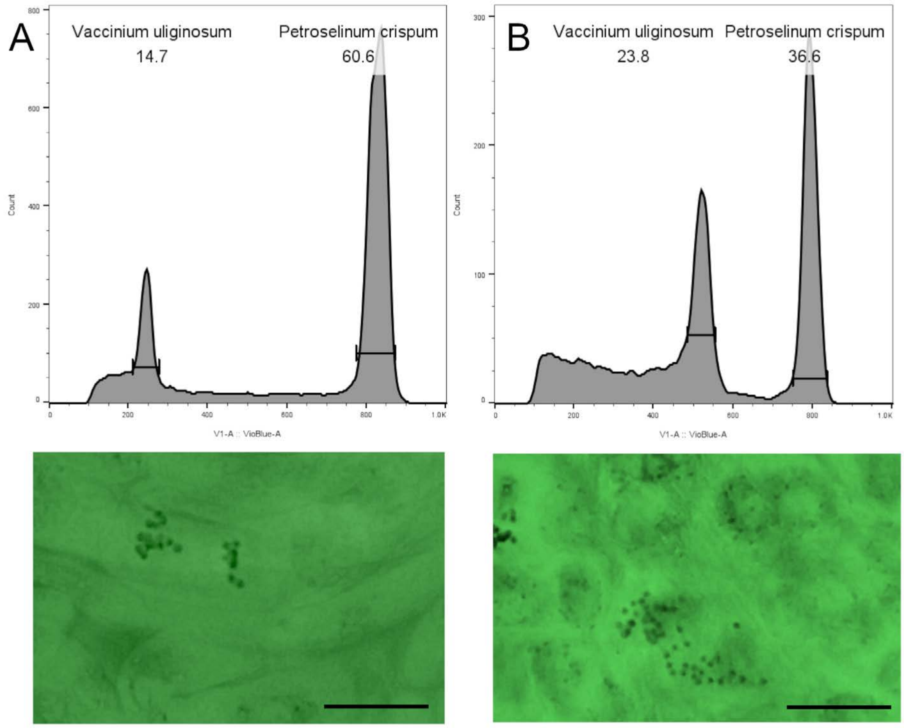

Fig. 2. Flow cytometry histograms (A and B) and root tip squashes stained with basic fuchsin and viewed using FITC filter (C and D). A and C, diploid individual (2n = 24), B and D, tetraploid individual (2n = 48). Scale bar: 20 µm.

and revealed the presence of two relative genome size classes with average RGS values of 0.313 ± 0.009 and 0.661 ± 0.011, corresponding to diploid and tetraploid karyotypes, respectively. Ploidy within populations was uniform, no mixed stands were found; no triploids or hexaploids were detected.

Morphometrics. The variation in leaf shape observed in the sampled plants was well represented in the reconstructed "mean leaf" and its principal components (Fig. 3 inset). A total of 97.94% of the variation was captured within the first four principal components. PC1 represents the leaf size and accounts for 86.58% of the variance, PC2 represents variation in the leaf width while maintaining its obovate shape, representing 7.31% of the variance. PC3 accounts for 2.64% of the variance, representing the leaf curvature with the petiole aligned either to the left or right. This variation in the position of the petiole was because the primary vein was often curved and our method oriented all leaves so that the leaf tip (i.e. the distal end of the primary vein) was vertically aligned with the centroid. Lastly, PC4 reflects 1.41% of the variance, reflecting the symmetry of the distal leaf tip to the proximal petiole: negative SD produces a broadly obovate shape, while positive SD produces a symmetrical lanceolate leaf shape (Weight *et al.* 2007).

Both the LeafAnalyser PCA and the PCA based on 8 morphological characteristics in Fig. 3 show a similar trend towards the separation of the diploid and tetraploid samples. However, in both cases the two clusters overlap and the separation is not complete. The clusters overlap even more on exclusion of the highly correlated morphological characteristics related to the absolute leaf size. The main parameters influencing the separation in all the PCAs appear to be the absolute size of the leaves including leaf length, width and area, as well as the plant height. Other parameters, such as leaf circularity and other leaf shape variations (lanceolate/orbicular, leaf tip acute/obtuse/obcordate) do not seem to contribute to the separation between diploids and tetraploids (Table 2; Fig. 3).

Table 2. The Kruskal-Wallis test comparing the morphological characteristics between diploid and tetraploid individuals of *Vaccinium uliginosum s.l.* from the Central Western Carpathians (n = 67).

Parameter	**H**	**p**
Leaf area	34.687	<0.001
Leaf perimeter	37.543	<0.001
Leaf circularity	0.050	0.822
Leaf width	41.018	<0.001
Leaf length	39.510	<0.001
Plant height	40.849	<0.001
Stomatal size	3.131	0.077
Stomatal density	6.975	0.008

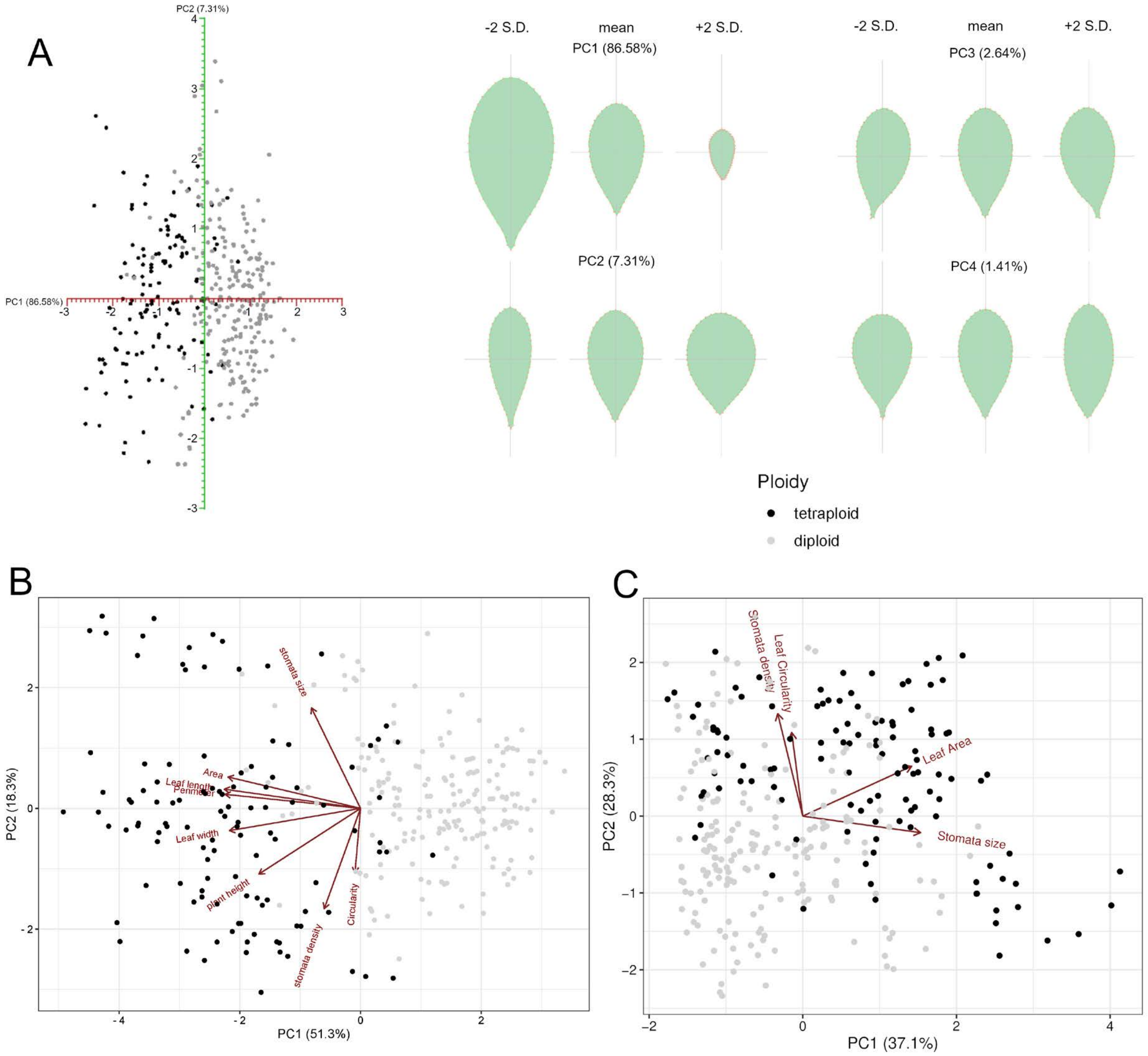

Fig. 3. A) variation in leaf shape of diploid (grey) and tetraploid (black) individuals of *Vaccinium uliginosum s.l.* from the Central Western Carpathians based on LeafAnalyser PCA. The inset shows the variation of reconstructed leaf shapes along the first four principal components. B) principal component analysis of the same individuals based on 8 morphological characteristics. Arrows in the inset represent the contribution of the characters to the overall explained variation. The methods for obtaining the morphological characters is provided in the main text (n = 335, five leaves per individual). C) principal component analysis of the same individuals based on the same morphological characteristics, with the highly correlated parameters excluded.

Table 3. Spearman's correlation matrix between morphological characteristics and elevation (n = 67). * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.

Leaf area	1								
Leaf perimeter	0.823 ***	1							
Leaf circularity	0.093	-0.072	1						
Leaf width	0.818 ***	0.846 ***	0.264 *	1					
Leaf length	0.817 ***	0.918 ***	-0.131	0.839 ***	1				
Plant height	0.542 ***	0.749 ***	0.106	0.785 ***	0.756 ***	1			
Stomatal size	0.593 ***	0.358 **	-0.035	0.237	0.274 *	0.038	1		
Stomatal density	0.118	0.235	0.084	0.224	0.136	0.372 ***	-0.116	1	
Elevation	-0.664 ***	-0.725 ***	0.081	-0.676 ***	-0.716 ***	-0.719 ***	-0.351 **	-0.486 ***	1
	Leaf area	Leaf perimeter	Leaf circularity	Leaf width	Leaf length	Plant height	Stomatal size	Stomatal density	Elevation

As can be seen in Table 3, the parameters related to the absolute leaf size showed strong and significant pairwise correlations. All parameters, with the sole exception of leaf circularity, were also strongly correlated with elevation. The stomatal density was significantly correlated with plant height and the stomatal size with leaf length, area and perimeter (Table 3). While stomatal density differed significantly between the diploids and tetraploids, stomatal size did not reach statistical significance (Table 2). Both were, however, strongly correlated with elevation.

DISCUSSION

Ploidy of the *Vaccinium uliginosum* samples and population analysis. We determined the ploidy of our samples using flow cytometry and chromosome counting. The flow cytometry results are in agreement with previously published values for *V. uliginosum* in Scandinavia (Eidesen *et al.* 2007) and the Alps (Regele *et al.* 2017).

A distinct pattern to the populations of *V. uliginosum s.l.* in the Central Western Carpathians can be observed in Fig. 1. The high elevations of the Low and High Tatra mountain ranges serve as glacial refugia for the diploid lineage; the tetraploid lineage occurs between and around them on the forelands. The elevation range of approx. 1600–1650 m a.s.l. is the cutoff value below which the plants are tetraploid, and above diploid (Alsos *et al.* 2005; Eidesen *et al.* 2007). This value only applies to this specific region of the Central Western Carpathians – the cut-off in the Alps determined by Regele *et al.* (2017) was approximately 1500 m a.s.l., above which both cytotypes co-occurred. Unlike in the Alps, the lineages in this region overlap less and show a higher degree of niche separation. In our sample set, tetraploids are exclusively found in peat bogs and diploids in alpine dwarf shrub communities (see Table 1).

There is a single notable exception, sample SK9, which is diploid, despite being sampled at an elevation of 1220 m a.s.l. in an alpine shrub community slightly below the timberline. This location offers a possible explanation for this unusually low occurrence of a diploid *V. uliginosum* individual as the Žiarska valley in the High Tatras experiences regular avalanches including a record-breaking, so-called „hundred-year-avalanche“ on 25.3.2009 which tore sheets of snow several kilometres long from the ridges on the north-western side above the valley (sample locations SK10 and SK11). The avalanche devastated the vegetation on the valley slopes and the snow took two years to melt completely before the shrub and tree cover were able to recover (Lizúch 2009). Avalanches have been previously observed to transport seeds or other propagules of alpine plants to lower zones. Once these alpine plants become established at lower elevations, they require space in order not to be outcompeted by taller species from lower elevations which are better adapted to such conditions. The destructive effects of avalanche disturbances enable small alpine plants to grow under such temporarily reduced competition (Rixen *et al.* 2007). Sample location SK9 lies at the convergence of several avalanche tracks from both sides, under the steepest slopes of the entire valley (Klaučová 2007). It is therefore entirely possible that an avalanche transported *V. uliginosum* propagules from the ridges above with it, which then took root in the exposed soil of the valley.

It is important to add that sample SK9 consists of a single clonal colony on a single spot in the lower elevations of the valley and no other specimens were found within a 200 m radius, which makes it a clear outlier. The avalanche transfer theory may be confirmed by surveying other similar avalanche-prone spots in the High Tatras, requiring further field work and research.

The extent of *V. uliginosum s.l.* in this region has been greatly diminished by peat mining, the draining of wetlands and highland pasturing (Stanová 2020). During the field work phase of this research, there were several locations where no *V. uliginosum s.l.* was observed, despite it being reported by www.biomonitoring.sk (Fig. 1, locations marked with X). The retreat affects tetraploid populations more severely, as they depend on lowland peat bog ecosystems. Shrub communities in peat bogs are most endangered by changing microclimate conditions and the growth of tree cover (Baranec *et al.* 2000). There appears to be only a few of these locations left in Slovakia where the tetraploid lineage survives and even fewer where it thrives. Among the locations observed in this study, the only ones where the tetraploid bushes bore abundant fruits were SK1 and SK2.

The alpine environment suitable for the diploid lineage has not undergone such a dramatic agricultural change, even though cattle pasturing on alpine meadows continues to influence the alpine heath layer. A stark contrast was observed between the eastern and western half of the Low Tatra mountains; while the populations of *V. uliginosum* in the western half appeared lush and well-established, sample location SK18 in the eastern half contained only a single small clonal colony, despite the fact that a large area along the 4 km length of the mountain ridge (which was reported as a growth site by biomonitoring.sk) was searched. Klinkovská *et al.* (2023) noted that the alpine shrub layer is significantly damaged by sheep and cattle grazing, preventing alpine heathland regeneration. This may also be the case for the eastern Low Tatra region, where the terrain allows for easier pasturing than in the western half.

Morphometrics. The results of the leaf morphometry are consistent with Regele *et al.* (2017) and with the populations of *V. uliginosum s.l.* in the Alps. Our study agrees with their conclusion that the morphological differences detected between the diploid and the tetraploid lineage are also inversely correlated with elevation and therefore cannot be attributed to ploidy alone. It has previously been shown that leaf characters provide no discriminating power between cytotypes when sampled at the same elevation (Silbernagl & Schönswetter 2019). It was unfortunately not possible to assess the discriminating power of the flower and berry characteristics in our sample set, since many of the *V. uliginosum s.l.* communities in this study did not bear any flowers or berries at all. This phenomenon has been previously observed in *V. uliginosum* populations in high elevation stands and may be due to harsh weather conditions, unfavourable fruiting years or herbivory. (Bertová & Futák 1982; Jansen *et al.* 2000). While proving a considerable limitation of our study, the common occurrence of sterile *V. uliginosum* communities also shows that the flower and berry characteristics cannot be relied upon to distinguish between the diploid and the tetraploid lineages.

It has been suggested that the size and density of the leaf stomata differ significantly between populations of different ploidy levels (Chavez & Lyrene 2009; Lattier *et al.* 2019), even when sampled in the field (McGoey *et al.* 2014), while also being influenced by factors such as weather, microclimate and water availability (Casson & Gray 2008). However, this was not the case in our study, since the stomatal size did not differ significantly between the diploid and tetraploid samples. Moreover, both stomatal parameters were strongly inversely correlated with altitude. It is possible that in our dataset the stomatal size and density were more influenced by weather and water availability in specific sampling years, which in turn may have skewed the pairwise correlations and ANOVA results. This is important to consider when analysing deciduous perennials such as *V. uliginosum*.

The Slovak approach to the taxonomy of *Vaccinium uliginosum s.l.* The cornerstone taxonomical literature in Slovakia considers the diploid lineage of *V. uliginosum* in the Carpathians a separate species, *V. gaultherioides* Bigelow (Bertová & Futák 1982; Marhold & Hindák 1998). In 1998, the old Cronquist classification was updated according to the Angiosperm Phylogeny Group system (APG) and has been revised with each subsequent APG update. This revision facilitated the update of the family, from the old Vacciniaceae to the new Ericaceae, but not of the species itself (Mártonfi 2022). Therefore, the distinction between the diploid and tetraploid *V. uliginosum* in the Slovak literature remains unclear. Considering the ongoing retreat of both diploid and tetraploid populations of *V. uliginosum* in Slovakia, it would be beneficial to extend the protective measures applied to *V. uliginosum s.s.* to also include the diploid lineage occupying the alpine habitats in Slovakia.

CONCLUSION

According to our data, it is not possible to clearly distinguish between the diploid and tetraploid lineages in the region of the Central Western Carpathians based on vegetative morphology alone, even though the lineages show a higher degree of niche separation than in the Alps.

We documented the case of a diploid individual at an unusually low elevation, as well as a range of elevations (approx. 1600–1650 m a.s.l.) where the two cytotypes overlap. Therefore, it is recommended to analyse the cytotype of *V. uliginosum s.l.* before making any assumptions regarding its affiliation with any specific lineage, or “subspecies” in the traditional sense.

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REZIME

Varijacije veličine genoma i morfologije lista *Vaccinium uliginosum* u centralno-zapadnim Karpatima

Zuzana Vaneková i Haider Sami

Ukupno 67 jedinki koje pripadaju vrsti *Vaccinium uliginosum sensu lato* uzorkovano je sa različitih lokacija u centralno-zapadnim Karpatima kako bi se analizirala njihova ploidnost, veličina genoma i morfologija lista korišćenjem tečne citometrije, brojanja hromozoma i različitih morfometrijskih metoda, uključujući terenska merenja, grafičku analizu skeniranih listova i merenje stomata. Visoke nadmorske visine Niskih i Visokih Tatri iznad 1600 m nadmorske visine predstavljaju glacijalne refugijume za diploidnu liniju; između i oko njih na predgorjima, javlja se tetraploidna linija. Suprotno ustaljenoj praksi u slovačkoj botanici, nije bilo moguće razlikovati diploidne i tetraploidne jedinke samo na osnovu morfologije lista, iako loze pokazuju veći stepen razdvajanja niša nego u Alpima. Varijacije u obliku lista izgleda da se javljaju nasumično, a na varijacije u veličini lista i biljke utiču i citotip i nadmorska visina. Demonstrirali smo slučaj diploidne jedinke na neuobičajeno niskoj nadmorskoj visini, kao i u rasponu nadmorskih visina (približno 1600–1650 m nadmorske visine) gde se dva citotipa preklapaju. Ova studija prvi put sveobuhvatno mapira pojavu, morfologiju i citotip *V. uliginosum* u Slovačkoj.

Ključne reči: tečna citometrija, morfologija lista, morfometrija, ploidija, stome, *Vaccinium uliginosum*