Contribuţii Botanice – 2025, LX: 85-95 DOI: 10.24193/Contrib.Bot.60.7
Grădina Botanică "Alexandru Borza"
Cluj-Napoca

CROSS-GENUS TRANSFERABILITY OF SSR MARKERS FROM *GENTIANA* SPP. TO *SWERTIA PERENNIS* AND *S. PUNCTATA* (GENTIANACEAE)

***Ioan BĂCILĂ*[1], *Dana ŞUTEU*[1], *Bogdan-Iuliu HURDU*[1], *Zoltán R. BALÁZS*[2,3,4], *Leonard CALISTRU*[1], *Ana COSTE*[1]**

[1] National Institute of Research and Development for Biological Sciences, Institute of Biological Research, Department of Experimental Biology, 48 Republicii St., **RO-400015 Cluj-Napoca, Romania**

[2] Babeş-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, 1 Kogălniceanu St., **RO-400084 Cluj-Napoca, Romania**

[3] Center for Systematic Biology, Biodiversity and Bioresources - 3B, Faculty of Biology and Geology, Babeș-Bolyai University, 1 Kogălniceanu St., **RO-400084, Cluj-Napoca, Romania**

[4] Doctoral School of Integrative Biology, Babeș-Bolyai University, 1 Kogălniceanu St., **RO-400084 Cluj-Napoca, Romania**

e-mail: dana.suteu@icbcluj.ro

Abstract: The transferability of simple sequence repeats (SSRs) markers among closely related species is a common strategy in population genetics; however, cross-genus transferability is less frequent. We evaluated cross-genus SSR amplification in Gentianaceae by testing 30 heterologous primer pairs developed for *Gentiana* across two *Swertia* taxa, *S. punctata* and *S. perennis*. Consistent, single-locus marker amplification was obtained for 11/30 (36.7%) primer pairs; the remainder yielded multiple, non-specific bands. Owing to limited polymorphism and low reproducibility, six SSR loci were retained for downstream characterization of the *Swertia* populations. Across these loci, eight alleles were detected in total (each SSR primer pair amplifying 1–2 alleles). Among the retained markers, LAW12 and LAW25 exhibited heterozygous profiles, whereas the remaining four loci were effectively homozygous in the analyzed material. The resulting data matrix was used to infer a Neighbor-Joining tree and to perform PCoA; both analyses recovered a clear separation between the two *Swertia* species, supported by bootstrap values.

Despite the low polymorphism of the six SSRs, the markers were sufficient to distinguish the analyzed populations along taxonomic lines. Further work, ideally the development of *Swertia*-specific SSRs or genome-wide SNP assays, would provide higher resolution and broader transferability across the genus.

Keywords: conservation genetics, endemic species; genetic diversity; glacial relict; polymorphism; South-Eastern Carpathians

Introduction

The genus *Swertia* was established by Linnaeus in *Species Plantarum* (1753) and now represents one of the most species-rich herb genera in the family Gentianaceae, comprising roughly 165 accepted species worldwide according to Kew's Plants of the World Online (POWO) [40]. Its native range spans the subarctic and subalpine zones of the Northern Hemisphere to the tropical mountains of the Old World, with diversity centres in temperate and subtropical regions, and in the montane tropics [40].

S. perennis L. was among the first species described and assigned to this genus when Linnaeus established *Swertia*, alongside *S. difformis*, *S. rotata*, *S. corniculata*, and *S. dichotoma* [15]. It was later designated the lectotype of the genus on the basis of Herb. Linn. 327.1 [3], thereby anchoring the application of the generic name to *S. perennis* in modern taxonomy. In Romania, *S. perennis* is one of two recognized *Swertia* species present, the other being *S. punctata* Baumg.; *S. perennis* is a vulnerable and rare glacial relict [27] occurring in montane–subalpine springs and fens, whereas *S. punctata* is a similarly rare, sub-endemic plant species of the South-Eastern Carpathians, occurring in both humid habitats (swampy meadows, eutrophic fens/peatlands, and springs), as well as rocky grassland and cliffs (siliceous substrate) [14, 40].

Molecular analyses on *Swertia* spans classic population surveys and newer omics approaches. Early studies on the Himalayan medicinal plant *S. chirayita* used dominant markers (RAPD/ISSR) and reported appreciable within-population diversity with geographically patterned variation [e.g., 7, 13, 18]. Plastid genomics has expanded rapidly, with complete chloroplast genomes now available for multiple species (e.g. *Swertia mussotii*, *S. japonica*, *S. bifolia*, *S. tetraptera*, *S. franchetiana*, *S. przewalskii*), and comparative plastome analyses have clarified genome architecture, variability hotspots useful for barcoding, and phylogenetic relationships within the genus [6, 34, 36, 37, 38]. Beyond organelles, transcriptome-level studies, especially in *S. mussotii*, have identified candidate genes in the secoiridoid/iridoid pathway (e.g., geraniol-10-hydroxylase, iridoid synthase) underlying key pharmacological metabolites [16, 35]. For temperate fen species, nuclear datasets are beginning to emerge: regional studies on *S. perennis* indicate structured diversity and phylogeographic lineages that complement cpDNA inferences [31, 32].

Microsatellites (SSRs) are highly polymorphic, codominant markers that are exceptionally informative for assessing genetic diversity, distinguishing closely related varieties, and managing core/germplasm collections [4, 10]. Their cross-species transferability depends on the conservation of flanking regions, typically higher among closely related taxa [2, 23], and is often limited for genomic SSRs located in less conserved DNA; EST-derived SSRs tend to transfer more readily [19, 24].

Against this backdrop, and to our knowledge, no population-level studies in *Swertia* have employed validated nuclear genomic SSR panels across multiple species or populations. Aside from transcriptome mining (EST-SSRs) and plastid repeats, conservation-genetics applications using nuclear SSRs in *Swertia* are essentially absent; most work has relied on ISSR or plastid-derived markers [16, 36].

To fill this gap for *S. punctata* and *S. perennis*, we tested the cross-genus transferability of 30 SSR markers developed for *Gentiana lawrencei* var. *farreri* and *Gentiana kurroo* [17, 26] and provided a preliminary assessment of genetic relationships between the two species.

Materials and Methods

Study species and sampling of biological material

***Swertia perennis* L.** (Figure 1, left) is a perennial, glabrous herb ~15–50 (–70) cm tall with short rhizomes and simple erect stems; leaves opposite, basal narrowly to linear-lanceolate

and cauline ovate-lanceolate, 3–7-nerved; inflorescences paniculate to few-flowered; flowers actinomorphic, usually 4–5-merous, with a rotate-campanulate, deep blue-violet corolla bearing darker spotting and conspicuous nectary fields on each lobe; stamens 4–5; capsule ellipsoid-ovoid; seeds minute, reticulate [8, 25, 30]. It flowers (June) July–September and is predominantly outcrossing/entomophilous [25]. Biogeographically, the species has a circumboreal distribution across the temperate to boreal zones of the Northern Hemisphere, extending from Scandinavia and the Alps/Carpathians to Siberia and North America [25, 40]. It is considered to be a glacial relict in the Carpathians [9, 27].

***Swertia punctata* Baumg**. (Figure 1, right) is a perennial, glabrous herb ~20–60 cm tall with a short rhizome and erect, usually branched stems; leaves opposite, lower narrowly lanceolate to lanceolate and upper ovate-lanceolate, several-nerved; inflorescence paniculate; flowers 4–5-merous with a pale blue-violet to lilac corolla densely spotted (“punctate”) on the lobes, each lobe bearing a conspicuous nectary field; capsule ellipsoid-ovoid; seeds numerous, minute [8, 25, 30]. Flowering mainly July–August [30]. The species is sub-endemic to the South-Eastern Carpathians (Ukraine and Romania), with only two small occurrences outside this mountain range, in Bulgaria and Kosovo [29].

Fig. 1: Left: ***Swertia perennis* on the humid cliffs from Cetățile Ponorului, Bihor Mountains;** Right: ***S. punctata* near the Valea Cepelor Spring, Bihor Mountains.** Photos: B.-I. Hurdu.

Seven populations of *Swertia punctata* and six of *S. perennis* were sampled in the Carpathian Mountains, Romania (Table 1). Within each population, 10 individuals were randomly selected along transects, maintaining a ≥10 m spacing where possible to minimize resampling of clones/close relatives. Young, healthy leaves from each individual were collected

into tubes prefilled with silica gel. Representative voucher specimens for each population were pressed and deposited in the herbarium of Babeș-Bolyai University, Cluj-Napoca (CL Herbarium), with identifications verified against regional floras. Upon return to the laboratory, tubes were kept dry at room temperature until DNA extraction.

Table 1: Summary of sampled populations (*S. punctata*, *S. perennis*): taxon, population code, mountain range, locality, geographical coordinates, and altitude.

Taxon	Pop. code	Range	Locality/Massif	Coordinates (Longitude E/ Latitude N)	Altitude (m)
Swertia punctata	SPunCM	Apuseni Mts.	Bihor Peak (Cucurbăta Mare)	22.69555/46.44184	1635
	SPunMM	Apuseni Mts.	Muntele Mare, Molhașul lui Tomoiu	23.25264/46.49347	1705
	SPunR	SW Carpathians	Retezat Mts.	22.9316/45.35485	2100
	SPunT	SW Carpathians	Bistra Cirque, Țarcu Mts.	22.53061/45.28638	1799
	SPunF	S Carpathians	Făgăraș Mts.	24.63148/45.59422	1935
	SPunS	S Carpathians	Șureanu Mts.	23.65038 /45.508	1338
	SPunP	S Carpathians	Parâng Mts.	23.65183/45.36484	2075
Swertia perennis	SPerMB	Apuseni Mts.	Bihor Mts.	22.70271/46.56359	980
	SPerRG	E Carpathians	Rodnei Mts.	24.80768/47.57557	1692
	SPerR	E Carpathians	Rarău Mts.	25.61035/47.4418	1350
	SPerB	E Carpathians	Pârâul Ruşilor fen (Bilbor area)	25.47634 47.10181	958
	SPerF	E Carpathians	Borsároș Fen (Sâncrăieni)	25.9015/46.4513	892
	SPerP	E Carpathians	Prejmer Peat Bog	25.73498/45.72737	512

DNA extraction

Total genomic DNA was extracted from 13–15 mg of silica gel–dried leaf tissue using the innuPREP DNA Mini Kit (Analytik Jena AG, Jena, Germany) according to the protocol provided by the manufacturer; the final elution was performed in 60 µL to increase DNA concentration. DNA quality was assessed on a 1% agarose gel stained with ethidium bromide, and DNA concentration was measured with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). To monitor handling accuracy, blind duplicates (~9% of individuals) were included and negative controls were implemented during downstream genotyping.

SSR fingerprinting

Thirty microsatellite markers developed for *Gentiana lawrencei* var. *farreri* and *G. kurroo* [17, 26] were tested for transferability to *Swertia punctata* and *S. perennis*. Each primer pair required optimization, as suboptimal amplification or nonspecific bands were otherwise obtained. PCR conditions are listed in Table 2. Following amplification and inspection of agarose-gel profiles, only six SSR primer pairs were retained; these were fluorescently labeled (6-FAM) and used in subsequent reactions. The characteristics of the six primer pairs, including the annealing temperature, are provided in Table 3.

Table 2: Thermal cycling conditions for SSR amplification (T_a - annealing temperature).

No.	PCR step	Temperature (°C)	Time
1	Initial denaturation	94	5 min
2	Denaturation	94	30 s
3	Annealing	T_a	45 s
4	Extension	72	45 s
5	Repet steps 2-4 (x35 cycles)		
6	Final extension	72	10 min
7	Hold	4	∞

PCR products were purified using a 1:1 Sephadex–Sephacryl mixture (GE Healthcare Bio-Sciences AB, USA) and then diluted 1:50. An aliquot of 1.5 μL of the dilution was added to 10 μL of Hi-Di formamide mixed with GeneScan™ 500 ROX™ Size Standard (Applied Biosystems, Thermo Fisher Scientific, USA) and analysed by capillary electrophoresis on an ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA).

Table 3: Characteristics of the six microsatellite primers selected after testing/optimization and used for cross-transferability in *Swertia* spp. T_a = primer specific annealing temperature.

Locus	Primer sequence (5′–3′)	Observed allele size(s), bp	T_a (°C)	Reference
G23	F: GATGCACGGCCTTCATTAGC R: ATCAATGGTGGAGGTCCTGG	117 (monomorphic)	57	[17]
LAW12	F: AGTGGCACAAAAACGGACTC R: AGCTCGGATTTTGGTTGATG	127–129	58	[26]
LAW19	F: ATCAGATGGTTCGACAAGGG R: GGCCTCTCTCTTCCCAATTC	215 (monomorphic)	50	
LAW24	F: TGATGCACTCTTCCCATGAA R: GGGTTTTGTGTGCGAAGTTT	139 (monomorphic)	53	
LAW25	F: CCGAGGTCGATCCTACAGAG R: AAACGCTTTTGGTTTGGTTG	128–129	54	
LAW45	F: CCAGTTTTGTAAGCTCTTCTAGGC R: TATGATCCTGGTCCCAGAGG	153 (monomorphic)	55	

Microsatellite data analysis

Allele scoring was performed in GeneMapper v4.0 (Applied Biosystems, Thermo Fisher Scientific, USA). Experimental reliability was evaluated using duplicate samples from different populations; only unambiguous, repeatable peaks concordant between duplicates were retained. Descriptive statistics, number of alleles (Na), observed heterozygosity (Ho), and expected heterozygosity (He) were calculated per population with GenAlEx 6.5 [20, 21]. An allele-frequency matrix was then generated and analysed in POPTREE2 [28] to infer a Neighbor-Joining tree based on Shared-Allele distance, with 1,000 bootstrap replicates. Relationships among individuals were further explored by Principal Coordinates Analysis (PCoA) computed in PAST v4.13 [12].

Results and Discussion

Cross-transferability and polymorphism

The SSR markers developed for *Gentiana* that were tested in *Swertia* showed limited cross-transferability. Of the 30 loci screened in *S. punctata* and *S. perennis*, 11 (36.7%) showed consistent single-locus amplification; while the rest produced multiple or nonspecific bands. Owing to low polymorphism and/or poor reproducibility, only six loci were retained for downstream analyses (Table 3). A total of eight alleles was detected across the six SSR loci, with 1–2 alleles observed per locus (Table 4). Only two loci (LAW12, LAW25) showed clear polymorphism, and heterozygous individuals were identified mainly in *S. punctata*. The remaining four loci, including LAW19 (for which scorable PCR products were obtained only in *S. perennis*), were effectively monomorphic in the present dataset (Table 4).

Genetic variability

Consistent with the low allelic diversity, observed heterozygosity (Ho) and expected heterozygosity (He) were very low across populations (mean Ho ~ 0.063–0.071; mean He ~ 0.101–0.102) (Table 4). Only LAW12 and LAW25 showed heterozygous genotypes; the remaining four loci were homozygous (fixed) in the analysed samples.

Table 4: Genetic characterization of six microsatellite markers tested across 13 *Swertia* populations.
Ho, observed heterozygosity; **He**, expected heterozygosity.

Locus	H_o	H_e	Number of alleles
G23	0	0	1
LAW12	0.063	0.102	2
LAW19	0	0	1
LAW24	0	0	1
LAW25	0.071	0.101	2
LAW45	0	0	1

The resulting matrix was used to infer a Neighbor-Joining tree, which revealed a clear separation between the two *Swertia* species (Fig. 2), supported by robust bootstrap values.

The pattern recovered by the Neighbor-Joining analysis was essentially confirmed by the PCoA results (Fig. 3).

Cross-transferability and locus performance

Interspecific transfer of SSRs relies on the conservation of primer-flanking regions, which decays with phylogenetic distance; accordingly, transfer success generally tracks genomic homology among taxa [2, 23]. Of the 30 Gentiana loci tested, only 11 (36.7%) produced consistent PCR products in *Swertia*, and just six were suitable for downstream analyses, a result consistent with expectations for cross-genus assays, which commonly show reduced success compared to intra-generic tests [19, 24]. Published examples show similarly modest cross-genus yields, e.g., *Medicago*→*Trifolium* ~18–22%, *Glycine*→other legumes ~1–3%, *Daucus*→*Foeniculum* ~23%, and ~35% across heterologous primers in *Cereus*, with family-level legume screens reporting ~30% reproducible cross-genus amplicons [1, 5, 19, 33, 39], and, in grasses, cross-species transfers from several Poaceae donors yielded only 7.3% success in *Trichloris crinita* [22].

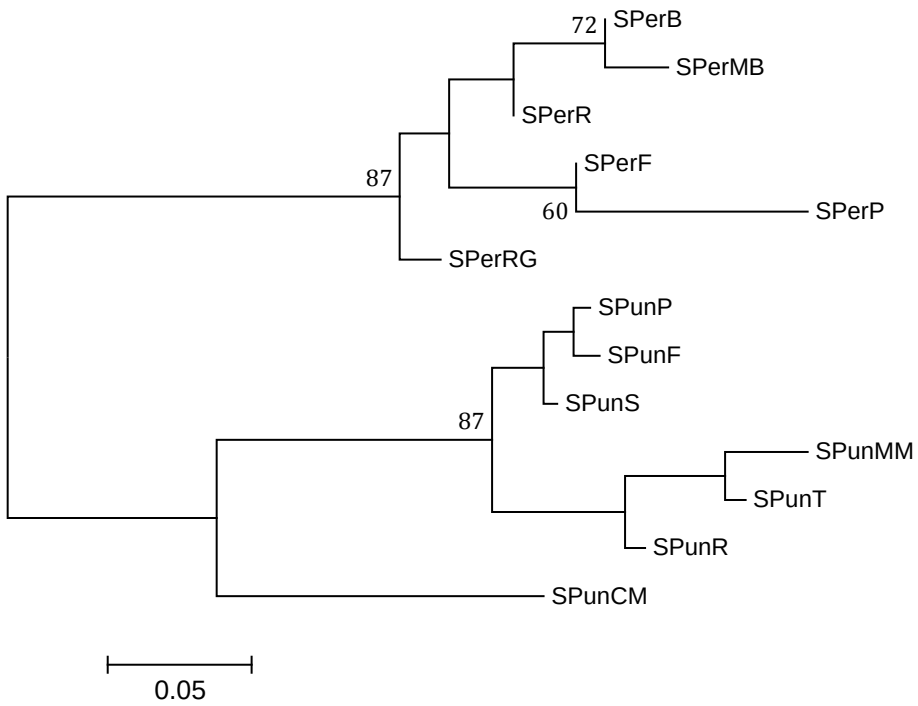

Fig. 2: Neighbor-Joining tree for populations of *Swertia punctata* and *S. perennis*. Bootstrap values ≥60% are shown on major branches. Population codes as in Table 1. Abbreviations: SPer, *S. perennis*; SPun, *S. punctata*.

The low allelic output (8 alleles total; 1–2 per locus) and the fact that only LAW12 and LAW25 proved polymorphic are also consistent with expectations for transferred genomic SSRs: primer–site mismatches can reduce amplification efficiency and introduce null alleles, while motif or copy-number conservation limits detectable variability [19]. In addition, ascertainment bias (markers isolated in a different genus), allelic dropout (preferential amplification of

common alleles), and homoplasy can all depress apparent heterozygosity and allele counts in the target genus [2, 23].

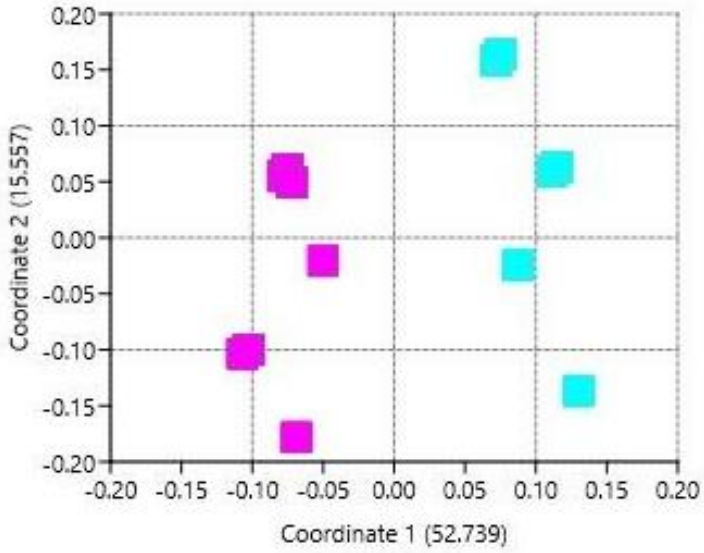

Fig. 3: Principal Coordinates Analysis (PCoA) of *Swertia* populations based on Simpson's similarity coefficient. Symbols: blue squares = *S. perennis* populations; magenta squares = *S. punctata* populations.

Given the moderate cross-genus amplification but limited polymorphism, conservation-genetics in *Swertia* would benefit from (i) developing taxon-specific SSRs (genomic or EST-derived) to avoid flanking-region mismatches, or (ii) shifting to SNP-based approaches (e.g., GBS/RAD or targeted capture), which typically deliver higher marker densities and transferability across close congeners [2, 23]. Until such resources exist, carefully curated heterologous loci (e.g., LAW12, LAW25) can still provide preliminary structure signals, but downstream inference should acknowledge potential null alleles and low *He* biases intrinsic to cross-genus SSR panels [11, 19].

Conclusions

Across 13 populations of *S. punctata* and *S. perennis*, cross-genus transferability of Gentiana SSRs was moderate: consistent PCR products were obtained for 11 of the 30 loci (36.7%), but only six met the defined criteria for polymorphism and reproducibility. These six loci revealed a total of eight alleles (1–2 per locus). Despite the low allelic diversity, the retained markers were sufficient to separate the two species in both NJ and PCoA analyses, providing a taxonomically coherent signal at the population level.

Methodologically, the present findings highlight the limitations of heterologous genomic SSRs in *Swertia* (low polymorphism, potential null alleles) and emphasize the need for taxon-specific marker development.

In summary, while cross-genus SSRs provided a workable, low-cost starting point, robust population genetics in *Swertia* will require species-tailored marker sets or genome-wide SNP data to capture meaningful variation across the Carpathian range.

Acknowledgements: The financial support was provided through the Nucleu Program as part of the 2022–2027 National Research, Development and Innovation Plan, funded by MCID, project no. PN23020401, contract no. 7N/03.01.2023.

Conflict of Interests: The authors declare that there are no conflicts of interest related to this article.

REFERENCES

1. Aiello, D., Ferradini, N., Torelli, L., Volpi, C., Lambalk, J., Russi, L., Albertini, E., 2020, Evaluation of cross-species transferability of SSR markers in *Foeniculum vulgare*, *Plants*, **9:** 175. https://doi.org/10.3390/plants9020175.
2. Barbará, T., Palma-Silva, C., Paggi, G.M., Bered, F., Fay, M.F., Lexer, C., 2007, Cross-species transfer of nuclear microsatellite markers: potential and limitations, *Molecular Ecology*, **16:** 3759-3767.
3. Barrie, F.R., 1993, Lectotype for *Swertia*: Herb. Linn. 327.1. In: Jarvis, C.E., Barrie, F.R., Allan, D.M., Reveal, J.L. (Eds.), *A list of Linnaean generic names and their types*. *Regnum Vegetabile*, Koeltz Scientific Books, Königstein, Germany, **127:** 92.
4. Ben-Ari, G., Lavi, U, 2012, Marker-assisted selection in plant breeding. In: Altman, A., Hasegawa, P.M. (Eds.), *Plant Biotechnology and Agriculture*, Academic Press, San Diego, CA, USA: 163-184.
5. Bombonato, J.R., Bonatelli, I.A.S., Silva, G.A.R., Moraes, E.M., Zappi, D.C., Taylor, N.P., Franco, F.F., 2019, Cross-genera SSR transferability in cacti revealed by a case study using *Cereus* (Cereeae, Cactaceae), *Genetics and Molecular Biology*, **42:** 87-94.
6. Cao, Q., Gao, Q., Ma, X., Zhang, F., Xing, R., Chi, X., Chen, S., 2022, Plastome structure, phylogenomics and evolution of plastid genes in *Swertia* (Gentianaceae) in the Qing-Tibetan Plateau, *BMC Plant Biology*, **22: 195**. https://doi.org/10.1186/s12870-022-03577-x.
7. Chhipi Shrestha, J.K., Bhattarai, T., Sijapati, J., Rana, N., Maharjan, J., Rawal, D.S., Raskoti, B.B, Shrestha, S., 2013, Assessment of genetic diversity in Nepalese populations of Swertia chirayita (Roxb. Ex Fleming) H. Karst using RAPD-PCR technique, *American Journal of Plant Sciences*, **4**(8): 1617-1628. 10.4236/ajps.2013.48196.
8. Ciocârlan, V., 2009, *Flora ilustrată a României – Pteridophyta et Spermatophyta*, Ed. Ceres, București.
9. Dítě, D., Hájek, M., Svitková, I., Košuthová, A., Šoltés, R., Kliment, J., 2018, Glacial-relict symptoms in the Western Carpathian flora, *Folia Geobotanica*, **53**: 277-300.
10. Ellegren, H., 2004, Microsatellites: simple sequences with complex evolution, *Nature Reviews Genetics*, **5**: 435-445. https://doi.org/10.1038/nrg1348.
11. Gutierrez, M.V., Vaz Patto, M.C., Huguet, T., Cubero, J.I., Moreno, M.T., Torres, A.M., 2005, Cross-species amplification of *Medicago truncatula* microsatellites across three major pulse crops, *Theoretical and Applied Genetics*, **110**: 1210-1217. https://doi.org/10.1007/s00122-005-1951-6.
12. Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001, Paleontological statistics software package for education and data analysis, *Palaeontologia Electronica*, **4(1):** 1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.
13. Joshi, P., Dhawan, V., 2007, Axillary multiplication of *Swertia chirayita* (Roxb. Ex Fleming) H. Karst., a critically endangered medicinal herb of temperate Himalayas, *In Vitro Cellular and Developmental Biology – Plant*, **43**: 631-638. https://doi.org/10.1007/s11627-007-9065-2.
14. Kliment, J., Turis, P., Janišová, M., 2016, Taxa of vascular plants endemic to the Carpathian Mts., *Preslia*, **88**: 19-76.
15. Linnaeus, C., 1753, *Species plantarum*, Impensis Laurentii Salvii, Holmiae, Stockholm, **1**, pp. 226-227.
16. Liu, Y., Wang, Y., Guo, F., Zhan, L., Mohr, T., Cheng, P., Huo, N., Gu, R., Pei, D., Sun, J., Tang, L., Long, C., Huang, L., Gu, Y.Q., 2017, Deep sequencing and transcriptome analyses to identify genes involved in

secoiridoid biosynthesis in the Tibetan medicinal plant *Swertia mussotii*, *Scientific Reports*, **7**: 43108. https://doi.org/10.1038/srep43108.

17. Malhotra, E.V., Jain, R., Bansal, S., Mali, S.C., Sharma, N., Agrawal, A., 2021, Development of a new set of genic SSR markers in the genus *Gentiana*: in silico mining, characterization, and validation. *3 Biotech*, **11**: 430. https://doi.org/10.1007/s13205-021-02969-4.
18. Neupane, S., Sijapati, J., Bhattarai, T., Shrestha, S., 2017, Genetic diversity in Nepalese population of *Swertia chirayita* (Roxb. Ex Fleming) H. Karst based on inter-simple sequence repeats (ISSR) markers, African Journal of Biotechnology, **16**(16): 895-907. https://doi.org/10.5897/AJB2016.15858.
19. Peakall, R., Gilmore, S., Keys, W., Morgante, M., Rafalski, A., 1998, Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants, *Molecular Biology and Evolution*, **15**(10): 1275-1287. https://doi.org/10.1093/oxfordjournals.molbev.a025856.
20. Peakall, R., Smouse, P.E., 2006, GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research, *Molecular Ecology Notes*, **6**(1): 288-295.
21. Peakall, R., Smouse, P.E., 2012, GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update, *Bioinformatics*, **28**: 2537-2539.
22. Randazzo, C.P., Ferri, A.M., Carabajal Paladino, L., Andres, A.N., Ingala, L.R., 2019, Cross-species transfer of SSR markers in *Setaria sphacelata* and *Trichloris crinita* sp. *Agronomía Colombiana*, **37**(2): 112-119.
23. Simko, I., 2009, Development of EST-SSR markers for the study of population structure in lettuce (*Lactuca sativa* L.), *Journal of Heredity*, **100**(2): 256-262. https://doi.org/10.1093/jhered/esn072.
24. Sourdille, P., Tavaud, M., Charmet, G., Bernard, M., 2001, Transferability of wheat microsatellites to diploid Triticeae species carrying the A, B and D genomes, *Theoretical and Applied Genetics*, **103**: 346-352. https://doi.org/10.1007/s00122-001-0542-4.
25. Struwe, L., Kadereit, J.W., Klackenberg, J., Nilsson, S., Thiv, M., von Hagen, K.B., Albert, V.A., 2002, Systematics, character evolution and biogeography of Gentianaceae, including a new tribal and subtribal classification. In: Struwe, L., Albert, V.A. (Eds.), *Gentianaceae – Systematics and Natural History*, Cambridge University Press, Cambridge: 21-309.
26. Sun, S.-S., Fu, P.-C., Cheng, Y.-W., Zhou, X.-J., Han, J.-M., 2018, Characterization and transferability of microsatellites for *Gentiana lawrencei* var. *farreri* (Gentianaceae), *Applications in Plant Sciences*, **6**(1): e1015. https://doi.org/10.1002/aps3.1015.
27. Szatmari, P.M., Hurdu, B.-I., 2022, Low altitude glacial relicts in the Romanian flora, *Contrib.Bot.* **57**: 19-51.
28. Takezaki, N., Nei, M., Tamura, K., 2010, POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows-interface, *Molecular Biology and Evolution*, **27**(4): 747-752. https://doi.org/10.1093/molbev/msp312.
29. Tan, K., Vladimirov, V., 2001, *Swertia punctata* (Gentianaceae) in Bulgaria, *Bocconea*, **13**: 461-466.
30. Tutin, T. G., 1972, *Swertia* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), *Flora Europaea*, Vol. 3, *Diapensiaceae to Myoporaceae*, Cambridge University Press, Cambridge: 67.
31. Urbaniak, J., Kwiatkowski, P., 2023, The molecular population structure of *Swertia perennis* (Gentianaceae) in Central Europe, *Scientific Reports*, **13**: 17059. https://doi.org/10.1038/s41598-023-43731-5.
32. Urbaniak, J., Kwiatkowski, P., Pawlikowski, P., 2018, Phylogeography of *Swertia perennis* in Europe based on cpDNA markers, *PeerJ,* **6**: e5512. https://doi.org/10.7717/peerj.5512.
33. Wang, M.L., Gillaspie, A.G., Newman, M.L., Dean, R.E., Pittman, R.N., Morris, J.B., Pederson, G.A., 2004, Transfer of simple sequence repeat (SSR) markers across the legume family for germplasm characterization and evaluation, *Plant Genetic Resources*, **2**(2): 107-119. https://doi.org/10.1079/PGR200441.

34. Xiang, B., Li, X., Qian, J., Wang, L., Ma, L., Tian, X., Wang, Y., 2016, The complete chloroplast genome sequence of the medicinal plant *Swertia mussotii* using the PacBio RS II platform, *Molecules*, **21**(8): 1029. https://doi.org/10.3390/molecules21081029.
35. Xiang, B., Li, X., Wang, Y.,Tian, X., Yang, Z., Ma, L., Liu, X., Wang, Y., 2017, Cloning and characterization of two iridoid synthase homologs from *Swertia mussotii*, *Molecules*, **22**(8): 1387. https://doi.org/10.3390/molecules22081387.
36. Yang, L., Li, J., Zhou, G., 2022, Comparative chloroplast genome analyses of 23 species in *Swertia* L. (Gentianaceae) with implications for its phylogeny, *Frontiers in Genetics*, **13**: 895146. https://doi.org/10.3389/fgene.2022.895146.
37. Yang, L., Xiong, F., Xiao, Y., Li, J., Chen, C., Zhou, G., 2020, The complete chloroplast genome of *Swertia tetraptera* and phylogenetic analysis. *Mitochondrial DNA Part B: Resources*, **5**(1): 164-165. https://doi.org/10.1080/23802359.2019.1698368.
38. Yoichi, W., 2023, The complete chloroplast genome sequence of *Swertia japonica* (Schult.) Makino (Gentianaceae), *Mitochondrial DNA Part B: Resources*, **8(11):** 1179-1182. https://doi.org/10.1080/23802359.2023.2275335.
39. Zhang, Y., Sledge, M.K., Bouton, J.H., 2007, Genome mapping of white clover (*Trifolium repens* L.) and comparative analysis within the Trifolieae using cross-species SSR markers, *Theoretical and Applied Genetics*, **114**: 1367-1378. https://doi.org/10.1007/s00122-007-0523-3.
40. POWO. Plants of the World Online, 2023, Facilitated by the Royal Botanic Gardens, Kew, Available online: https://powo.science.kew.org/ (accessed on October 10, 2025).

TRANSFERABILITATEA INTERGENERICĂ A MARKERILOR SSR DE LA *GENTIANA* SPP. LA *SWERTIA PERENNIS* ȘI *S. PUNCTATA* (GENTIANACEAE)

(Rezumat)

Swertia este unul dintre genurile cele mai bogate în specii din Gentianaceae (~165 specii acceptate) și în flora României este reprezentat de doi taxoni: *S. punctata* Baumg. (subendemică, rară) și *S. perennis* L. (vulnerabilă, relict glaciar în Carpați).

În acest studiu, am urmărit să testăm transferabilitatea intergenerică a markerilor microsateliți (SSR) dezvoltați pentru *Gentiana* către *Swertia punctata* și *S. perennis* și, simultan, să oferim o evaluare preliminară a relațiilor genetice dintre ele.

Am eșantionat 13 populații din Carpații României (7 *S. punctata*, 6 *S. perennis*) și am analizat 30 loci SSR heterologi (din *Gentiana lawrencei* var. *farreri* și *G. kurroo*), optimizând condițiile de amplificare pe fiecare locus. Genotiparea a fost urmată de statistici descriptive (Na, Ho, He) și de analize de structură (arbore Neighbor-Joining cu 1.000 bootstrap; PCoA). Dintre cei 30 de loci, 11 (36,7%) au amplificat consistent, iar 6 au îndeplinit criteriile de polimorfism și reproductibilitate. În ansamblu s-au identificat 8 alele (1–2/locus), cu polimorfism clar doar la LAW12 și LAW25; ceilalți loci au fost practic monomorfici în materialul analizat. Heterozigoția a fost redusă (Ho medie ~ 0,063–0,071; He medie ~ 0,101–0,102), dar matricea SSR a permis o separare coerentă a celor două specii în NJ și PCoA, cu bootstrap adecvat.

Transferabilitatea intergenerică a SSR-urilor din *Gentiana* către *Swertia* a fost moderată ca rată de amplificare și limitată ca putere informativă din cauza polimorfismului redus. Pentru studii robuste de genetică a conservării la genul *Swertia* recomandăm dezvoltarea de SSR specifici genului (ideal derivați din resurse NGS și validați pe populații) și/sau adoptarea unor abordări bazate pe SNP (de exemplu GBS/RAD) care să ofere o rezoluție superioară.

Received: 20.10.2025; Accepted: 20.11.2025