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THE *POA GRANITICA* GROUP IN THE CARPATHIAN MOUNTAINS: SOME MOLECULAR INSIGHTS

***Ioan BĂCILĂ*[1], *Dana ŞUTEU*[1], *Ana COSTE*[1], *Liviu FILIPAŞ*[1], *Tudor URSU*[1], *Ilie-Adrian STOICA*[1], *Bogdan-Iuliu HURDU*[1], *Mihai PUŞCAŞ*[2], *Gheorghe COLDEA*[1]**
[1]Institutul de Cercetări Biologice, str. Republicii, nr. 48, **RO-400015 Cluj-Napoca, Romania**
[2] Universitatea Babeş-Bolyai, Grădina Botanică A. Borza, str. Republicii, nr. 42,
RO-400015 Cluj-Napoca, România
e-mail: suteudana@yahoo.com

Abstract: *Poa deylii* Chartek & Jirasek is a grass taxon endemic to the South-Eastern Carpathians, which is of unclear taxonomic status, with no molecular research on the structure of its populations. Many authors have considered, based on morphological traits, this taxon to be a valid species or a subspecies included within *P. granitica* Br.-Bl. In the present study we have used three molecular markers – one nuclear (ITS1) and two chloroplastic (introns *trnG* and *trnL*) – to explore the variability within the *P. granitica* group. No genetic difference was detected, in spite of the morphological variation that distinguishes these two taxa (*P. granitica* subsp. *granitica* and *P. deylii*). Other approaches, such as the AFLP technique, that address the entire genome, might be required for a better understanding of the genetic variation within this group.

Keywords: *Poa granitica* group, taxonomy, endemic species, cpDNA, ITS

Introduction

The genus *Poa* includes annual and perennial grass species native to temperate regions of both hemispheres. The genus *Poa* belongs to the *Pooideae* subfamily of the family *Poaceae* [1].

The *Poa granitica* group comprises two distinct taxonomic units*: P. granitica* Br.-Bl. and *Poa deylii* Chrtek & Jirasek. The morphological differences between them are minute and both have approximately the same habitat preferences (alpine and subalpine acidophilic communities of *Salicion herbaceae*) [2, 7]. The taxonomic history of the group is obscure because *P. deylii* has been ascribed to different taxonomic categories (var. *disparilis* Nyar., subsp. *disparilis* (Nyar.) Nyar.), reviewed by Filipaş *et al*. (2009) [3]. Due to the low level of morphological differentiation, the presence of both taxa in the South-Eastern Carpathians was questionable. Filipaş *et al*. (2009) pointed out that *P. granitica* is not present in the flora of the SE Carpathians, being endemic to the Western Carpathians. Also the authors clarified the chorology of *P. deylii*. The presence of *P. deylii* is certified through herbarium material in the following massifs: Bucegi, Făgăraş, Maramureş, Retezat, Iezer and Rodna Mountains. In this last mountain range the species is more frequent and abundant then in the other parts of the SE Carpathians.

Molecular markers have proved a powerful tool to resolve many taxonomic issues, especially where the morphological boundaries between very close taxa are obscure or critical [4, 8, 9, 10, 12, 13].

The main goal of this present study has been to investigate whether there is a genetically sound basis for supporting *Poa deylii* as a distinct taxonomic unit. Furthermore, we aim to identify a molecular marker polymorphic enough to discriminate between *P. granitica* and *P. deylii* and to investigate taxonomic boundaries within the *P. granitica–P. deylii* group in the SE Carpathians.

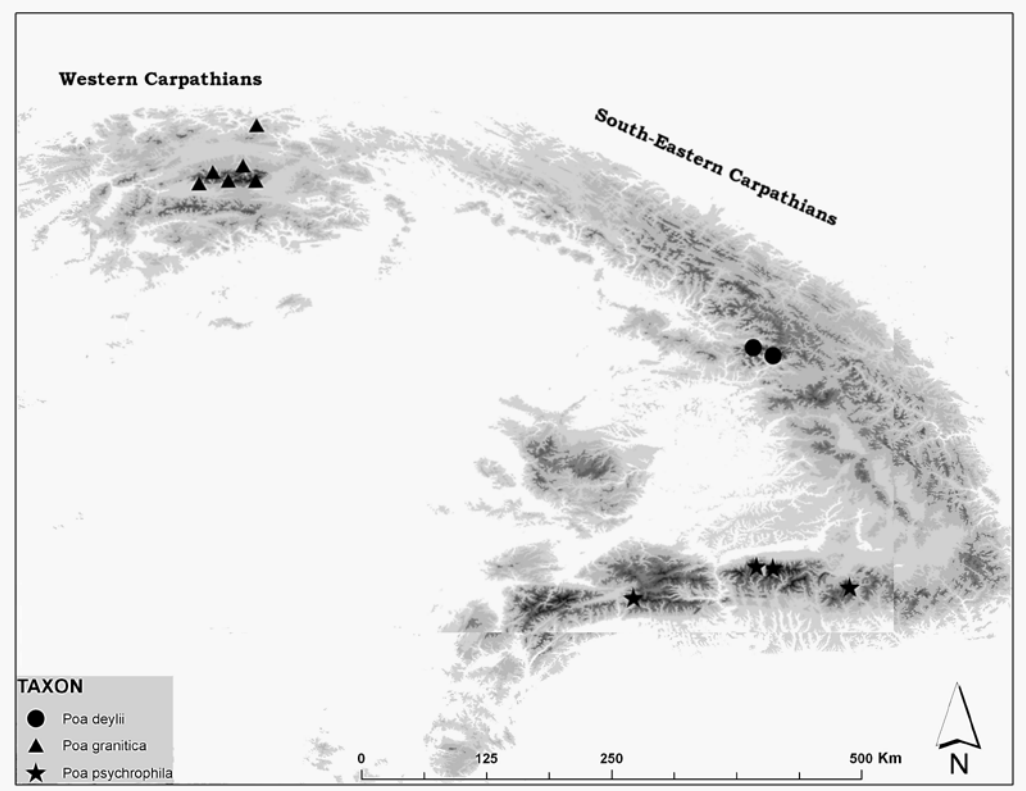

Fig. 1: Distribution map of sampled populations of *Poa* spp. used in this study

Materials and Methods

Sampling. We sampled 12 populations from the Carpathians (see Table 1, Fig. 1): 6 populations from the Western Carpathians (Tatra Mts) ascribed to *Poa granitica*, 2 populations of *Poa deylii* from the South-Eastern Carpathians (Rodna Mts) and 4 populations of *Poa psychrophila* as the outgroup, also from the SE Carpathians (Bucegi, Făgăraş and Parâng). Young green leaves of five random individuals were collected from each population and stored in silica gel.

DNA extraction

Total DNA was extracted from roughly 13 mg of silica gel-dried leaf tissue, using DNeasy 96 Plant Mini Kit (Qiagen) according to the manufacturer's protocol, except the final elution which took place in 80 µl in order to increase the DNA concentration.

cpDNA and nrDNA analysis

Two chloroplast DNA regions (introns *trnG* and *trnL*) were initially amplified and sequenced for 5 individuals from the different sampling areas, in order to look for nucleotide variation. The nuclear region ITS1 was also amplified and sequenced for the same purpose. Primers used were as follows: *trnG*: trnG and trnG2G [11, 14]; *trnL*: C and D [16]; ITS1: ITS2 and ITS5 [18].

Amplifications were performed in a 50 µl total reaction volume with 1X Taq Buffer (10X Taq Buffer, Fermentas), 2.5 mM $MgCl_2$, 0.5 mM of each dNTP, 0.12 µM of each primer, 0.16 mg/ml BSA, 2 U of Dream Taq Polymerase (Fermentas) and 10 µl of diluted genomic DNA. Amplification condition were those described by Shaw *et al*. (2005, 2007) [14, 15] except for the alignment temperatures, which were as follows: *trnG* (66 ^{o}C), *trnL* (56 ^{o}C) and ITS1 (52 ^{o}C). PCR products were purified using the commercial kit Wizard^{R} SV Gel and PCR Clean-Up System, according to the manufacturer's protocol (Promega Corporation, USA). Sequencing was performed in a 20 µl volume using BigDye Terminator Cycle Sequencing Ready Reaction Kit, v.

3.1 according to the manufacturer's suggestions (Applied Biosystems), using the following thermal cycle parameters 96 °C, 10 sec, 35 cycles of 96 °C, 10 sec, 50 °C (60 °C for ITS1 and *trnL*), 10 sec, 66 °C, 4 min. Both DNA strands were sequenced. Excess primers and labeled ddNTPs were removed by purification with Sephadex and Sephacryl (1:1) (GE Healthcare Bio-Sciences AB). The samples were prepared prior sequencing by adding 10 µl of HiDi formamide. The samples were run on an ABI PRISM® 3130 Genetic Analyzer, Applied Biosystems. Sequences were assembled and edited using BioEdit v.7.0.9.0 [5].

Table 1: Population locations and elevation of the 12 sampled populations

Loc. nr.	Designate taxa	Mountain System	Massif	Location name	Altitude (m)
1	*Poa granitica*	Western Carpathians	Tatry Wysokie	Kasprowy Wierch	1985
2	*Poa granitica*	Western Carpathians	Tatry Wysokie	Czarny Staw pod Rysami	1575
3	*Poa granitica*	Western Carpathians	Tatry Wysokie	Velicke Pleso (Velicka Dolina)	1837
4	*Poa granitica*	Western Carpathians	Wysoké Tatry	Mlynica Dolina	1821
5	*Poa granitica*	Western Carpathians	Západné Tatry	Smutna Dolina	1580
6	*Poa granitica*	Western Carpathians	Západné Tatry	Smutna Dolina	1580
7	*Poa deylii*	South-Eastern Carpathians	Rodna Mts.	Pietrosul	2190
8	*Poa deylii*	South-Eastern Carpathians	Rodna Mts	Rebra	2261
9	*Poa psychrophila*	South-Eastern Carpathians	Făgăraş	Valea Doamnei	2200
10	*Poa psychrophila*	South-Eastern Carpathians	Bucegi	Baba Mare	2266
11	*Poa psychrophila*	South-Eastern Carpathians	Făgăraş	Bâlea Lake	2160
12	*Poa psychrophila*	South-Eastern Carpathians	Parâng	Cârja	2354

Data analysis

The relationships among detected cpDNA and nrDNA haplotypes were analysed using the programme Mega 4.0 [17]. Tree construction was carried out through Neighbour Joining method (based on Kimura 1980 genetic distance [6]).

Results and Discussions

The sequences of the introns: *trnG* and *trnL* had 654 bp and 528 bp respectively. The nuclear ITS1 sequence had for *Poa* group 327 bp. 42 informative nucleotides were found in the combined alignment of all sequence consisting of 1059 bp.

After aligning the sequences obtained for one nuclear and two chloroplast markers we obtained two haplotypes. Haplotype one was represented by the populations of *Poa psychrophila*, while haplotype two was represented by the populations of *Poa granitica* and *Poa deylii*. Between the populations of *Poa granitica* and *Poa deylii*, none of the markers used was polymorphic.

Table 2: DNA barcoding utility of single-locus combinations of plastid and nuclear regions for *Poa granitica* and *Poa deylii*

Plastid/nuclear region	Aligned length (bp)	Variable characters
trnG	654	13
trnL	528	19
ITS1	327	10

In figure 2, Neighbour Joining analysis shows the population separation of *Poa psychrophila* from populations of *Poa granitica* and *Poa deylii* at a maximum bootstrap value.

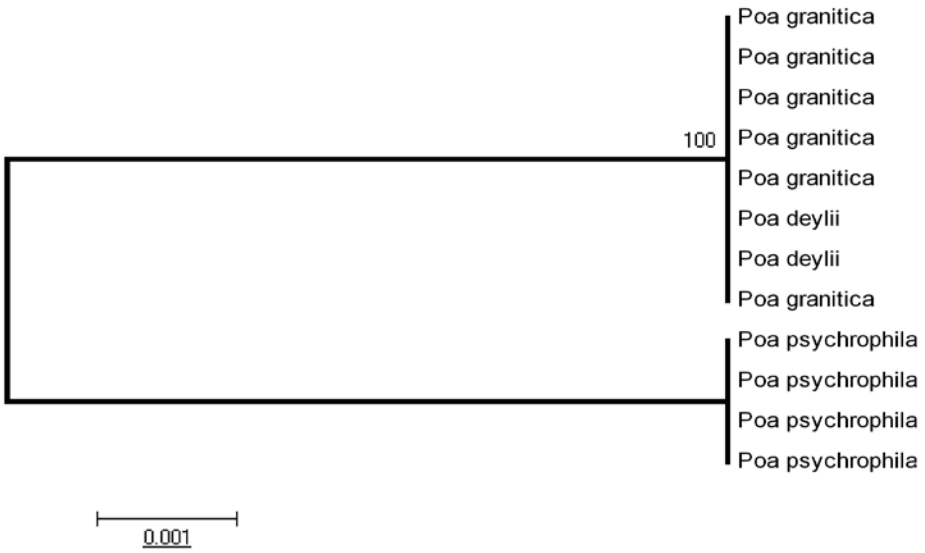

Fig. 2: Neighbour-Joining analysis, based on nuclear and cpDNA data (ITS1-*trnG-trnL* fragments) based on Kimura genetic distance (1980). The numbers above the branches are bootstrap values (% of 1000 replicates).

The analysis does not reveal any taxonomic pattern inside this group, the populations of *P. granitica* from the Western Carpathians and populations of *P. deylii* from the SE Carpathians sharing the same haplotype.

The lack of striking differences is not only restricted to the genetic level, but it also applies to morphological characters. The principal morphological traits that separate *P. granitica* from *P. deylii* are represented mainly by the more dense indumentum at the spikelets level, and the width of leaf present on the lower stems, 3–3.5 cm in the case of *P. granitica* and 2–3 cm in the case of *P. deylii* [3]. In the case of *Poa granitica* sometimes the spikelets proliferate; this phenomenon was never observed in the case of *P. deylii*.

Conclusions

The nuclear and chloroplastic markers analysed detected no genetic variability within the sampled populations of *Poa granitica* and *P. deylii*. These markers are not applicable as diagnostic taxonomic markers for possible identification and delimitation of *P. granitica* from *P. deylii*. Auxiliary DNA molecular markers might be used for finding polymorphic loci to identify the taxonomic status of *P. deylii*.

Additionally, it should be very useful to use AFLP genotyping to obtain a more detailed insight of the genetic structure of populations within the *P. granitica* group.

Finally, we emphasise that this study is the first designed to investigate the genetic differentiation between taxa described within the *P. granitica* group in the South-Eastern Carpathians.

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GRUPUL *POA GRANITICA* ÎN MUNȚII CARPAȚI: O ABORDARE MOLECULARĂ

(Rezumat)

Lucrarea prezintă o analiză din punct de vedere molecular asupra statutului taxonomic incert al taxonului *Poa deylii* din cadrul grupului *Poa granitica*.

Analizele moleculare au fost conduse în direcția identificării unui marker molecular suficient de polimorf pentru a face o distincție clară între taxonii *Poa granitica* subsp. *granitica* şi *Poa deylii*. În acest scop au fost încercați atât markeri cloroplastici cât şi un marker nuclear (ITS1).

Întrucât rezultatele nu au dus la o concluzie satisfăcătoare este necesară continuarea eforturilor de identificare la nivel molecular, fie a unui marker cloroplastic sau nuclear polimorf, fie utilizarea tehnicii AFLP adresată întregului genom.

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