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R E V I E W

of the dissertation

by

Evgenii Baiakhmetov, MSc

entitled

“Inferring hybridisation and introgression processes within *Stipa* (Poaceae)”

The PhD dissertation of Evgenii Baiakhmetov, which I am reviewing, was written under the supervision of prof. dr. hab. Marcin Nobis (from Jagiellonian University, Krakow) and dr. Polina Gudkova (from Tomsk State University, Russia). This PhD dissertation addresses significant scientific problems linked with hybridisation, one of the fundamental mechanisms driving the plant genome evolution. Natural hybridisation is a frequent phenomenon among angiosperms and can have various impacts on plant evolution. The allopolyploidisation (hybridisations with the whole genome duplication), homoploid speciation (hybridisation and gene flow without WGD), the formation of various kinds of hybrid zones and adaptive or neutral introgression comprise just some of the many possible evolutionary outcomes of interspecific genetic exchange. Although these mechanisms have been studied for many years, they are still far from sufficient understanding in many ways.

The dissertation starts with a short introduction to familiarise the readers with the subject of the analyses – species from the genus *Stipa* - showing the present state and the major challenges of taxonomical classification in this genus. The next part defines the aims of the research, such as assessing the utility of the restriction-based high-throughput technique DArTseq for genome-wide SNPs genotyping and detection of hybridisation and introgression processes within *Stipa*. The results





of these studies were further combined with morphological analyses to create a very integrative approach to delimiting pure and admixed taxa. Another objective was to develop the first nuclear and mitochondrial draft genomes of *Stipa* species. The last aim somehow summarised the other research goals, testing the utility of genus *Stipa* as a good model to study hybridisation and introgression events in nature. These goals are clearly defined and are both scientifically and technically very ambitious.

The next part consists of a very brief description of the methods used in this project. It is worth mentioning that in his analyses, Mr Baiakhmetov applied classical methods, such as morphological analyses but also scanning electron microscopy and very recent NGS methods, such as restriction-based high-throughput technique DArTseq, or the sequencing of the second and third generation.

The core element of this dissertation consists of the three papers, all published in high-impact journals, which contain, present and discuss the results. These publications are as follows:

1. **Baiakhmetov E.**, Nowak A., Gudkova P.D., Nobis M. 2020. Morphological and genome-wide evidence for natural hybridisation within the genus *Stipa* (Poaceae). *Scientific Reports* 10:13803. (*JCR IF in 2020* = 4.380; *JCR 5 Year IF* = 5.133; *MEiS* = 140)
2. **Baiakhmetov E.**, Guyomar C., Shelest E., Nobis M., Gudkova P.D. 2021, The first draft genome of feather grasses using SMRT sequencing and its implications in molecular studies of *Stipa*. *Scientific Reports* 11: 15345. (*JCR IF in 2020* = 4.380; *JCR 5 Year IF* = 5.133; *MEiS* = 140)
3. **Baiakhmetov E.**, Ryzhakova D., Gudkova P.D., Nobis M. 2021. Evidence for extensive hybridisation and past introgression events in feather grasses using genome-wide SNP genotyping. *BMC Plant Biology* 21: 505 (*JCR IF in 2020* = 4.215; *JCR 5 Year IF* = 4.960; *MEiS* = 140)

Although all of them are multi-author papers, Mr Baiakhmetov is always their first author and one of the corresponding authors, which clearly indicates his prominent role. It is also worth to notice that these analyses were done in international research collaboration (Russia, Germany, France), which is a very reasonable approach for doing high-quality science nowadays. Since all these papers have already been assessed by the panels of competent reviewers and editors, there is no point to do it in this report. Instead, I will briefly point out their most prominent findings and conclusions.

In publication #1, Mr Baiakhmetov and co-workers analysed the extent of hybridisation between selected *Stipa* species using morphological and molecular markers (SilicoDArT and SNPs





markers). This integrative taxonomy approach proves that taxon *S.x lazkovii* is a hybrid between *S. krylovii* and *S. bungeana*. The chloroplast and mitochondrial genomes of *S.x lazkovii* was inherited from *S. bungeana*. The analyses showed that introgression could be an important mechanism in the evolution in *Stipa*. The application of NGS techniques for investigations of genomes of wild taxa is still not very common, although the number of such reports recently has been growing relatively fast.

The second publication reported the first draft genome sequence of *S. capillata*, the most widespread species among Eurasian *Stipa*. The obtained data showed that *S. capillata* has a small genome size and may be of allotetraploid origin. The draft genome sequence provides an essential source of genetic data and likely will be a valuable tool in further analyses of *Stipa* evolution, phylogeny, and comparative studies of genome structure in this group of plants. These newly obtained data have been already used for estimation of divergence time of *Stipa* species based on rDNA sequences. It shows that the Author can successfully implement the obtained data and skills to design subsequent experiments

The last publication focused on detecting hybridisation events among several *Stipa* species across several hybrid zones in Russia and Central Asia. The analyses of SNPs markers (obtained with DArTseq techniques) were applied to numerous specimens and allowed to identify a relatively high number of hybrid individuals. The data suggested a contemporary gene flow between analysed populations and indicated that introgression might have played an essential role in the evolution of *Stipa*.

In the last paragraph of the dissertation, mgr Evgenii Baiakhmetov discussed the utility of the various molecular methods in taxonomic and phylogenetic studies in *Stipa*, a genus which underwent many hybridisations and introgression events during its evolution. PhD student also described in this section the future perspective and possibilities that “the Era of Open Sciences” can bring to the phylogenetic and taxonomic studies.

In terms of content, I rate the work highly. The assumptions of the doctoral project are well thought out. I consider the choice of the research topic to be right - the undertaken topic is very important and future-proof. It is worth emphasising that the PhD student's scientific achievements are not limited to the indicated publications being the basis for the dissertation. Additional achievements of Mr Baiakhmetov include as many as six scientific papers.





Finally, I ask the PhD student a few questions about the dissertation. I am asking for answers to them during the public part of the doctoral defence.

1. Publication #1; The data on chromosome number is known only for *S. krylovii*, which is tetraploid with $2n = 44$. The chromosome numbers of other taxa (*S. x lazkovii* and *S. bungeana*) are unknown. I would like to ask about the methods the authors used to prove that the *S. x lazkovii* is a hybrid, not an allotetraploid, which has one genome common with *S. bungeana* and the other with *S. krylovii*.
2. In publication #3, the taxa described as “pure specimens” (*S. capillata*, *S. krylovii* and *S. baicalensis*) are tetraploids of unknown origin or, as it was shown in publication #2, an allotetraploid. Is it possible that the “admixed individuals” are not the hybrids between two tetraploids but the new allotetraploid species? For example, *Brachypodium distachyon* was previously regarded as a single species with three cytotypes ($2n = 10, 20, 30$). Recently, based on molecular and cytogenetic data, it has been shown that the cytotypes $2n = 10$ and $2n = 20$ represent two different diploid species whereas a cytotype $2n = 30$ is an allotetraploid derived from them (doi:10.1093/aob/mcr294).
3. In publication #, 2 Author claims that the genome size established in his analyses was two times smaller than expected 2.355 Mb and two times larger than the expected monoploid size 589 Mb estimated using flow cytometry (both values come from <https://doi.org/10.23855/preslia.2019.117>). I would like PhD student to explain how to compare the data on genome size estimated using flow cytometry and obtained from a genome sequencing project. What are the advantages and disadvantages of these two methods?
4. Publication #2 Authors used the term “NORs” for rDNA locus. NOR- Nucleolus Organizing Region – define only transcriptionally active 35S rDNA locus. In polyploids, especially in allopolyploids, and hybrids some of their DNA loci could be preferentially silenced. Are any data on transcriptional activity of 35S rDNA loci available in *Stipa*?
5. Author claimed that the new high-throughput technique DArTseq allows for better resolving of the taxa in *Stipa* genus than the more traditional markers (chloroplast markers and nrITS or IGS sequences from 35S rDNA). I would like the PhD student to discuss shortly the advantages and disadvantages of the new and traditional markers in analyses of polyploid and hybrid taxa.





Concluding my review, with no doubt, the Author has done a very good doctoral project. This is a great achievement of both the Author and his supervisor, Professor Marcin Nobis (Jagiellonian University) and co-supervisor dr. Polina Gudkova (Tomsk State University). The first paper has already attracted significant attention of the community, being cited nine times (7 excluding self-citations of the selected author; data according to Web of Sciences accessed on the 25th of May, 2022), which is the best and most objective showcase of the significance of this research.

I, hereby, declare that the reviewed PhD thesis entitled “Inferring hybridisation and introgression processes within *Stipa* (Poaceae)” by Evgenii Baiakhmetov meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended) and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts Evgenii Baiakhmetov for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences

Bożena Kolano

I, hereby, request that the thesis is accepted with distinctions due to the valuable, innovative and published results. The underinvestigated wild taxa, especially polyploids and/or hybrids, are challenging for phylogenetic and genomic study. The presented three publications published in very good journals are the best proof that PhD candidate fulfilled his research project in an outstanding way.

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