# Részletes beszámoló – Detailed research report (grant nr. OTKA PD109686)

## Speciation in the genus *Epipactis*

The main aim of the current research was the understanding of the underlying evolutionary mechanisms underpinning speciation in the genus *Epipactis* (Orchidaceae), most specifically that of section *Euepipactis*. The sampling for this study involved the sampling of the supposed progenitor species, *E. helleborine* sensu stricto (s.s.), which is a strictly outcrossing species, and its supposed derivate (daughter) species in the same geographic area. The main idea was to use the genomic method, RAD-seq (Restriction-site associated DNA sequencing), to reconstruct the evolutionary history of the progenitor species and the daughter species with a special interest in the relationship between these two groups of species, which was impossible before the advent of genomic methods.

At the beginning of the grant period, I devoted three larger expeditions to sample *Epipactis*: we had an expedition to the Balkans and to the Caucasus Mts. in 2014, one to Western Europe in 2015. We benefited also from our priori collection of samples from the Carpathian Basin. This has supplied us with 112 samples of the supposed progenitor species from the Caucasus Mts. to Wales (Fig. 1.)

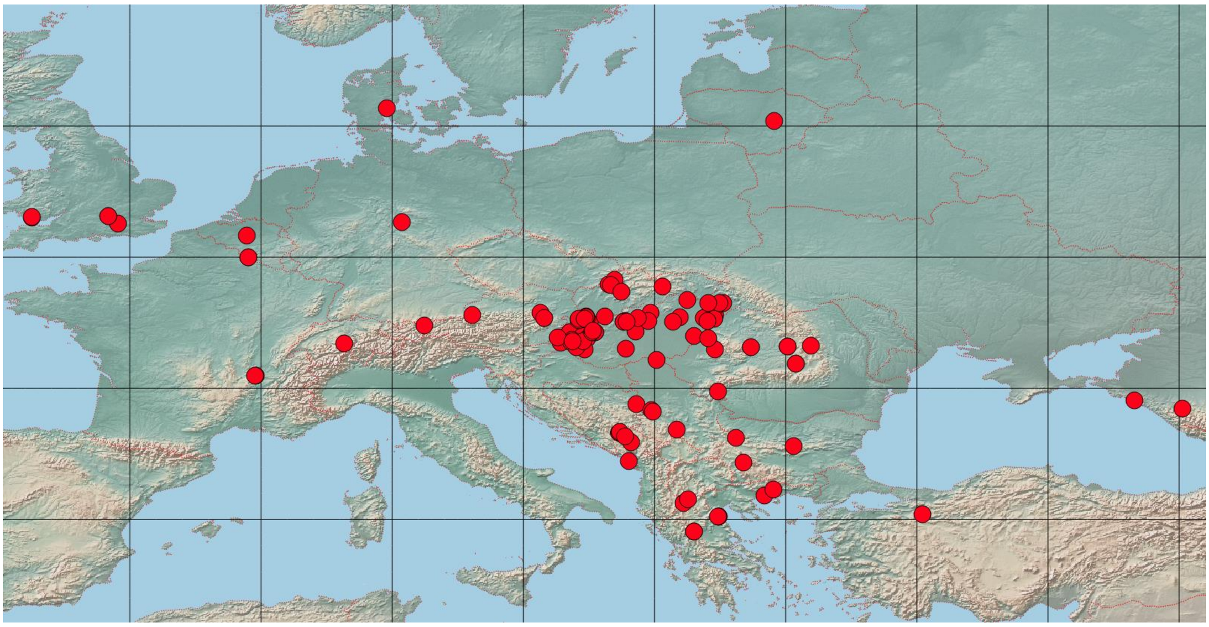


Fig. 1. Sampling of *E. helleborine* s.s. for the study of speciation in the genus *Epipactis*.

This was supplemented by sampling supposed daughter species on the above area. Altogether, I have managed to collect 286 samples of 48 microspecies. When all samples were put together in 2015, we started to extract gDNA from selected samples that were selected (i) to represent geographically *E. helleborine* s.s., (ii) to include several samples of the microspecies from different geographic regions.

Here, we faced a technical problem that is unprecedented in the orchid literature – the total genomic DNA extracted from older samples (usually those collected in 2014, but some also collected in 2015 alike) showed highly degraded nature on the gel images (Fig. 2).

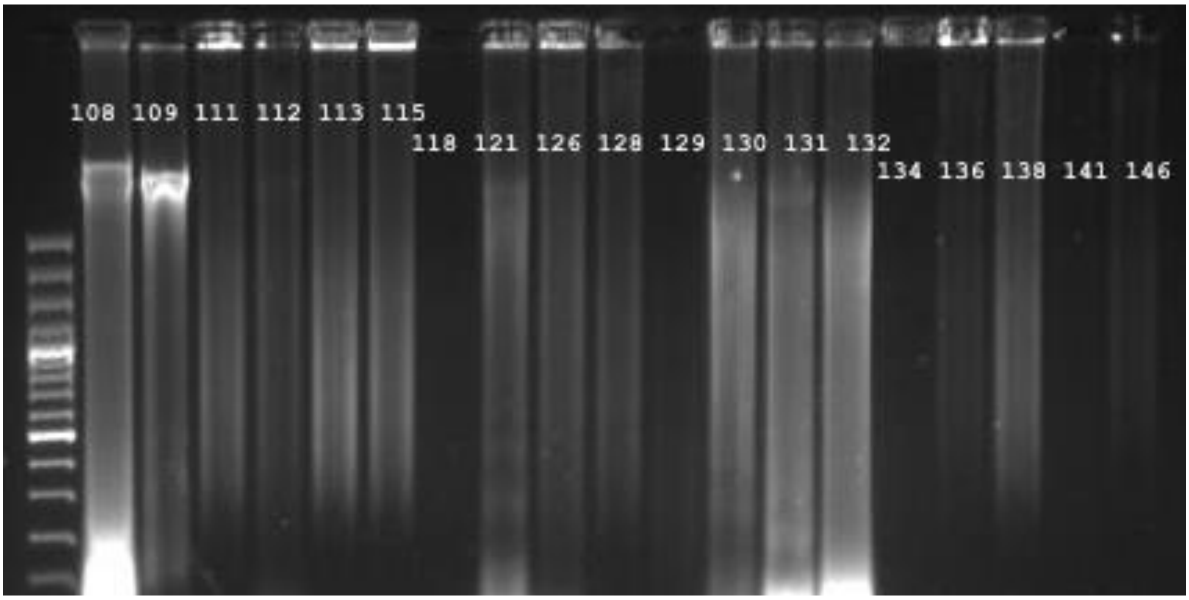


Fig. 2. A typical gel image of gDNA extracts of *Epipactis* samples. The total DNA shows significant degradation in most of the samples.

This degradation of gDNA affected most of our samples and unfortunately forced us to reduce the sample set significantly. I even made some re-collections for samples that were available at geographically close regions, but some precious samples were ultimately lost (e.g. from N Greece). We cannot give an ultimate answer to what has happened to our tissue samples – they were kept together with all the other samples of species from different orchid genera, and e.g. in *Ophrys* we did not experience the same degradation of gDNA. Nevertheless, we managed to get good-quality gDNA of a representative set of samples of *E. helleborine* s.s., and also for 28 microspecies selected based on the above criteria.

Before the application of RAD-seq in *Epipactis*, we also tested seemingly variable DNA-regions including the *rbc*L-*acc*D spacer of the plastid genome (reported to show complete lineage sorting in Italian samples of *Epipactis*, see Tranchida-Lombardo *et al.*, 2011). This was surprising as none of the previous reports of using candidate-genes have ever reported to show complete lineage sorting, and thus they provided limited insight into the phylogenetic relationships of *Epipactis* section *Euepipactis* (Bateman *et al.*, 2005; Drouzas *et al.*, 2017; Zhou & Jin, 2018). To test if this unusual performance of the above DNA-region still exist at a larger geographic scale, we sequenced this region in several accessions of *Epipactis* species from our collection. We put extra effort into including samples of *E. leptochila* from a large geographic area (Russia, Hungary, Germany, UK) to test the performance of this region as – according to the Italian study – a unique haplotype (E3) was shared in all Italian samples of *E. leptochila*. Our pilot sample set included also several samples of different microspecies and several accessions of the same microspecies of *Epipactis* to test for both the utility of this marker at the species level and below that in this genus. We sequenced the 760 bp long *rbc*L-*acc*D plastid IGS and compared it to the haplotypes reported by Tranchida-Lombardo *et al.* (2011).

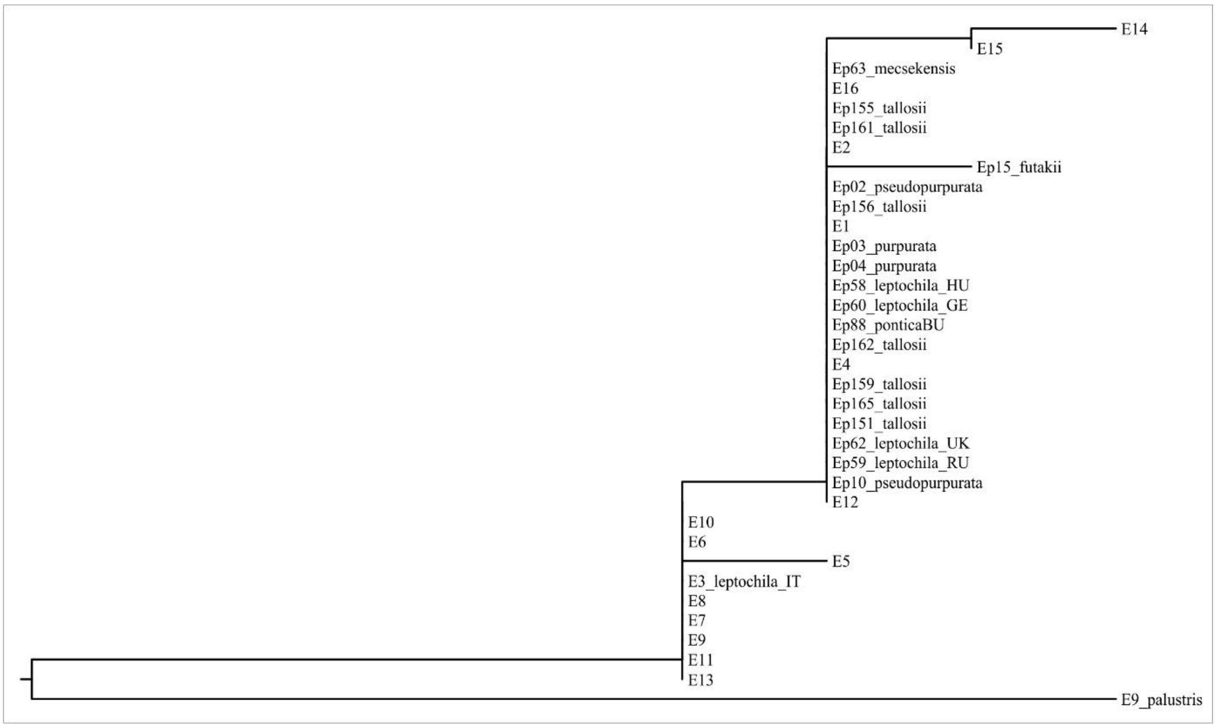


Fig. 3. The genetic relationship between haplotypes of *rbc*L-*acc*D spacer (coded as E1-E16) reported by Tranchida-Lombardo *et al*. (2011) and our pilot sample set (coded as 'Ep'X_'species name'_'country') as depicted by a neighbour-joining phylogram based on uncorrected p-distance.

Our pilot results (Fig. 3) showed different pattern on a larger geographic scale than those reported for Italy. Most importantly, our *E. leptochila* samples all fell into the same clade where all of our other samples were grouped (the clade containing the most widespread haplotype – E1 – of the Italian peninsula). Similarly, we could not obtain resolution neither between the species studied, nor between samples of the same species. Therefore, we have concluded the uselessness of this DNA region on a larger geographic scale and abandoned further study applying this plastid spacer. It is interesting, however, that the Italian samples showed more genetic isolation from the rest of the samples, and this differentiation would certainly worth further studying.

Similarly to the above, we also tested the variability of one of the most widely used phylogenetic marker in plants, the nuclear ribosomal internal transcribed spacer (nrITS) using the same sampling philosophy as described above. As expected, our pilot results showed (Fig. 4) significant incomplete lineage sorting (i.e. the lack of a specific ribotype typical of a given species due to shared ancestral polymorphism of the molecular genetic marker studied). This is evidenced on Fig. 4 by the general splitting of *E. tallosii* samples (all coming from the territory of Hungary) between the two main nrITS lineages. Also, several different microspecies shared the same ribotype, which is – in the absence of significant gene flow in these autogam species – attributable to incomplete lineage sorting. In conclusion, at the beginning of the work we have validated the use of genomic methods as the only alternative to bring phylogenetic resolution to this group, since we were unable to use the previously reported DNA-regions as being useful in the section *Euepipactis*.

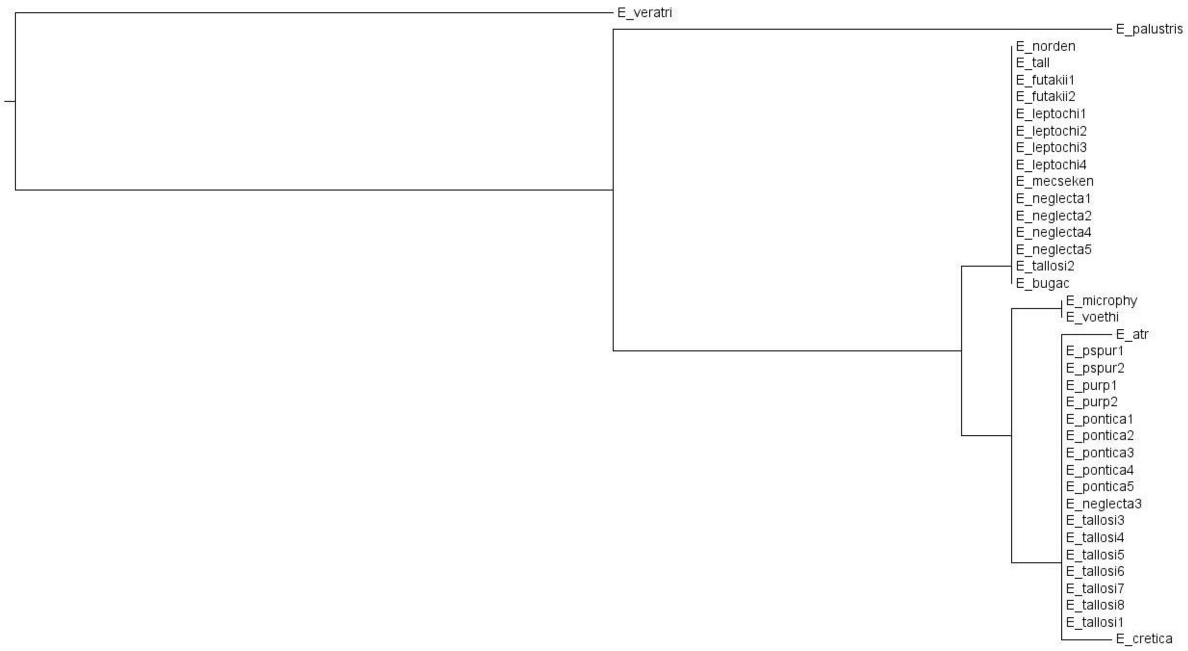


Fig. 4. The genetic relationship between ribotypes of nrITS in our pilot sample set as depicted by a neighbour-joining phylogram based on uncorrected p-distance.

In light of the above pilot results, we started to generate DNA-extracts for the RAD-seq method in 2015. After overcoming the problems with the gDNA in the extracts, we had another problem with RAD-seq. Our collaborator who intended to teach me the technique in Vienna had no space in the lab in 2015. So, to save the project, I decided to use an external service provider to have them prepared the RAD-seq libraries and make the libraries sequenced. We made a contract in 2016 February (with the consent of NKFIH) but it turned out that the company requires much more and better gDNA than what we were prepared for. After half year of work, we put together all the necessary, good quality gDNA samples, but the company has resigned from the work quoting an expired contract between them and one of their contractors. This whole story is well-documented in the history of this grant. Luckily, when the company resigned from the work, we managed to go to Vienna to work on the libraries, but this caused one year delay in the work. I prepared two libraries in the second half of 2016, then a third one in the first part of 2017. However, this one year delay made it necessary to apply for an extension of this grant, which was successful.

After overcoming on all the above obstacles, we got a large RAD-seq dataset for 29 species of *Epipactis*, including two species as outgroup. For the remaining samples, we have selected 29 samples of *E. helleborine* s.s. – the putative progenitor of the autogamous species – extended west–east from Wales to the eastern shore of the Black Sea. Within this area, we selected a further 77 ingroup samples, together encompassing 26 named taxa; between one and six samples (mean 2.9 ± 1.4) representing each taxon, with a concentration of sampling in eastern Europe. Taxa were selected to encompass the full range of breeding systems from putative obligate allogams to putative obligate autogams. Field-collected samples of leaf tissue were immediately immersed in sachets of fine-grained silica gel, and an open flower from each plant was immediately placed in 96%

ethanol as a voucher for each studied plant (deposited in the herbarium of the University of Debrecen – DE).

Three single-digest RAD libraries were prepared of between 40 and 60 individuals (including repeats where judged necessary). Each analysis began with 210 ng of dsDNA digested with SbfI-HF enzyme, reflecting the comparatively large (Leitch *et al.*, 2009) genome size of *E. helleborine* (2C = *ca* 28 pg). The protocol of library preparation largely followed Paun *et al*. (2016) but with the minor modifications as described by Trucchi *et al*. (2017). The only deviation from these past protocols was applying a different regime of sonication using Bioruptor Pico, which in this case involved three cycles of 45 s 'on' and 45 s 'off' at 6 °C. After library control, the libraries were submitted to be sequenced on an Illumina HiSeq as 100 bp single-end reads.

After allowing for the presence of 10% missing values using IUPAC symbols to account for heterozygosity, the 108 *Epipactis* accessions representing 29 species collectively produced 12,543 filtered SNPs for use in downstream phylogenetic analyses. As we were specifically interested in the relationship between *E. helleborine* s.s and its supposed derivate species, this dataset was used in phylogenetic tree reconstruction and phylogenetic network building (Fig. 5).

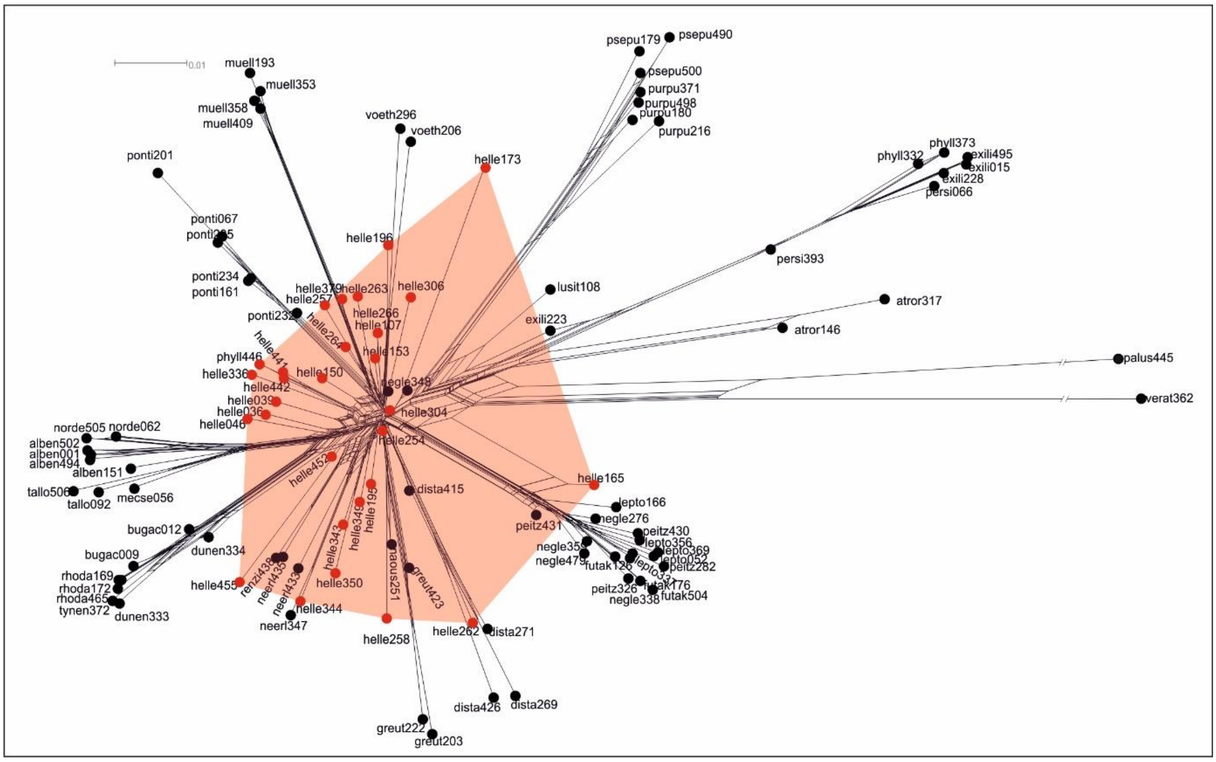


Fig. 5. Phylogenetic network depicting the genetic relationship of the studied *Epipactis* samples presented as a NeighbourNet network based on uncorrected-p distance. The dots represent an edge with a sample, where the red dots represent samples of the supposed progenitor species. The branches leading to the two outgroup samples are severely truncated to facilitate viewing.

Our genomic results gained unprecedented phylogenetic resolution to the studied species aggregate, and showed – for the first time in the history of studying this genus – clean phylogenetic evidence for *E. helleborine* s.s. as the progenitor of most derived, autogamous species in section *Euepipactis*. Since some autogamous lineages of mostly obligate autogamous nature (e.g. *E. muelleri*, *E. leptochila*, *E. greuterii*) show comparatively large branches stemming from the ancestral plexus that is *E. helleborine*. Also, we can clearly see a tendency for having a faster deviation from that ancestral plexus in case of species more prone to autogamy. On the contrary, species that are rather facultative autogamous (e.g. *E. neerlandica*, *E. distans*) are mixed more with samples of the ancestral species, and they are also on comparatively shorter branches. Therefore, we can conclude to autogamy as the most important driver of the evolutionary radiation section *Euepipactis* is displaying, and describing it as being correlated to the level of autogamy: the more prone a species to autogamy, the more effective and fast the isolation of an *Epipactis* microspecies is from the genetic background *E. helleboine* s.s. provides.

These results were presented at the most significant botanical event on this globe, the International Botanical Congress 2017 in Shenzhen, China. I was the opening speaker of the section T2-50-02, Systematics of Orchidaceae organised by Prof. Keith Cameron (USA). Based on this presentation, I was invited to give a talk at the 6th International Orchid Workshop 2018 in Białystok, Poland. The manuscript describing these details in length has been submitted to New Phytologist. I copy here the journal-specific questions for their preliminary evaluation:

1.) What hypotheses or questions does this work address?
We are seeking phylogenomic evidence for the autogamy driven speciation in the orchid genus *Epipactis* – specifically: (a) the universality of the widespread allogamous species as the progenitor of the autogams arisen iteratively across Europe; (b) irreversibility of transition from allogamous to autogamous lineage; (c) whether autogams are evolutionary dead-ends?
2.) How does this work advance our current understanding of plant science?
We report the fine-scale structure of an autogamy driven, incipient speciation, where we prove the universality of an allogamous species as progenitor several autogamous lineages, and report a case where an autogamous lineage is further split into isolated autogam species, thus violating the universality of evolutionary dead-ends.
3.) Why is this work important and timely?
Autogamy driven speciation and evolutionary dead-ends are current topics in plant evolutionary science (reviewed recently in *New Phytologist*). Our study unravels the anatomy of such a speciation using an appropriate phylogenomic approach (RAD-seq). Additionally, we use our well-resolved phylogeny to guide insightful decisions in taxonomy of such recent evolutionary diversifications.

Also, I am copying here the abstract of our submitted work:

Summary

- The terrestrial orchid genus *Epipactis* has become a model system for the study of several evolutionary and ecological questions of general interest, most notably speciation via a transition from allogamy to autogamy, but close phylogenetic relationships have proven difficult to resolve through Sanger sequencing.

- We analysed using RAD-seq 108 plants representing 29 named taxa that together span the genus, focusing on section *Euepipactis*.
- The resulting filtered matrix of 12,543 single nucleotide polymorphisms generated an unrooted network, a rooted, well-supported likelihood tree. The 27 named taxa of the ingroup were resolved as 11 genuine, geographically widespread species: four dominantly allogamous and seven dominantly autogamous. An assessment of shared ancestry showed lack of hybridisation.
- A single allogamous species, *E. helleborine*, is the direct ancestor of most of the remaining species, though one of the derived autogams has generated one further autogamous species. *Epipactis helleborine* has also frequently generated many local variants showing inclinations toward autogamy (and occasionally cleistogamy) best viewed as incipient speciation. This clade is currently undergoing an evolutionary radiation, albeit generating fewer species than some authors believe, driven by a wide spectrum of genotypic, phenotypic and environmental factors. Autogams are on average as widespread and ecologically successful as allogams.

Key words: allogamy, autogamy, *Epipactis* section *Euepipactis*, evolutionary dead-end, evolutionary radiation, orchid, phylogeny, phylogeography, RAD-seq; speciation

### RAD-seq based phylogenomics of the genus *Ophrys*

This is a forerunner study of the current grant, where a RAD-seq library was prepared in frame of an Austrian-Hungarian research grant. It was necessary to add more libraries in course of the current grant as it deals with the phylogenomics of an orchid genus with similar taxonomic problems caused by unique speciation as a result of recent evolutionary radiation. Therefore, I applied for the extension of this grant to cover also *Ophrys*, that we started earlier. In the framework of the current grant, I have expanded the first *Ophrys* library with three consecutive libraries, so, we could analyse the results of a rather large data and sample set.

The first result of these analyses focuses on the phylogenetic relationship and taxonomy of the whole genus based on the RAD-seq dataset. It was published just before the closing of the grant:

> Bateman RM, Sramkó G, Paun O (2018) Integrating restriction site-associated DNA sequencing (RAD-seq) with morphological cladistic analysis clarifies evolutionary relationships among major species groups of bee orchids. ANN BOT-LONDON 121(1) pp. 85-105 (IF$_{2016}$: 4.041). Please note that the three authors of this work contributed equally to the work.

Without going into the details, I copy here the abstract of the above paper to summarise the results of those studies:

Background and Aims: Bee orchids (*Ophrys*) have become the most popular model system for studying reproduction via insect-mediated pseudo-copulation and for exploring the consequent, putatively adaptive, evolutionary radiations. However, despite intensive past research, both the phylogenetic structure and species diversity within the genus remain highly contentious. Here, we integrate next-generation sequencing and morphological cladistic techniques to clarify the phylogeny of the genus.

Methods: At least two accessions of each of the ten species groups previously circumscribed from large-scale cloned nuclear ribosomal internal transcibed spacer (nrITS) sequencing were subjected to restriction site-associated sequencing (RAD-seq). The resulting matrix of 4159 single nucleotide polymorphisms (SNPs) for 34 accessions was used to construct an unrooted network and a rooted maximum likelihood phylogeny. A parallel morphological cladistic matrix of 43 characters generated both polymorphic and non-polymorphic sets of parsimony trees before being mapped across the RAD-seq topology.

Key Results: RAD-seq data strongly support the monophyly of nine out of ten groups previously circumscribed using nrITS and resolve three major clades; in contrast, supposed microspecies are barely distinguishable. Strong incongruence separated the RAD-seq trees from both the morphological trees and traditional classifications; mapping of the morphological characters across the RAD-seq topology rendered them far more homoplastic.

Conclusions: The comparatively high level of morphological homoplasy reflects extensive convergence, whereas the derived placement of the *fusca* group is attributed to paedomorphic simplification. The phenotype of the most recent common ancestor of the extant lineages is inferred, but it post-dates the majority of the character-state changes that typify the genus. RAD-seq may represent the high-water mark of the contribution of molecular phylogenetics to understanding evolution within *Ophrys*; further progress will require large-scale population-level studies that integrate phenotypic and genotypic data in a cogent conceptual framework.

One more MS is under active development for the results focusing on the most recently radiated group, the *Ophrys fuciflora* aggregate. We will make use of all samples processed in the four RAD-seq libraries we prepared during this grant. I am only attaching the preliminary key figure here (Fig. 6) to demonstrate the wealth and scope of the MS.

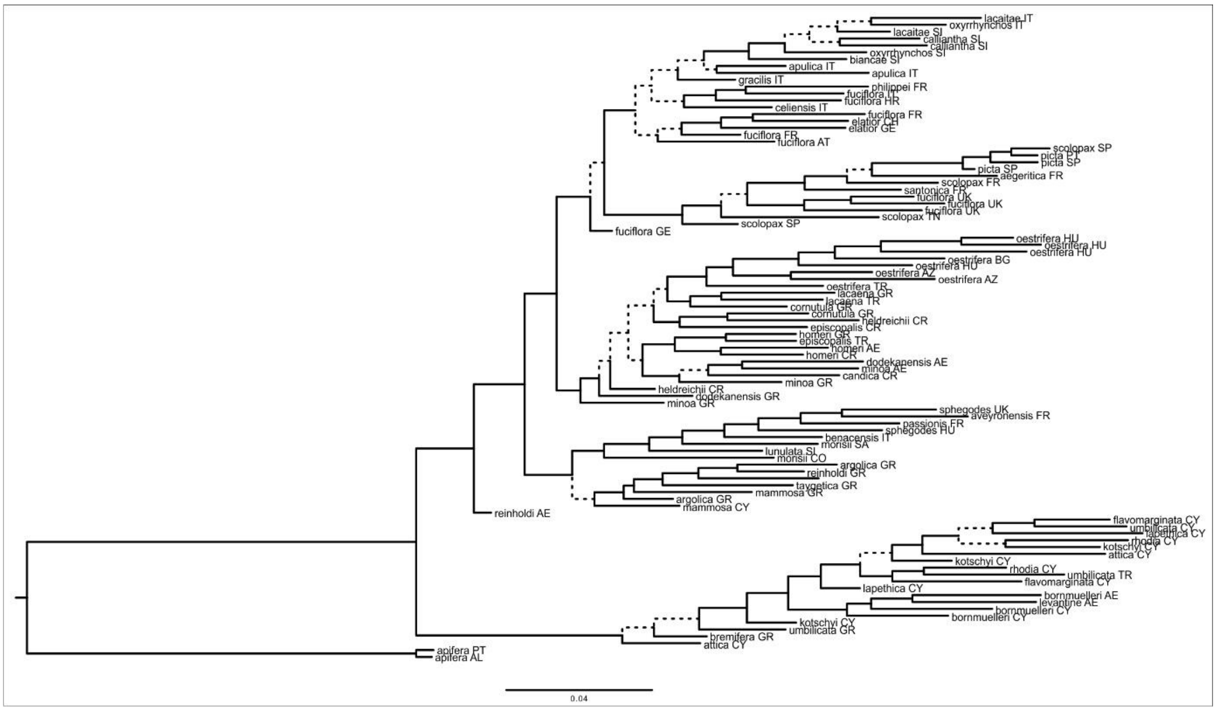


Fig. 6. RaxML-based phylogeny of 42 microspecies of *Ophrys* based on 3883 highly filtered, high-quality SNPs. The sample names refer to microspecies' names, whereas geographic convenience of the sample is abbreviated by ISO country codes.

## Other projects during the grant period in phylogenetics and orchid ecology

As I was enjoying the salary of this PD grant, plus I also had time during the grant period (during data collection and the delay of RAD-seq libraries), I was involved in several studies using phylogenetic methods and orchid phylogeny. In these studies, mostly orchid ecological studies using phylogenetic control, I used the genetic data to provide phylogenetic background for the ecological analyses. Alternatively, some are purely phylogenetic studies providing genetic background for basic taxonomic and evolutionary conclusions.

Two papers were produced from the phylogenetic analysis of the genus *Himantoglossum* using an Eurasian sampling. The first one describes the evolutionary relationships based on nuclear and plastid markers, whereas the second one provides an exhaustive description of the taxonomic conclusions from these data.

> Sramkó Gábor, Molnár V Attila, Hawkins Julie A, Bateman Richard M (2014): Molecular phylogeny and evolutionary history of the Eurasiatic orchid genus *Himantoglossum* s.l. (Orchidaceae), ANN BOT-LONDON 114: 1609-1626. IF: 3.654

> Bateman Richard M, Molnár V Attila, Sramkó Gábor (2017) *In situ* morphometric survey elucidates the evolutionary systematics of the Eurasian *Himantoglossum* clade (Orchidaceae: Orchidinae). PEERJ 5: e2893. $\text{IF}_{2016}$: 2.177

I was also included in the phylogenetic analysis of a rare, Icelandic orchid, *Platanthera hyperborea*, which allowed us to draw several additional taxonomic conclusions and to gain new insight for the evolutionary history of this orchid genus.

> Richard M Bateman, Gábor Sramkó, Paula J Rudall (2015) Floral miniaturisation and autogamy in boreal-arctic plants are epitomised by Iceland's most frequent orchid, *Platanthera hyperborea*. PEERJ 3:e894. IF: 2.183

I was included in an ecological research aiming at the study of seed number of autogamous orchids, where I provided the phylogenetic background for the phylogenetic control in the analyses.

> Sonkoly Judit, Vojtkó E Anna, Tökölyi Jácint, Török Péter, Sramkó Gábor, Illyés Zoltán, Molnár V. Attila (2016) Higher seed number compensates for lower fruit-set in deceptive orchids, JOURNAL OF ECOLOGY 104: 343–351. IF: 5.521

One of the main studies during the early period of this research grant was devoted to the study of phylogenetic relationshiphs within the poorly known plant genus *Elatine*. I have played a major role in the study describing the evolutionary history of this genus.

> Gábor Sramkó, Attila Molnár V, János Pál Tóth, Levente Laczkó, Anna Kalinka, Orsolya Horváth, Lidia Skuza, Balázs András Lukács, Agnieszka Popiela (2016) Molecular phylogenetics, seed morphometrics, chromosome number evolution and systematics of European *Elatine* L. (Elatinaceae) species. PEERJ 4: e2800. IF: 2.183

I similarly played a leading role in the phylogenetic work related to the little-known genus *Sicista*, where we produced two influential papers during the grant period. The first one summarises all information available on the *Sicista subtilis* species group (in which the Hungarian species is included) and adds solid genetic data for a current taxonomy of the group. The second one is analysing the evolutionary relationship of the whole genus with a focus on past evolutionary events in the genus.

> Tamás Cserkész, Mikhail Rusin, Gábor Sramkó: An integrative systematic revision of the European southern birch mice (Rodentia: Sminthidae, *Sicista subtilis* group), MAMMAL REV 46: 114–130. IF: 3.286
>
> Cserkész Tamás, Fülöp Attila, Almerekova Shyryn, Kondor Tamás, Laczkó Levente, Sramkó Gábor: Phylogenetic and Morphological Analysis of Birch Mice (Genus *Sicista*, Family Sminthidae, Rodentia) in the Kazak Cradle with Description of a New Species, J MAMM EVOL: DOI 10.1007/s10914-017-9409-6

Three papers follow which are using phylogenetic data as phylogenetic control in analyses addressing orchid ecological questions.

> Attila Molnár V, Attila Takács, Edvárd Mizsei, Viktor Löki, Zoltán Barina, Gábor Sramkó, Jácint Tökölyi (2017) Religious differences affect orchid diversity of Albanian graveyards, PAK J BOT 49: (1) 289-303. $\mathrm{IF}_{2016}$: 0.69
>
> Molnár V Attila, Löki Viktor, Takács Attila, Schmidt Júlia, Tökölyi Jácint, Bódis Judit, Sramkó Gábor (2015) No evidence for historical declines in pollination success in Hungarian orchids. APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 13(4): 1097 - 1108. IF: 0.557
>
> Löki Viktor, Tökölyi Jácint, Süveges Kristóf, Lovas-Kiss Ádám, Hürkan Kaan, Sramkó Gábor, Molnár V Attila: The orchid flora of Turkish graveyards – a comprehensive field survey. WILLDENOWIA - ANNALS OF THE BOTANIC GARDEN AND BOTANICAL MUSEUM BERLIN-DAHLEM 45: 231-243. IF: 0.721

I also contributed to a review of phylogeographic studies of steppic species, where I have contributed with the review of plant phylogeographies of the Carpathian Basin. This review has already been cited 12 times since its publication in 2016:

> Łukasz Kajotch, Cieślak Elżbieta, Varga Zoltán, Paul Wojciech, Mazur Milosz A, Sramkó Gábor, Kubisz Daniel: Phylogeographic patterns of steppe species in Eastern Central Europe: a review and the implications for conservation, BIODIVERS CONSERV 25: 2309–2339. IF: 2.265

Finally, I was included in the taxonomic and conservation genetic analyses of plant taxa as a phylogeneticist expert. My role in these studies were data generation, data analyses and writing of the final manuscript.

> Mosolygó-L Á, Sramkó G, Barabás S, Czeglédi L, Jávor A, Molnár V. A, Surányi Gy (2016) Molecular genetic evidence for allotetraploid hybrid speciation in the genus Crocus L. (Iridaceae). PHYTOTAXA 258: (2) 121-136. IF: 1.24

Szczecińska Monika, Sramkó Gábor, Wołosz Katarzyna, Sawicki Jakub (2016) Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens* (L.) Mill in East Central Europe. PLOS ONE 11: (3) e0151730. IF: 2.806

Bartha L, Sramkó G, Volkova PA, Surina B, Ivanov AL, Banciu HL (2015) Patterns of plastid DNA differentiation in *Erythronium* (Liliaceae) are consistent with allopatric lineage divergence in Europe across longitude and latitude. PLANT SYST EVOL 301: (6) 1747-1758. IF: 1.361

Altogether, these published papers total 15 items gathering an impact factor of 34.464 with the direct or indirect support of the OTKA PD109686 grant.

## Implementation of the RAD-seq method in Hungary

An important aim of the grant proposal was to learn and import the RAD-seq technique, this very powerful genomic method in ecological genetics, to Hungary. As the RAD-seq library preparation is much more complex than simply following a laboratory protocol, a joint research with a researcher practised in RAD-seq was inevitable. My Viennese colleague provided the necessary practice in RAD-seq library preparation, and I prepared six libraries with him – a period that helped me to meet most of the pitfalls of this method and learn how to avoid them.

During the completion of this grant, I have applied for a grant of the Hungarian Academy of Sciences that is focusing on the recent evolutionary history of steppic plants and animals using the RAD-seq method. As we saw in light of our RAD-seq work in orchids, only such genomic approach can resolve so recent evolutionary events like recent migration of plant and animal species. This grant application was successful, and I could start this grant after the end of my OTKA PD grant. By now, we have produced RAD-seq libraries for several dozen samples of four species (*Salvia nutans*, *Aster oleifolius*, *Sicista subtilis*, *Adonis volgensis*) in Hungary including the Illumina sequencing of the libraries and the bioinformatics analyses. The results from these analyses were partly presented at the last 'Hungarian Botanical Conference' in Debrecen (Sramkó Gábor (2018) Filogenomikai módszerek a botanikai kutatásban [Phylogenomics in Botanical Researches] XII. Aktuális Flóra- és Vegetációkutatás a Kárpát-medencében: Program és összefoglalók. Debrecen, 2018.). Therefore, I think we can say with confidence that this very powerful phylogenomic method was successfully implemented in Hungary, and thus the main background aim behind the current grant has successfully been achieved.

## Literature cited

Bateman RM, Hollingsworth PM, Squirrell J, Hollingsworth ML 2005. Tribe Neottieae. Phylogenetics. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN eds. Genera Orchidacearum. Volume 4. Epidendroideae (Part One). Oxford: Oxford University Press, 487–495.

Drouzas AD, Charitonidou M, Tsiftsis S. 2017. Chloroplast DNA variation in Epipactis atrorubens populations from northern Greece. Botany Letters 164(1): 55-62.

Leitch IJ, Kahandawala I, Suda J, Hanson L, Ingrouille MJ, Chase MW, Fay MF. 2009. Genome size diversity in orchids: consequences and evolution. Annals of Botany 104(3): 469-481.

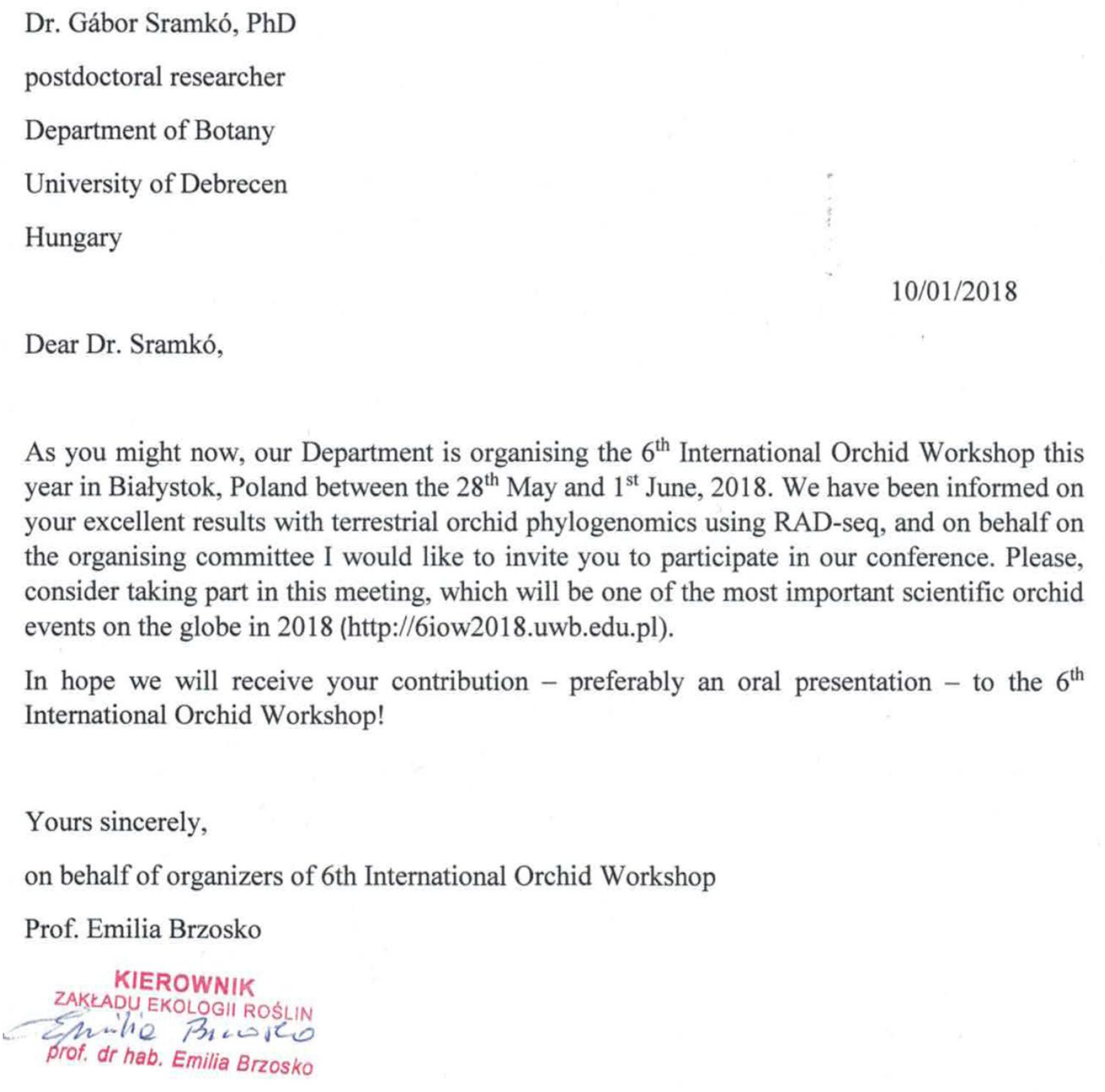
Paun O, Turner B, Trucchi E, Munzinger J, Chase MW, Samuel R. 2016. Processes driving the adaptive radiation of a tropical tree (Diospyros , Ebenaceae) in New Caledonia, a biodiversity hotspot. Systematic Biology 65(2): 212-227.

Tranchida-Lombardo V, Cafasso D, Cristaudo A, Cozzolino S. 2011. Phylogeographic patterns, genetic affinities and morphological differentiation between Epipactis helleborine and related lineages in a Mediterranean glacial refugium. Annals of Botany 107(3): 427-436.

Trucchi E, Frajman B, Haverkamp THA, Schönswetter P, Paun O. 2017. Genomic analyses suggest parallel ecological divergence in Heliosperma pusillum (Caryophyllaceae). New Phytologist 216(1): 267-278.

Zhou T, Jin X-h. 2018. Molecular systematics and the evolution of mycoheterotrophy of tribe Neottieae (Orchidaceae, Epidendroideae). PhytoKeys 94: 39-49.

## Invitation letter from Prof. Emilia Brzosko to participate with the orchid RAD-seq results at the 6th International Orchid Workshop, Białystok, Poland.



Dr. Gábor Sramkó, PhD
postdoctoral researcher
Department of Botany
University of Debrecen
Hungary

10/01/2018

Dear Dr. Sramkó,

As you might now, our Department is organising the 6th International Orchid Workshop this year in Białystok, Poland between the 28th May and 1st June, 2018. We have been informed on your excellent results with terrestrial orchid phylogenomics using RAD-seq, and on behalf on the organising committee I would like to invite you to participate in our conference. Please, consider taking part in this meeting, which will be one of the most important scientific orchid events on the globe in 2018 (http://6iow2018.uwb.edu.pl).

In hope we will receive your contribution – preferably an oral presentation – to the 6th International Orchid Workshop!

Yours sincerely,

on behalf of organizers of 6th International Orchid Workshop

Prof. Emilia Brzosko

KIEROWNIK
ZAKŁADU EKOLOGII ROŚLIN
prof. dr hab. Emilia Brzosko