



**Alexander Kilchevsky**, Chief Scientist, the Laboratory of Ecological Genetics and Biotechnology, the Institute of Genetics and Cytology, NAS of Belarus, Academician, Professor



**Natalya Savina**, Research Scientist, the Laboratory of Ecological Genetics and Biotechnology, the Institute of Genetics and Cytology, NAS of Belarus



**Svetlana Kubrak**, Lead Scientist, the Laboratory of Ecological Genetics and Biotechnology, the Institute of Genetics and Cytology, NAS of Belarus, PhD



**Elena Makeyeva**, Head of the National Coordination Centre on Access to Genetic Resources and Benefit-Sharing, the Institute of Genetics and Cytology, NAS of Belarus, PhD, Associate Professor

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# Genetic Research in the Field of Biological Diversity

The **National Strategy for the Sustainable Development of the Republic of Belarus** covering the period up to 2030 brings to the fore the special role of biological and landscape diversity. Animal and Plant Kingdoms are indispensable components of agriculture and forestry, fishery, tourism, health care etc. [1]. About 27 100 species of living organisms are registered in the territory of our country, including 4450 plant species, 16 thousand of animals, 2 thousand of protists (single-celled) and 4800 fungi [2].

In 1993, Belarus ratified the UN Convention on Biological Diversity and, in 2014, acceded to the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization to the Convention on Biological Diversity, and it implements policy in the field of environmental protection in accordance with its objectives. The National Coordination Centre on Access to Genetic

Resources and Benefit-Sharing (ABS NCC) established at the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus exercises the main activity in this field [3].

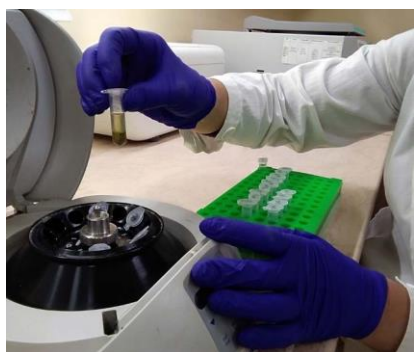
Its employees, as part of a Working Group established by order of the Minister of Natural Resources and Environmental Protection of the Republic of Belarus, in 2021-2022, were involved in the development of the concept and draft Law “On Genetic Resources Management”, which was approved on February 19, 2024, at the Meeting of the Council of the National Assembly of the Republic of Belarus. It is aimed at regulating access to genetic resources and their conservation as the national wealth of the country; deriving, on a fair and equal basis, benefits from their utilization by foreign researchers and other stakeholders. Its adoption is relevant and timely right now, when, as a result of the complex influence of various factors, especially anthropogenic ones, over the past 200 years, about 70 previously encountered native plant species have disappeared from the flora of Belarus. In the country, 303 species of wild plants and 202 species of wild animals are included in the Red Book as rare and endangered [4].

The importance of legal regulation in this segment is also reflected in Chapter 8 of the Concept of the National Biosafety System approved in March 2022 by the Council of Ministers of the Republic of Belarus, which outlines the need to adopt a set of measures *“to protect the population, animals and the environment from the effects of hazardous biological factors and the prevention of biological threats, including improving legislation in the field of regulating access to genetic resources and information on nucleotide sequences; mechanisms for monitoring their use, as well as supervision and penalties for a violation of the country’s sovereign rights to benefits arising from their utilization”* [ 5].

Anthropogenic interference in natural systems has led to a crisis in the diversity of living organisms at the global level. This has become the reason for the formation of a multidisciplinary science – conservation biology, the goal of which is to maintain the species composition of modern biological communities. It combines both fundamental disciplines (botany, zoology, population biology, genetics etc.) and purely applied ones, studying natural resources management and their utilization. Conserving biological diversity requires combining highly specialized skills to study the entire ecosystem so that scientists from different disciplines work together to resolve common problems, and therefore, an interdisciplinary approach to this problem is more effective than a reductionist one.

The 6th National Report on the implementation of the Convention on Biological Diversity in the Republic of Belarus announced the following target: *“Maintaining the genetic diversity of natural flora and fauna, cultivated plants, farm and domestic animals; establishing and replenishing a bank of genetic resources of a human, animals, plants, and microorganisms of the Republic of Belarus...”* [2]. This emphasizes the importance of preserving and expanding bioresource (genetic) collections – a necessary tool for studying Plant and Animal Kingdoms. The collections represent long-term repositories of biomaterials of any organism making it possible for a researcher to select the objects of interest for work.

A mandatory condition for the work of botanical institutions is compiling and maintaining a collection of plants representing various levels of organization – from organismal to molecular (collections of living plants, seeds, cell cultures, herbariums, cryocollections etc.). Such depositories become an important source of knowledge and provide an opportunity to apply it in practice addressing environmental issues and rational nature management. Modern scientific approaches require scientists not to limit themselves to studying one type of biological material (cells, seeds, herbarium specimens), but to conduct comprehensive studies of the entire plant organism, including at the genetic level. Since 2013, the Republican DNA Bank of a Human, Animals, Plants and Microorganisms, where DNA and biological material samples of various organisms are collected, cataloged and stored, has been operational at the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus. The Bank’s scientific activity is multifaceted. One of its areas is the study and inventory of plant genetic resources based on the modern methods of molecular biology. Thus, as part of the “DNA Bank of Plants” section, in 2017, a collection of wild flora was compiled and is annually replenished, including the samples of rare and endangered species included in the 4th edition of the Red Book with the aim of collecting, preserving, and studying the unique genetic information of plant genotypes with low adaptive capacity.





Ятрышник  
клопоносный



Тайник  
яйцевидный



Венерин башмачок  
настоящий



Любка  
зеленоцветковая

In this case, the collection of material is carried out without removing objects from their habitats, that is, the number of natural populations of protected species is not disturbed. In this regard, the Republican DNA Bank may act as a resource center accumulating material for environmental and population studies, thereby contributing to the conservation of the biological diversity of our country's flora. To date, the DNA Collection of Rare Plant Species includes almost 800 DNA and biological material samples. Representatives of 101 species of categories I-IV of national environmental significance from the main and preventive protection lists of the Red Book have been deposited.

The collection of flowering plants (Angiospermae) includes 81 species from 34 families, and among them, Ranunculaceae and Orchidaceae are most widely represented. In addition to the taxonomic division, the samples are classified according to the geographical principle: the collections, including protected plants from the National Parks "Narochansky", "Belovezhskaya Pushcha", "Braslav Lakes", "Polessye State Radiation-Ecological Reserve", as well as rare species growing in protected and non-protected territories of Gomel, Grodno, Vitebsk and Minsk Regions, have been identified. The Republican DNA Bank is in close collaboration with the Flora and Plant Taxonomy Laboratory of the Institute of Experimental Botany named after V.F. Kuprevich, NAS of Belarus; the Faculty of Biology, the Brest State A.S. Pushkin University; and scientific departments of environmental institutions of Belarus.

The first and important step in developing a strategy for the preservation of the plant gene pool is an inventory of resources carried out with the aim of accounting and collecting data on the quantitative and qualitative characteristics of populations. At that, information on the species composition of natural objects, their life activity, and a degree of morphological and genetic variability is collected. As a result, existing biodiversity is assessed, and the objects that require special attention and study are identified.

The main method for plant species identification is still a morphological one. However, molecular genetic analysis is used as a reliable additional approach, and in some cases, the only one possible. Thanks to advances in sequencing and software technologies, DNA sequences have become a source of new information for a better understanding of the evolutionary and genetic relationships of living organisms. To confirm affiliation at the species level, the most convenient method is DNA barcoding, which allows determining the nucleotide sequences of variable taxonomically significant regions, the so-called DNA barcodes. This method is based on the assumption that a short standardized sequence of DNA barcodes can help distinguish species because interspecies genetic variation exceeds that within a species. The nucleotide sequence of the sample under study is compared with a reference data library, and in the case of fixation of an exact match with the barcodes available there, it is identified. The discrepancy may indicate the emergence of a new haplotype or a geographic variant, or the existence of a recently discovered species.

Molecular identification system is evolving into a global network the essential element of which is reference libraries of reference DNA barcodes for known species. To create them, large-scale campaigns for the sampling and sequencing of collections take place in nature museums, herbariums and other resource centers across the globe, and due to that, the heritage of more than a century-long natural history can be used in modern scientific research. According to the recommendations of the International Center for Barcoding (CCDB), collection specimens must contain information as follows: species name; voucher details; a collection record; sample ID; a DNA barcode sequence; and PCR primers to obtain an amplicon.

Employees of the Republican DNA Bank verify the species affiliation of plant samples using 4 main markers: nuclear ITS2 and chloroplast *rbcL*, *psbA-trnH*, and *matK*. Genetic data obtained during genotyping of rare species are accumulated in a local DNA barcode database, which allows, if necessary, a comparative analysis of samples from the different regions of their growth.

In addition to its applied value (excluding the cases of incorrect species identification, control over the purity of a sample, inventorying the genetic resources of a particular region), DNA barcoding has become an effective tool in fundamental disciplines – molecular phylogeny and population genetics. Research on the former concerns evolutionary relationships between the taxonomic levels of the supraspecific order, while research on the latter focuses on variation within and between the populations of the same species. DNA barcoding is applicable to both disciplines and allows the characterization of different levels of organization since it aims to identify species and is focused on their delimitation [6].

Over the past decade, there has been an integration of the method as a routine procedure into taxonomy, leading to a number of methodological advances in this science. Taxonomic research requires the collection of morphological and ecological data and may vary for different complexes (i.e. it requires the application of different methods and skills). DNA barcoding has opened up perspectives with regard to species identification and has simplified this process due to relatively standardized protocols for data sequencing and labeling. On the other hand, the acceleration of the inventory of living organisms means the simplification of procedures, which does not always have a positive effect on the results, and therefore, in scientific circles, an integrative approach combining classical taxonomy and genetic analysis is considered optimal [7]. In poorly studied taxonomic groups, DNA barcoding may precede conventional taxonomic work to sort specimens by genetically divergent groups [8]. In addition, the method is an important tool in the discovery of new species that can be discovered as a result of mass screening using genetic methods. If an unknown specimen is identified that does not have an exact match in the DNA barcode library, this is not a reason to interpret it as a new species. Such a sample is considered in the context of traditional taxonomic analysis, which is usually much slower but produces more reliable results, especially when taking into account preobtained genetic information [6]. Using DNA barcoding, you can find micropopulations that are unique from a genetic point of view, rare biotypes and forms, and conduct a taxonomic revision of different systematic groups [8].

Modern taxonomy is an active area of research since hypotheses for species delimitation are constantly revised and refined. In this context, molecular genetic studies are expedient not only for the purpose of reliable species identification, but also for resolving some controversial issues of relationships between them within families. Employees of the Republican DNA Bank, using the analysis of nuclear ITS marker variability as an example, have verified the taxonomic status of 16 orchid species – representatives of the flora of Belarus; 13 out of them are included in the Red Book. It was shown, based on the classical systematics data, that their phylogenetic relationships are fully consistent with the existing taxonomy of this family [9].

In addition, 11 rare species of the Ranunculaceae family, belonging to 2 subfamilies and 7 genera, turned out to be clearly distinguishable based on the comparison of nucleotide sequences of the ITS region. The Ranunculaceae family is one of the largest species in terms of its number and diversity, which presents difficulties in elucidating the relationships among subfamilies and their phylogeny. ITS region variability turned out to be completely consistent with the existing taxonomy and made it possible to effectively establish the status of individual taxonomic groups



within the family [10]. Using the ITS2 DNA barcode, the genetic polymorphism of water caltrop was assessed – a controversial taxon, which is considered either as one polymorphic species or 10 or more individual species according to fruit morphology. Analysis of three morphologically different groups of water caltrop did not reveal genetic differences between these types by the ITS2 DNA barcode, which can be considered as the fact that all plant samples from different populations of Belarus belong to the same species or as evidence that they have descended from a common ancestor over a short period time and are phylogenetically close relatives [11].

Thus, DNA barcoding has established itself as a mature field of biodiversity science, bridging the conceptual gap between traditional botanical disciplines and new research in molecular genetics. And broad specialization and the ability to involve specialists from different fields of knowledge is an ideal combination for achieving real results in conservation biology.

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***\*Non-binding translation***

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