

## REVIEW



on a dissertation work for obtaining the educational and scientific degree "PhD" in: field of higher education 6. "Agricultural sciences and veterinary medicine: professional direction 6.3 "Livestock breeding" the scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction"

**Author of the dissertation:** Yanka Ivanova Mihailova extramural PhD student at the Department of Animal Husbandry at the Agricultural University, Plovdiv

**Dissertation topic:** Molecular markers for genotyping and evaluation of genetic resources from local sheep breeds in Bulgaria.

**Reviewer:** Prof. Dr. Stayka Staneva Laleva, Agricultural Institute - Stara Zagora, field of higher education 6. "Agricultural sciences and veterinary medicine: professional direction 6.3 "Livestock breeding" scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction" appointed as a member of the scientific jury by order No. RD-RD-16-779/07.05.2022 by the AU Rector.

### 1. Brief introduction of the candidate

Yanka Ivanova Mihailova was born on 19.11. 1966. In 1989, she graduate the Technical University of Sofia, majoring in "Electronic Engineering and Microelectronics". In 1999, she completed her master's studies at UNSS-Sofia, being awarded the educational and qualification degree "Master" with the acquired professional qualification "Economic Management"

In 2020, at the same university, she completed a second master's program and acquired a professional qualification in "Accounting and Control". In 2016 completed a master's program at the Agricultural University - Plovdiv, being awarded the educational and qualification degree "Master" with an acquired

professional qualification in "Animal Husbandry".

## **2. Actuality of the problem.**

Effective management of livestock genetic resources is paramount to ensuring global and sustainable food security. The erosion of genetic resources in farm animals has been analyzed by many researchers, and within a few decades we may lose most of the extremely valuable genetic resources in animal husbandry.

Centuries of selection have led to changes in the genome of sheep breeds in response to environmental challenges and human needs related to various important economic traits, such as quality and quantity of wool, milk and meat. Therefore, native breeds are valuable sources of genomic variants through which it is possible to elucidate mechanisms of response to adaptation processes and artificial selection. On the other hand, the history and development of these breeds is related to the history of human migrations.

The geographical position of Bulgaria and the climatic features are prerequisites for the significant number of autochthonous breeds of sheep in our country. In recent years, thanks to the state's policies to protect biodiversity, the number of some of these breeds has increased several times. Considering that the differentiation of breeds is mainly based on phenotype, prerequisites are created for the inclusion of atypical animals in the selection process. This, on the one hand, calls into question the preservation of these genetic resources and, on the other hand, an inefficient use of a significant financial resource.

At this stage in our country, there is an urgent need to apply more precise, objective methods for determining the breed affiliation and differentiation of the local breeds in Bulgaria.

The development of next-generation sequencing technologies provides significant opportunities for the proper characterization of genetic resources in sheep breeding. Only on the basis of solid genetic characterization can urgent conservation measures be taken to avoid irreparable loss of genetic resources in sheep, integrating economic, sociological and political parameters.

Yanka Mihailova's dissertation provides information about the genetic diversity of a large part of the autochthonous breeds in Bulgaria, about their genetic uniqueness and differentiation. It can be the basis for the development of the breeding programs of these breeds, as well as for the development of future strategies for the development of native sheep breeding in our country.

### **3. Aim, tasks, hypotheses and research methods**

The goal is clearly formulated, and the set ten tasks ensure its implementation. For the development of this dissertation, data from the reports of the breeding organizations, analyzes and reports from IASRJ, annual reports of the Ministry of Agriculture, the EFABIS and DAD-IS information systems were used for a 13-year period from 2009 to 2021. The research included 12 local autochthonous breeds: Local Starozagora, Sredno-Staroplaninska, Dabenska, Sredno-Rodopska, Tetevenska, Koprivshtenska, Karakachanska, Local Karnobatska, Replianska, Sakarska, Breznishka and Kotlenska based on condition and trends of change, geographical distribution and degree of threat. A total of 600 animals from 50 herds were genotyped. Genotyping was performed with 15 microsatellite markers covering 20 of the 54 chromosomes, achieving genomic coverage within about 37% of the total



number of chromosomes. For the amplification of the 15 loci, 4 multiplexes were used.

The laboratory tests were carried out with modern equipment, guaranteeing accuracy in the relevant analyses. Statistical processing was performed with mathematical models and software used by leading scientists in the field. The methodological approaches used by the PhD student in the development of the dissertation work are correct, which, in my opinion, is an indicator of her very good theoretical preparation, acquired skills for planning and conducting experiments, for analysis and interpretation of the obtained results.

#### **4. Transparency and presentation of the obtained results**

The dissertation is written in a very good style and language and the most appropriate way of presentation is chosen. The significant volume of results obtained using microsatellite markers to assess and characterize the genetic diversity of 12 local Bulgarian sheep breeds are presented in 21 tables and 28 figures. For greater precision, some of the tables are included in Appendices 1 and 2.

The main part of them are the results of the microsatellite analysis of the selected breeds while optimizing the conditions for multiplex microsatellite analysis, as well as the results for basic genetic parameters - polymorphism in the microsatellite loci, genetic diversity, inbreeding and variation. A very important part, in my opinion, of the results in the dissertation work is the analysis of the correlations between the established genetic and phenotypic distances between the studied breeds.

The dissertation is written in a very good style and language, and the most appropriate way of presenting the obtained results has been chosen.

#### **5. Discussion of the results and used literature.**

The results of the large number of methodically correctly conducted studies are aimed at the main goal of the dissertation, namely the assessment of the population structure and characterization of the genetic diversity of local Bulgarian sheep breeds. The interpretation of the data is done professionally, thoroughly and very accurately. The results of the various analyzes are discussed and supported by data from leading scientists in recent years,

296 literature sources were used, of which 22 in Cyrillic and 274 in Latin. This, in my opinion, is an indicator of the doctoral student's good literacy awareness, her skills in handling scientific literature and very good scientific training.

On the basis of the obtained results, ten conclusions and three recommendations were formed, which are relevant for the development of strategies and policies for the preservation of the indigenous breeds reared in Bulgaria.

#### **6. Contributions of the dissertation work.**

With the development of the current dissertation work, Yanka Mihailova makes a number of scientific and research-related contributions. I would like to note the following:

Scientific contributions

1. An extended and systematic study of the genetic resources of 12 populations of local breeds of sheep in Bulgaria, of which 7 are new, was carried out, based on microsatellite analysis in 13 loci.

2. The levels of genetic diversity in the studied autochthonous breeds have been studied and established.

3. Unique alleles were found in the studied breeds, with the exception of Local Karnobatska, which is an indicator of a high level of allelic diversity

4. The genetic structure of the population, represented by 50 herds of the 12 studied breeds, was determined and each of the individuals belonged to the six genetic clusters.

#### Scientific and applied contributions

1. A correlation analysis between the genetic and phenotypic matrices was performed and the relationship between the studied genetic and phenotypic parameters was established, which is the basis for conducting a complex assessment of the genetic resources of sheep in Bulgaria.

2. A set of microsatellite markers suitable for characterizing the genetic structure, genetic diversity and analysis of genetic processes in indigenous sheep breeds in our country has been studied and established.

#### **7. Critical Notes and Questions.**

The PhD student complied with my critical notes from the prior discussion, which is why I do not have any for the submitted PhD thesis for review by me.

## 8. Published articles and citations

The dissertation student presents an independent scientific work with a title „Genetic diversity and structure of 2 indigenous sheep breeds (Kotel and Teteven) in Bulgaria using microsatellite markers“ in Biotechnologies and Biotechnological Equipment magazine in 2021.

The article reflects part of the research results in the dissertation work. With it, the requirements of a minimum of 30 points are met, according to LDASRB and the Regulations of the Agricultural University for its application.

The presented abstract reflects objectively the structure and content of the dissertation work.

### CONCLUSION:

Based on the different research methods learned and applied by the PhD student, the correctly performed experiments, the generalizations and conclusions made, I believe that the presented dissertation meets the requirements of the LDASRB and the Regulations of the Agrarian University for its application, which gives me the reason to rate it POSITIVELY

I allow myself to propose to the honorable Scientific Jury to also vote positive and award Yanka Ivanova Mihailova the educational and scientific degree "PhD" in the scientific specialty: "Breeding of agricultural animals, biology and biotechnology of reproduction", professional direction: 6.3. Animal husbandry, field of higher education: 6. Agricultural sciences and veterinary medicine

Date: 05.09.2022r. ....

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