REVIEW

on the thesis for obtaining the educational and scientific degree **"doctor"** in: field of higher education 6. Agricultural sciences and veterinary medicine, professional direction 6.3. Animal husbandry, the scientific specialty 'Breeding of agricultural animals, biology and biotechnology of reproduction'

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Thesis: "Molecular markers for genotyping and evaluation of the genetic resources of local sheep breeds in Bulgaria"

Reviewer: Prof. Ivan Iliev Atanassov, Agrobioinstitute, Agricultural Academy; field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological sciences, scientific specialty 'Genetics', designated as a member of the scientific jury by order No. RD-16-779 of 07/05/2022 by the Rector of AU-Plovdiv.

1. Brief introduction of the candidate.

Yanka Ivanova - Mihailova was born in 1966. In the period 1984 - 1999 she completed a master's program at the Sofia Technical University, in the period 1997 - 2000 she successively completed the master's programs "Economic Management" and "Accounting and Control" at the University of National and world economy Sofia. In the period 2014 - 2016, she completed a master's program at the Plovdiv Agricultural University and obtained a Master's degree in "Zooengineering". During the period 1998 - 2018, she was successively the Director of the APFSIO Directorate and the Chief Secretary of the Executive Agency for Selection and Reproduction in Livestock Breeding, and since 2018 she has been the Director of the Financial Management and Human Resources Directorate at the Agricultural Academy. Since 2019, she has been enrolled as a part-time doctoral student at the Department of Animal Husbandry Sciences at the Agricultural University, Plovdiv. In the framework of her current research activity, Yanka Ivanova – Mihailova is the author of 1 publication and 1 conference report. She speaks English and Russian.

2. Relevance of the problem.

Sheep breeding is one of the main sectors of animal breeding in Bulgaria with great economic and social importance. Traditionally, sheep farming in the country includes the breeding and maintenance of flocks of a large number of Bulgarian local autochthonous breeds of sheep distinguished by high adaptability to local conditions, and some of them also

with important ethno-cultural and historical significance. In addition to the breeding of such local breeds, a number of highly productive foreign breeds, as well as those created after selection in the country, were imported and are being bred in Bulgaria during the last century. Growing in recent decades in the country, a tendency of unsystematic crossing of cultivated high-productivity breeds with local autochthonous breeds reduces the breeding area of the latter and leads to a real danger of the disappearance of a part of them, and, accordingly, an irreversible loss of biodiversity and genetic resources for sheep breeding. Currently, the differentiation of the breeds grown in the country is done only by phenotype, which is not efficient enough and further complicates the monitoring and control by the breeding organizations on the maintenance and breeding of the autochthonous breeds. All this makes the development and real application of modern molecular genetic methods for the assessment of genetic diversity and differentiation in flocks and breeds of sheep relevant and very necessary, as a basis for the application in the country of modern objective and more precise approaches and procedures for real assessment of genetic diversity in native breeds and determination of breed affiliation. Taking into account the above, I consider that the topic of the dissertation work is very relevant and the implementation is expected to have a high scientific and scientific-applied effect.

3. Purpose, tasks, hypotheses and research methods.

The dissertation work includes a clearly and correctly formulated goal corresponding to the topic, as well as a set of clearly and precisely specified tasks, the implementation of which ensures the achievement of the set goal. The main hypothesis and research method involves using established highly polymorphic microsatellite markers for genotyping sheep from individual flocks of Bulgarian autochthonous breeds and using the obtained molecular genetic data to assess the genetic diversity and structure within and between the studied flocks and breeds. The data obtained from this study are additionally compared with phenotypic signs and differences between breeds, which significantly increases the informativeness and contribution of the research. Regarding the research methodology, it should be noted that it includes /a/ a very large set of researched herds and autochthonous breeds (a total of 600 sheep from 50 flocks of a total of 12 autochthonous breeds from all over the country) and /b/ a large set of 15 microsatellite markers providing relatively large genomic coverage. The experimental methods used include successful and effective application of the necessary for this type of research: correct collection and labeling of blood samples from individual sheep, DNA isolation, PCR amplification of the studied microsatellite regions, fragment analysis of the amplified DNA regions and complex processing of the obtained data. Within the framework of the dissertation work, the doctoral student successfully optimized procedures for multiplex (simultaneous) PCR amplification of several microsatellite regions, which reduces the costs and time for conducting the analyzes and can be successfully used directly in subsequent practical applications of the methods and results of the dissertation work. An important contribution to the success of the research included in the dissertation work is the appropriate selection and application of a set of statistical methods and software for analysis and evaluation of the obtained molecular genetic data and cluster analysis of phenotypic data.

4. Transparency and presentation of the obtained results.

The dissertation contains 187 pages and the presented information and results are illustrated with a total of 29 figures and 22 tables. The dissertation work is structured in the generally accepted way: literature review, aim and objectives, materials and methods, results and discussion. The presentation of the results of the dissertation begins with an in-depth analysis of the current state of the local autochthonous breeds of sheep in the country, the thresholds of danger have been determined using the methodology and current data from its application to individual breeds. Accordingly, the doctoral student uses the results of the analysis of the presented information to select a set of local autochthonous breeds subject to further research. A major part of the presentation of the results occupies the presentation of the experimental data from the large-scale microsatellite analysis of the selected breeds and flocks, including the optimization of the conditions for multiplex microsatellite analysis (14 SSRs in 4 multiplexes) and their applications for the analysis of a total of 600 sheep by 15 microsatellite markers. This part also includes the results for basic genetic parameters (polymorphism in the microsatellite loci, genetic diversity, inbreeding and variation) for the microsatellite markers used and the studied sheep populations. The presentation of the results is in detail necessary and sufficient for their further use, but on the other hand well systematized, which further facilitates familiarization with them and their use within the dissertation work, as well as a source of information for subsequent practical applications of DNA markers in sheep breeding. The other important part of the presentation of the results is the use of the data obtained from the microsatellite analysis to evaluate the genetic differentiation and structure of the studied breeds and flocks of sheep and the phylogenetic relationships between the breeds, and for this purpose, modern approaches and software for processing and computer analysis of the experimental data (AMOVA, PCoA, softwares GenAlEx, Structure, FAMD, etc.). The last part of results and discussion includes an analysis of the distance between the studied breeds of sheep based on their characteristic phenotypic traits and an original assessment of the correlation between the established genetic and phenotypic distances between the studied breeds. In general, the results of the dissertation work are presented complexly, concisely, but in the necessary details, in their logical sequence and excellently illustrated, which greatly facilitates familiarization with them and their use, both within the framework of the dissertation work and in subsequent applications of DNA markers in sheep breeding.

5. Discussion of the results and used literature.

The discussion of the results is also supported by an intelligently presented and well-structured literature review presenting a large volume of complex information, including the problems of sustainable agriculture and conservation of biological diversity and genetic resources in domesticated animals, the status of genetic resources in local sheep breeds in the country and applications of DNA markers for characterizing genetic diversity and structure in sheep. The discussion of each group of results is included within the separate parts of the dissertation work in which they are presented. The discussion of the results is analytical, well-argued and supported by new and up-to-date literature sources. As a result of the presented and discussed results, a total of 10 conclusions were formulated and 3

recommendations were made regarding further practical use of the applied set of microsatellite markers, and practical application of the obtained results for the preservation of the gene pool and effective management of genetic resources in the local sheep breeds in the country. A total of 296 literary sources are included and cited within the dissertation, of which 22 are in Bulgarian and the rest in English.

6. Contributions of the dissertation work.

The results, conclusions and contributions of the dissertation work can be attributed to two main groups.

Scientific contributions

The dissertation work is the first large-scale and systematic study of the genetic resources of local breeds of sheep in Bulgaria, including a large number of breeds and individual flocks of them (12 breeds and a total of 50 flocks) using a relatively large set of microsatellite DNA markers for a total of 13 loci, most located on different chromosomes. Accordingly, the obtained results and their bioinformatic analysis provide an opportunity to determine the main parameters and characteristics of the genetic diversity and population structure of the current genetic resources of autochthonous breeds of sheep in the country. Accordingly, the obtained results and information, together with the established effective multiplex procedures, can and should be used as a basis for systematic monitoring of the status and development of the genetic resources of local sheep breeds and effective management through breeding and conservation programs. The above is a major scientific contribution of the dissertation work with expected long-term benefits and effect. Another important scientific contribution is the conducted correlation analysis between the genetic and phenotypic matrices for the analyzed sheep breeds and established average correlation between the genetic and phenotypic parameters. Although the study used a relatively limited set of generally accepted phenotypic parameters, this approach can be successfully used for comprehensive evaluation of sheep genetic resources by expanding the set of phenotypic traits with quantitative and economically important traits.

Applied scientific contributions

The dissertation work has significant applied scientific contributions related to the application of modern molecular genetic methods for the characterization of genetic resources in sheep and their management through the implementation of breeding programs. An important applied scientific contribution of the dissertation work is the set of optimized protocols for multiplex microsatellite analysis with the markers used, the data from the analyzes for the studied breeds, the set of specialized software for bioinformatics processing of the obtained data and the obtained results and presented interpretation. Taken together, all this can be used as a methodological basis for direct application of the used microsatellite markers, protocols and software for routine periodic characterization of genetic diversity in local sheep breeds in the country and use of the results for decision-making in the conduct of breeding and conservation programs. Specific applied scientific contributions are also the recommendations included in the dissertation work regarding the need to apply measures to preserve two of the studied breeds (Mestna Karnobatska and Starozagorska) and use breeding schemes based on individual teams and strict observance of the selection

and exchange of typical the breed of rams between the herds of the other studied breeds.

7. Critical notes and questions.

I have no critical remarks on the presented dissertation work. I use the opportunity to pose the following question in connection with the practical application of the used methods and results of the dissertation work in the development and implementation of specific breeding programs for local breeds of sheep: "is it necessary to expand the used set of microsatellite markers and include markers for loci located on the sex chromosomes in order to effectively control and evaluate the effect of the exchange of rams in individual local breeds of sheep".
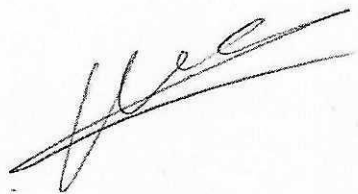
8. Published articles and citations.

Part of the results of the conducted research are presented in one scientific publication (Biotechnology & Biotechnological Equipment, 2021, Q3, IF 1.633), with the only author being a doctoral student, and one report at a scientific conference. So far, 3 citations of the publication have been found in the available scientific literature (https://scholar.google.com/scholar?cites=8909619642547501665&as_sdt=2005&sciodt=0,5&hl=en). 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 doctoral student, the correctly performed experiments, the generalizations and conclusions made, I consider that the presented dissertation meets the requirements of the ŽRASRB and the Regulations of the Agrarian University for its application, which gives me reason to evaluate it **POSITIVE.**

I take the liberty of proposing to the honorable Scientific Jury to vote positively and award Yanka Deneva Ivanova - Mihailova the educational and scientific degree **"Doctor"** in the field of higher education 6. Agricultural sciences and veterinary medicine, professional direction 6.3. Animal husbandry, the scientific specialty 'Breeding of agricultural animals, biology and biotechnology of reproduction'

Date: 31.08.2022 г.
Plovdiv

REVIEWER: ....
(prof. DSc Ivan Atanassov)