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DIFFERENCES AT MOLECULAR LEVEL BETWEEN *BOS TAURUS* L. AND *BUBALUS BUBALIS* L.

Dorin Alexandru VIZITIU¹ Oana Maria BOLDURA^{1*}, Simona MARC¹

¹Faculty of Veterinary Medicine, University of Life Sciences "King Mihai I" Timisoara, Calea
Aradului, no. 119, Timisoara, Romania
oanaboldura@usvt.ro

Abstract: This study explores molecular distinctions between cow (*Bos taurus*) and water buffalo (*Bubalus bubalis*) milk, targeting food fraud prevention in the dairy industry. Using DNA barcoding and protein fingerprinting, the research identified species-specific markers to differentiate these milks in products. DNA sequencing revealed about 87.39% similarity in mitochondrial COI genes, with unique single-nucleotide polymorphisms and deletions, while protein profiling uncovered casein and whey differences affecting cheese quality. These findings underscore a practical approach to ensure authenticity and quality in dairy products, particularly where premium products are at risk of fraudulent substitution.

• Introduction

In today's market, ensuring the authenticity and traceability of food products is critical due to increasing cases of food fraud and rising demand for high-quality products. This study focuses on identifying molecular differences between *Bos taurus* (cattle) and *Bubalus bubalis* (water buffalo) using DNA barcoding, a technique that amplifies short, conserved DNA sequences to enable precise species identification. Food fraud commonly involves substituting high-value products like buffalo or sheep milk with less costly cow milk, which impacts both consumer trust and economic value. DNA-based methods offer several advantages over traditional protein-based techniques, as DNA is more chemically and thermally stable, allowing species detection even after processing. By comparing DNA and protein profiles, this study aims to establish reliable tools for authenticating dairy products, reducing fraudulent substitutions, and enhancing food quality and safety in the dairy industry.

• Material and method

This study used milk samples collected from licensed farms in Timiș, Romania. Molecular techniques included DNA extraction and PCR amplification using primers dgLCO and dgHCO to amplify a 700 bp fragment.

For protein analysis, microfluidic electrophoresis was employed using an Agilent 2100 Bioanalyzer, with samples prepared by centrifugation and dilution. Agarose gel electrophoresis also analyzed nucleic acids, separating DNA fragments by size and visualizing them with UV transillumination, ensuring accurate species identification based on genetic and protein profiles.

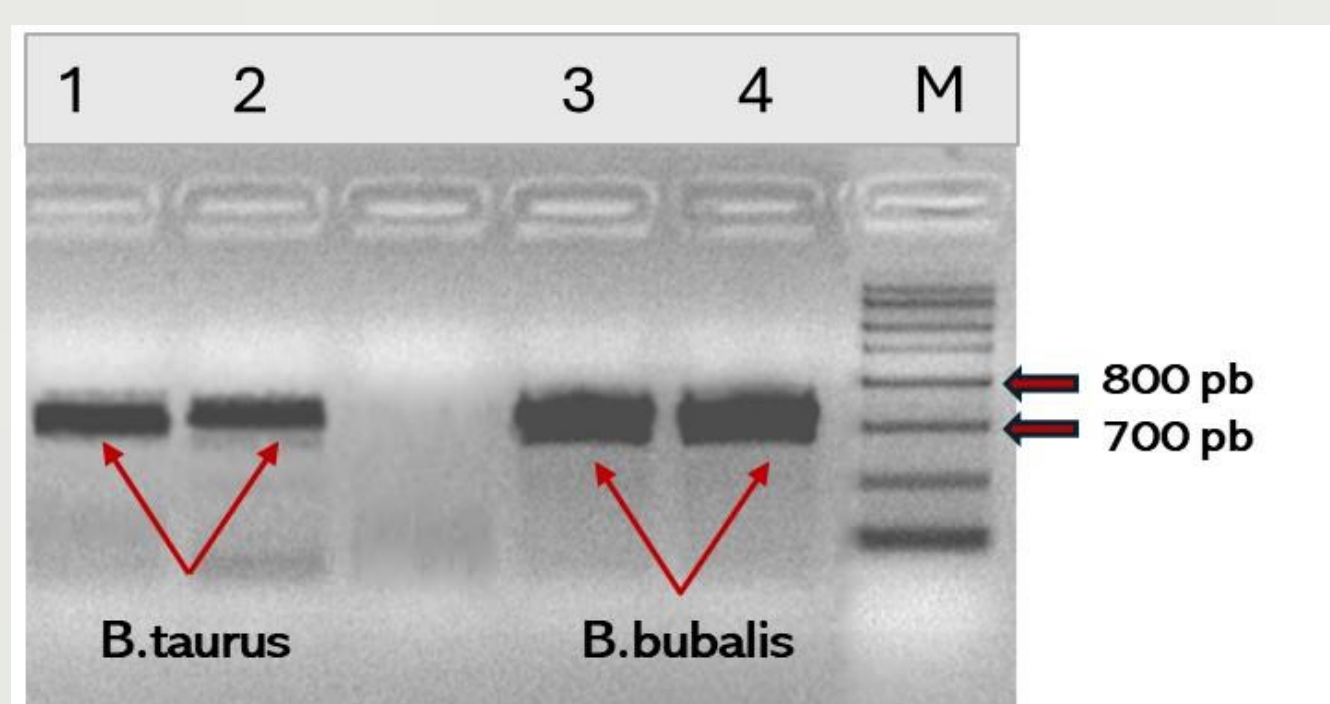


Figure 3. *B. taurus* and *B. bubalis* sequencing gel

• Results and discussions

DNA sequencing and proteomic profiling effectively differentiate milk from *Bos taurus* (cattle) and *Bubalus bubalis* (water buffalo). DNA analysis focused on the COI gene, using PCR with dgLCO and dgHCO primers, and confirmed species-specific sequences. The analysis demonstrated an 87.39% sequence similarity between the two species, with distinct SNPs and deletions allowing clear identification. Protein profiling through microfluidic electrophoresis highlighted variations in key milk proteins. *B. bubalis* milk displayed higher concentrations of β -casein and α -lactalbumin but lower levels of β -lactoglobulin and κ -casein compared to *B. taurus* milk. This approach supports accurate species differentiation, enhancing food traceability and allergen management.

Figure 2. DNA sequences of the two species alignment with highlight on differences

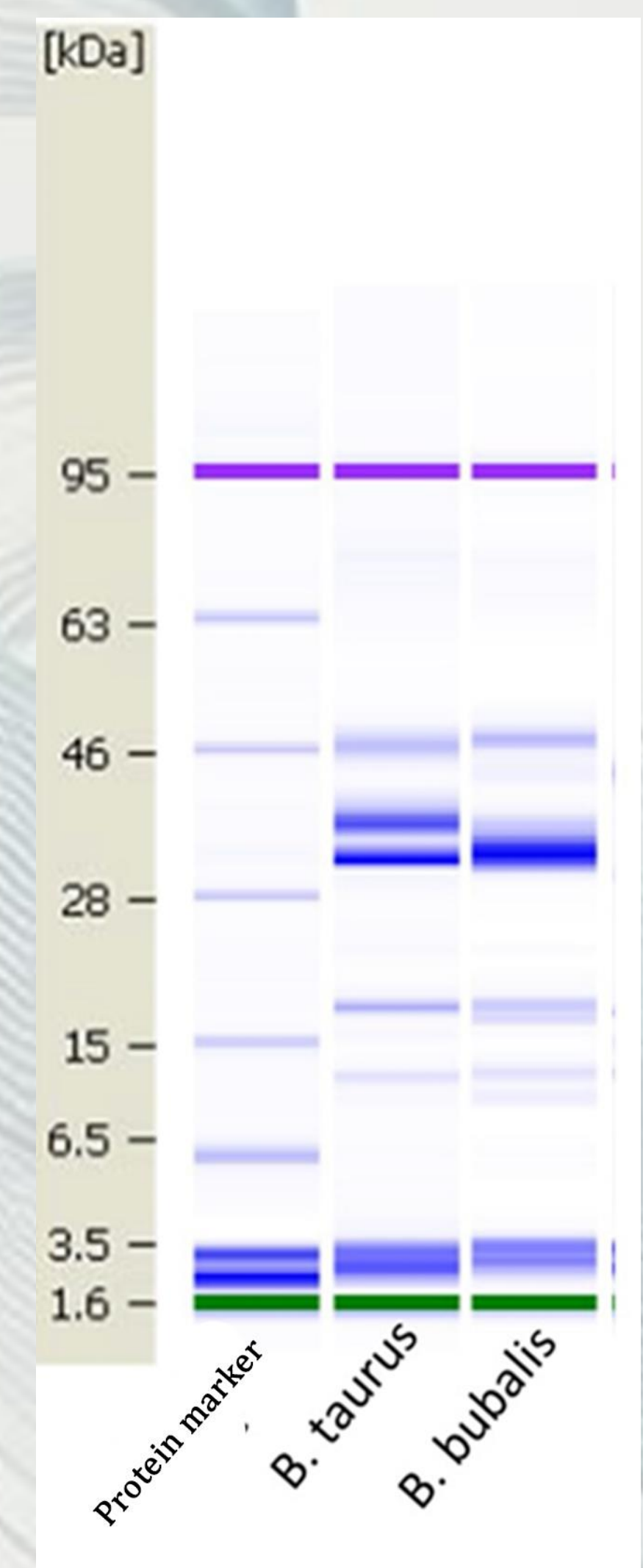
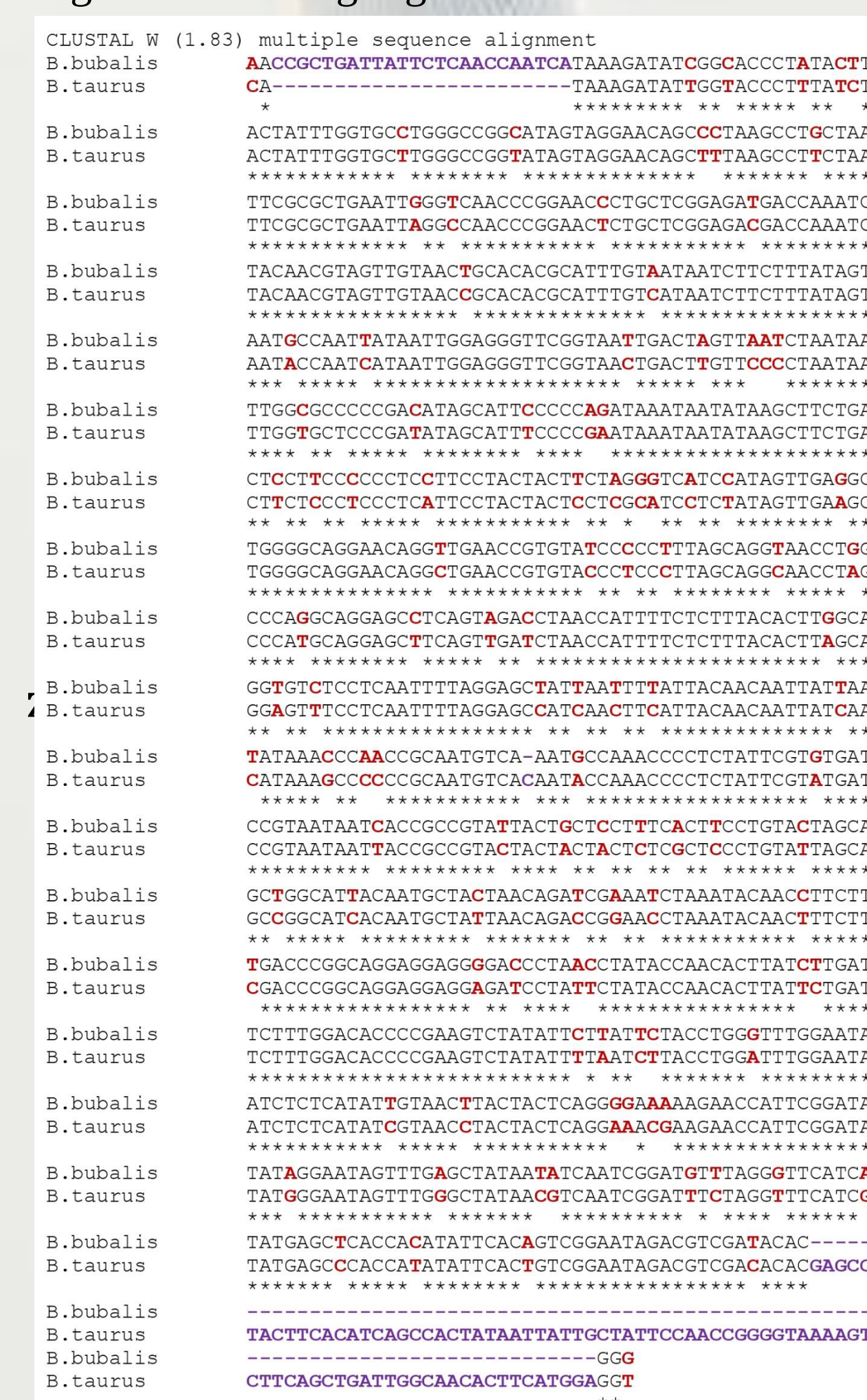


Figure 1. Gel electrophoresis of milk protein

• Conclusions

Results confirm that dgLCO and dgHCO primers effectively amplify the COI gene sequence for *Bos taurus* and *Bubalus bubalis*, enabling species identification via DNA barcoding in the BOLDSYSTEMS database. This method, applied to nucleic acids isolated from milk, reveals clear genetic distinctions between the species, supporting its use in various biological materials. Moreover, electrophoretic profiling of major milk proteins allows quantitative differentiation between milk from the two species when fresh samples are analyzed. These findings contribute advanced molecular methods for authenticating food products, ensuring the quality and safety of dairy and meat items, and protecting consumers and producers from fraud.

• References

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