

Abstract of Angelika's Maşior doctoral dissertation entitled:

'Identification of hybrids llama × alpaca based on DNA analysis'

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Alpacas (*Vicugna pacos*) and llamas (*Lama glama*) belong to the family of South American Camelids (Camelidae), also known as SACs. Their native habitat is the Altiplano region in South America. Both species have the same number of chromosomes ($2n = 74$) and can freely crossbreed, creating fertile offspring (llama x alpaca hybrids). The F₁ generation characterizes typically itself with medium body size and can be backcrossed with any type of parent, as well as other hybrid individuals. Subsequent generations of fertile hybrids are hard to distinguish by evaluating their phenotype alone.

Alpacas are famous for their fiber production, which is considered a luxury material. Hence, their breeding has also expanded to other continents. The first alpacas were brought to Poland in 2004, and since then interest in this species has been steadily growing. Unfortunately, many modern individuals tend to produce a significantly coarser fiber with high hairiness. It is assumed that one of the reasons for this adverse phenomenon is the hybridization with the llama species, ongoing since the 16th century. Currently, alpacas imported to Poland come from various countries, but most animals originate from Chile, due to this country's lenient laws regarding animal export. Regrettably, it is not uncommon that alpacas arriving in Poland do not have documented pedigrees, therefore lacking information about their origin. For this reason, the aim of this study was to evaluate microsatellite markers, the d-loop region of mitochondrial DNA, and the *DBY* gene located on the Y chromosome as tools for identifying hybridization among alpacas and determining the genetic diversity of alpacas kept in Poland. Also, an analysis involving whole-genome examination - Genotyping-By-Sequencing (GBS) was considered as an effective method for identifying hybrids and backcrosses.

The research material consisted of hair follicle and buccal swabs taken from the inner part of the cheek from alpacas and llamas kept in Poland. A first-generation hybrid was also obtained, along with its parents. Among the acquired individuals, two presumed llama x alpaca hybrids, showing phenotypic characteristics suggesting hybridization between the two species,

were also utilized for analysis. DNA was isolated from the collected biological material, and its quality and quantity were assessed on a spectrophotometer. Subsequently, molecular analyses were performed, which included the amplification of 17 microsatellite markers, the d-loop region, and the *DBY* gene. The microsatellite markers, after amplification, were subjected to STR fragment separation by capillary electrophoresis on a 3130xl sequencer, and then genotyped and submitted to bioinformatic analyses. The amplicons for the d-loop regions and *DBY* gene were sequenced using Sanger's method, and the obtained sequences were subjected to bioinformatic analyses.

Using microsatellite markers, the level of genetic admixture was estimated, but it was difficult to determine exactly when hybridization occurred. Based on this analysis, it was estimated that 8.8 % of the examined alpacas had a llama admixture, assuming a q coefficient ≥ 0.98 for purebred animals. The F_1 hybrid had only 7.4 % llama admixture, while its parents were assigned as a purebred llama (father) and purebred alpaca (mother). For the *DBY* region, no hybridization between the alpaca and llama was observed among the studied populations. Furthermore, four segregation sites, i.e., polymorphic changes characteristic only for alpacas or for llamas, were found. In the d-loop region, introgression was observed between the mitochondrial DNA of alpacas and llamas. Moreover, it was observed that hybridization disrupts cladogenesis by leading to the mixing of genetic information between two different evolutionary lines.

The genetic diversity of alpacas kept in Poland, based on conducted studies, was recorded at a high level, proving that these populations are not homogeneous and their genetic diversity is still being shaped. The obtained results also suggest that alpacas and llamas have not yet completely separated genetically, and even mtDNA introgression was observed in these two species. No evidence of hybridization in the male line was found after analyzing the *DBY* gene, however, the four variable sites identified in the current studies may possibly serve as diagnostic markers for identifying alpacas, llamas, and male hybrids. However, this hypothesis should be confirmed after examining a larger amount of male alpaca and llama population.