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**P18 ISAG Bursary Award: A new approach to the molecular differentiation of the wolf and the domestic dog in wildlife forensics.** A. E. Hrebianchuk\*<sup>1</sup> and I. S. Tsybovsky<sup>2</sup>, <sup>1</sup>*State Forensic Examination Committee of the Republic of Belarus, Minsk, Republic of Belarus*, <sup>2</sup>*Republican Unitary Service Enterprise «BelJurZabespechenne», Minsk, Republic of Belarus*.

The molecular differentiation of individuals of the wolf (*Canis lupus lupus*) and the dog (*Canis lupus familiaris*) represents a difficult problem in the study of the *Canidae* family. In wildlife forensics, the first step toward identification of materials of animal origin is to determine the species of the animal. The current panels of STR loci employed both in basic science and forensics, which use domestic dog DNA with a confirmed species origin, have not been tested for cross-applicability with DNA from any other wild canids. We developed a test system for DNA-based differentiation of wild and domestic representatives of the *Canidae* family. This real-time PCR-based system allows one to quickly and reliably distinguish test samples of a wolf and a domestic dog. The test system is designed to detect 2 targets, the pancreatic amylase gene (*Amy2b*) and oncogene *vMYC*. The differentiating parameter between the wolf and the domestic dog is the number of copies of the pancreatic amylase gene. For wild canids, the copy number of the amylase gene is a constant value of 2, whereas in a domestic dog, the number of copies of this gene is always greater than 2. The robustness of the differentiation of the wolf and the domestic dog using this test system was confirmed in a study of Belarusian populations of the wolf (121 samples) and the domestic dog (216 samples), while verification was carried out using biological samples of the raccoon dog (179 samples), the red fox (including its black-brown morph; 383 samples) and the Arctic fox (29 samples). The developed test system can be implemented using 2 types of thermal cyclers, as well as droplet digital PCR (ddPCR), and reagents from various manufacturers. All validations were carried out in accordance with the protocol of the SWGDAM and the ISO5725 standard. Successful identification of a biological trace as originating from a wolf or a domestic dog using this test system makes it possible to subsequently employ identifying panels of STR loci, allowing statistical evaluation of the possibility that a biological trace belongs to a certain *Canis lupus* individual.

**Key Words:** wolf, dog, *Canis lupus*, forensics, genetic differentiation