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COMPARATIVE *IN SILICO* ANALYSIS OF PRIMERS USED FOR VERTEBRATE METABARCODING

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DNA metabarcoding is a relatively new method of biomonitoring and refers to the molecular analysis of a mixture of intra- and extracellular DNA from various organisms. It allows the use of different target sequences for the simultaneous characterization of several organisms and has a big potential in biodiversity research. The environmental DNA (eDNA) analysis began to be applied for studying vertebrate species and like any metabarcoding research requires a correct selection of primers. In this study we aimed to perform *in silico* PCR and compare the degree of conservation, taxonomic coverage (Bc) and specificity (Bs) of 2 universal primers pairs used for vertebrate DNA analysis. The first pair (Kitano-16S) was proposed in 2006 by Takashi Kitano and co-workers as universal primers for identifying vertebrate species, based on 16S mitochondrial DNA sequencing. The second pair (12S-V5) targets the 12S mitochondrial region and was designed by Tiayyba Riaz and collaborators in 2011. The 12S-V5 has been validated using bioinformatics algorithms (*ecoPrimers*) and experimental laboratory approaches. The current study includes 191 NCBI mitochondrial genomes of 140 species of birds, 42 species of mammals, 5 species of amphibians and 4 species of reptiles, most species being part of the fauna of the Republic of Moldova.

In order to perform *in silico* analyses, there were used following bioinformatics platforms and tools: *NCBI*, *OBITools*, *ecoPCR*, *SO Ubuntu*. The assessment of the primers conservation level and graphical representation were performed using R language and ROBITools, ROBITaxonomy, ROBIBarcodes libraries.

The *in silico* PCR of the studied 191 mitogenomes showed that the pair Kitano-16S represents a higher degree of conservation compared to the second pair; howhever 12S-V5 demonstrated a clearly superior performance with a taxonomic coverage of 97.91% and a specificity of 76.47%. Respectively, for the Kitano-16S pair resulted a 21.99% taxonomic coverage and a 90.48% specificity. Also, the 12S-V5 pair has a maximum of 2 mismatches for both primers while the Kitano-16S - a maximum of one mismatch for the forward primer with a total lack of mismatches for most amplicons. The metabarcodes length is about 200 bp for Kitano-16S pair and varies between 96 and 106 bp for the 12S- V5. Finally, the *in silico* amplification of the studied vertebrate mitogenomes is more efficient with the use of universal primers 12S-V5. This pair can be used to perform metabarcoding research that targets above mentioned mitochondrial genomes.

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