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# Ability to obtain of ITS1 and ITS2 DNA barcode for metabarcoding of historical herbarium specimens

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*Motivation and Aim*: In the archival premises of the botanical gardens and academical herbariums, a large number of specimens are stored. This samples were collected many years ago. Some of them are valuable as a historical object, because they were collected by famous scientists of the past or they were collected during geographical expeditions of a one-two hundred years ago. However, often the specimens from these collections cannot be used for botanical investigations. Because they were not identified during the expedition and the transport storage conditions of the biomaterial in those ancient times did not allow it to be preserved in good enough quality for taxonomy identification of specimens.

Hugh Cuming's collection of flowering plants kept in the Moscow University Herbarium (MW) contains 420 type specimens, 403 non-type specimens and 106 specimens of unknown status. More than 120 specimens from the Philippines and Malaysia have not even been identified.

High throughput sequencing is increasingly being used by biological laboratories associated with field of plants in routine practice. DNA metabarcoding has been actively developing recently, allowing qualitative (to the level of species or genus for some taxa) analyze of complex biological mixes. It employs high-throughput sequencing (HTS) and comparative analysis of specific DNA sequences called "DNA barcodes" to discriminate species present in the mix. DNA barcoding has been widely used in various areas of botanical research, for example authentication and identification of medicinal or archeological plants [1, 2].

*Methods and Algorithms*: DNA from 122 herbarium samples (Cuming's collection from the Philippines and Malaysia) was extracted using modified CTAB-methods [3]. After quality (including DNA fragment length analysis) and quantity evaluation DNA samples were purified using AmPure magnetic beads (Beckman Counter, UK). A simplified two-step PCR using primers for DNA barcodes (ITS1 and ITS2) fused with Illumina adaptor sequences was performed for DNA library preparation as described elsewhere [4, 5]. Products of the first PCR for each barcode were mixed equimolar (or by volume if product concentration was below detection level) for each sample, indexed in the second PCR, and sequenced on the MiSeq platform with the MiSeq Reagent Kit v3 for 600 cycles (2 × 300 nt paired-end) (Illumina, USA).

Raw sequencing reads were trimmed using the trimmomatic software v.0.38 [6]. *De novo* assembled contigs (SPAdes 3.14) using for determinate of taxonomic classification by BLAST-based bioinformatic pipeline.

*Results*: The average concentration of the extracted DNA was 10.02 ng/μL (min 0,1 ng/μL, max 43,8 ng/μL). The average length of DNA fragments was in the 15–150 bp range and 38 % of samples contained DNA fragments about 2,500 bp long.
We were able to prepare the amplification product of the ITS1 and ITS2, the yield of which allowed us to use it for the preparation of libraries, for 59 samples. The amplification of ITS1 was more successful. The product was obtained for 59 samples, while ITS2 amplification was worse: only 29 samples were able to obtain the product. For these samples, the product corresponding to both (ITS1 and ITS2) marker was obtained as a result. There were no samples for which only the ITS2 was obtained. After treatment the sequencing data, assembled contigs length of 44 samples corresponded to the length of the product obtained. However, for 15 samples, it was not possible to assemble fragments using the applied pipelines. It is possible that the use of other sample assembled methods will make it possible to obtain DNA fragments suitable for further analysis.
*Conclusion*: Thus, we can say that ITS1 and ITS2 DNA metabarcoding can be used even for samples that have been stored for more than 185 years, even though DNA becomes highly fragmented during storage.
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