

Ancient Marine Metabarcoding – A new approach of stomach and microbiome analysis for historical fish

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The Antarctic and the surrounding Southern Ocean contain delicate and unique ecosystems. Despite its distance to congested areas, human influences on the ecosystems include direct impacts such as commercial fishing, tourism and research, as well as indirect impacts such as global warming or pollution. With the exception of fishing, the major increase of these impacts occurred within the last 100 years. There is a vast abundance of museum specimen stored in Natural History Museum (NHM). As such, it would be of major interest to make use of museum collections to potentially identify the impacts of such drivers. However, these samples are mainly inaccessible for genetic work based on the fact that they were for a prolonged amount of time stored in formalin. Over time formalin forms formic acid which hydrolyses DNA. Furthermore, formalin forms crosslinks between DNA strands and proteins. Here 8 different protocols are tested that should reverse the DNA hydrolysis and break the crosslinks between DNA and proteins, without causing too much fragmentation. We collected stomachs and guts from 400 specimens of the genus *Trematomus* from the Natural History Museum London. Specimen were between 20 and 120 years old, fixed in formalin and later transferred to ethanol. We evaluated various protocols for the suitability to infer sequence data from historically formalin-fixed samples and thus identified the interspecific prey and microbiome variation within the genus *Trematomus*. A 313 bp fragment of the cytochrome c oxidase subunit I (COI) was amplified and sequenced for prey item identification in the stomach and a 463 bp region of the 16S gene to investigate microbiome composition in the gut system. We were only able to obtain sequence data for ~20% of the samples for 16S and COI. Facilitating molecular analyses of museum stored fish holds enormous potential for microevolutionary insights that can benefit current efforts to prioritize conservation units in the Southern Ocean. However, potential benefits have to be put in contrast to high laboratory costs as well as high dropout rate of samples at every stage. Here, we show a case where both, prey item and microbiome information was obtained from NHM samples.

Keywords: Natural History Museum; metabarcoding; COI; 16S; prey item; microbiome