Historical DNA Barcoding of trematomid fishes using museum samples

Heindler Franz M.[1], Bart Hellemans[1], Henrik Christiansen[1], Anton Van de Putte[2], Gregory E. Maes[3] and Filip A. M. Volckaert[1]

[1] KU Leuven, Laboratory of Biodiversity and Evolutionary Genomics, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium
E-mail: franzmaximilian.heindler@kuleuven.be
[2] Royal Belgian Institute of Natural Sciences, OD Nature, Vautiersstraat 29, B-1000 Brussels, Belgium
[3] KU Leuven, Laboratory of Cytogenetics and Genome Research, Centre for Human Genetics, Genomics Core, Herestraat 49, PO Box 602, B-3000 Leuven, Belgium

The Antarctic and the surrounding Southern Ocean contain delicate and unique ecosystems, characterised by cold climate, seasonal photoperiods and remote location. 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. These drivers may have left imprints on the genetic structure. It has been argued that microevolution, evolution over an ecological timescale (<100 years), might be a yet underappreciated method of species to adapt to rapidly changing environments. Therefore, the adaptive potential of many species might be greatly underestimated or simply yet unknown. Purely ecological approaches often overpredict extinction risk and therefore rapid evolutionary processes should be taken into consideration in parallel. This is especially true when assessing the outcomes of migration into refugia during the Last Glacial Maximum and in in the context of predictive climate change scenarios.

This study focuses on species of the genus *Trematomus*, which are amongst the most abundant members of the Antarctic icefish community. The genus is not exploited commercially and has a circum-continental habitat range. *Trematomus* specimens collected during various research expeditions are preserved in natural history collections around the world.

Historical samples of marine species, however, are commonly preserved in formaldehyde which poses additional challenges. Over time, formaldehyde forms formic acid, which hydrolyses the DNA. Furthermore, formaldehyde causes the creation of crosslinks between DNA and proteins, which complicates/hampers DNA extraction.

We collected tissue from 400 specimens of the genus *Trematomus* from the Natural History Museum London. Specimen were between 20 and 100 years old, fixed in formalin and later transferred to ethanol. Tissue samples were taken from different regions (fin, muscle, liver) and DNA extraction was possible. A 450 bp fragment of the cytochrome c oxidase subunit I (COI) was amplified and sequenced to investigate if unscathed DNA fragments were successfully obtained. We thus evaluate various protocols for the suitability to infer sequence data from historically formalin-fixed samples and, based on results with one marker (COI), identify samples amenable to subsequent sequence-capture approaches, where multiple markers of interest can be analysed simultaneously. 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.