

Determination of the diversity of aquatic oligochaetes using DNA barcodes for the evaluation of the quality of fine sediment: a promising way

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Introduction: Oligochaetes are a common group of freshwater benthic invertebrates known to be sensitive to environmental changes. In Switzerland, they are used to assess the ecological quality of river and lake sediments in several cantons [1,2]. More extensive application of such indicators would require overcoming the difficulties related to the morphology-based identification of species. To circumvent this problem, genetic approaches for identifying species in biomonitoring are of great interest [3]. In this aim, a database of COI (cytochrome c oxidase subunit I) sequences of aquatic oligochaetes collected in Switzerland was established in 2012 [3]. Out of 185 sequenced specimens, 41 lineages were found, corresponding to 26 morpho-species, to unidentified species and to cryptic species within common species. In addition, the next-generation sequencing (NGS)-based metabarcoding was tested on samples composed of sorted oligochaete specimens. The results showed a good ability of NGS to detect the species present in the samples [4]. The objectives of this study were to 1) Continue to develop our COI barcode database of aquatic oligochaetes by analyzing specimens collected in rivers and lakes in different regions of Switzerland, 2) Compare morphology and NGS-based approaches to determine the species composition and 3) Evaluate the biological quality of sediments on the basis of the morphological and the NGS data.

Methods: 1) 160 oligochaete specimens were sampled in watercourses of the canton of Vaud and in the lake of Geneva. They were identified morphologically to the species level and DNA from each specimen was extracted using guanidine thiocyanate. The COI gene was amplified and sequenced using LCO 1490 and HCO 2198 primers. 2) Comparison between morphology and NGS-based approaches was performed on sediments from 10 sites. Each sample was sieved, then 100 specimens per sample were sorted for each analysis (morphology and NGS). For NGS analysis, a small part of each specimen was cut in a similar size and all the parts of a given sample were pooled. DNA was extracted from each pool using Qiagen, the COI gene

was amplified with the same primers as above and COI was sequenced using Illumina/Miseq technology. 3) The oligochaete index (IOBS) [5] was calculated using both, the morphological and genetic data.

Results: 1) The COI database was enriched with 27 new lineages. They corresponded to 10 morphospecies, to 3 cryptic species within common morphospecies and to unidentified species within the genera *Fridericia*, *Marionina*, *Achaeta*, *Lumbricillus* and *Tubifex*. Out of these 27 lineages, only five were present in Genbank database. 2) Per site, NGS allowed to detect more species or as many species as the morphological analysis. The absence of detection of some species by NGS could be partly explained by the lack of barcode (in our database and in Genbank) for these species. The majority of species detected by NGS and not by morphology corresponded to cryptic species. 3) For each sample, the ecological diagnostic established on the basis of the morphological and NGS data were generally in good agreement.

Discussion: This study confirms the potential of NGS to identify species in oligochaete communities and to assess the biological quality of sediments. The next steps of this research will be to pursue the enrichment of the COI database and to develop a NGS-based oligochaete index based on the direct analysis of sediment samples. The new Interreg Project "SYNAQUA" (2017-2018) should help to achieve these objectives.

References: [1] Flück et al. (2012) *Aqua & Gas* **4**: 18-22; [2] Vivien et al. (2015) *Archives des Sciences* **68**:105-116; [3] Vivien et al. (2015) *PLoS ONE* **10** (4) e0125485; [4] Vivien et al. (2016) *PLoS ONE* **11**(2):e0148644; [5] AFNOR (2016) NF T90-393.