

Determination of the diversity of aquatic oligochaetes using DNA barcodes for the evaluation of the quality of fine sediment

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Background

Improving practical aspects of biomonitoring...

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].

What has been already initiated for oligochaetes...

- ✓ A database of COI (cytochrome c oxidase subunit I) sequences of aquatic oligochaetes collected in Switzerland was established in 2012 [3] (Fig. 1).
- ✓ Next-generation sequencing (NGS)-based metabarcoding was tested on sorted oligochaete specimens. The results showed a good ability of NGS to detect the species present [4].

Objectives of the study...

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.
3. Assess the suitability of NGS-based index to evaluate the biological quality of sediments.

Implementation and Results

1 COI Database - 160 oligochaete specimens were sampled in watercourses of the canton of Vaud and in Lake 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.

- The COI database (Fig.1) 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.

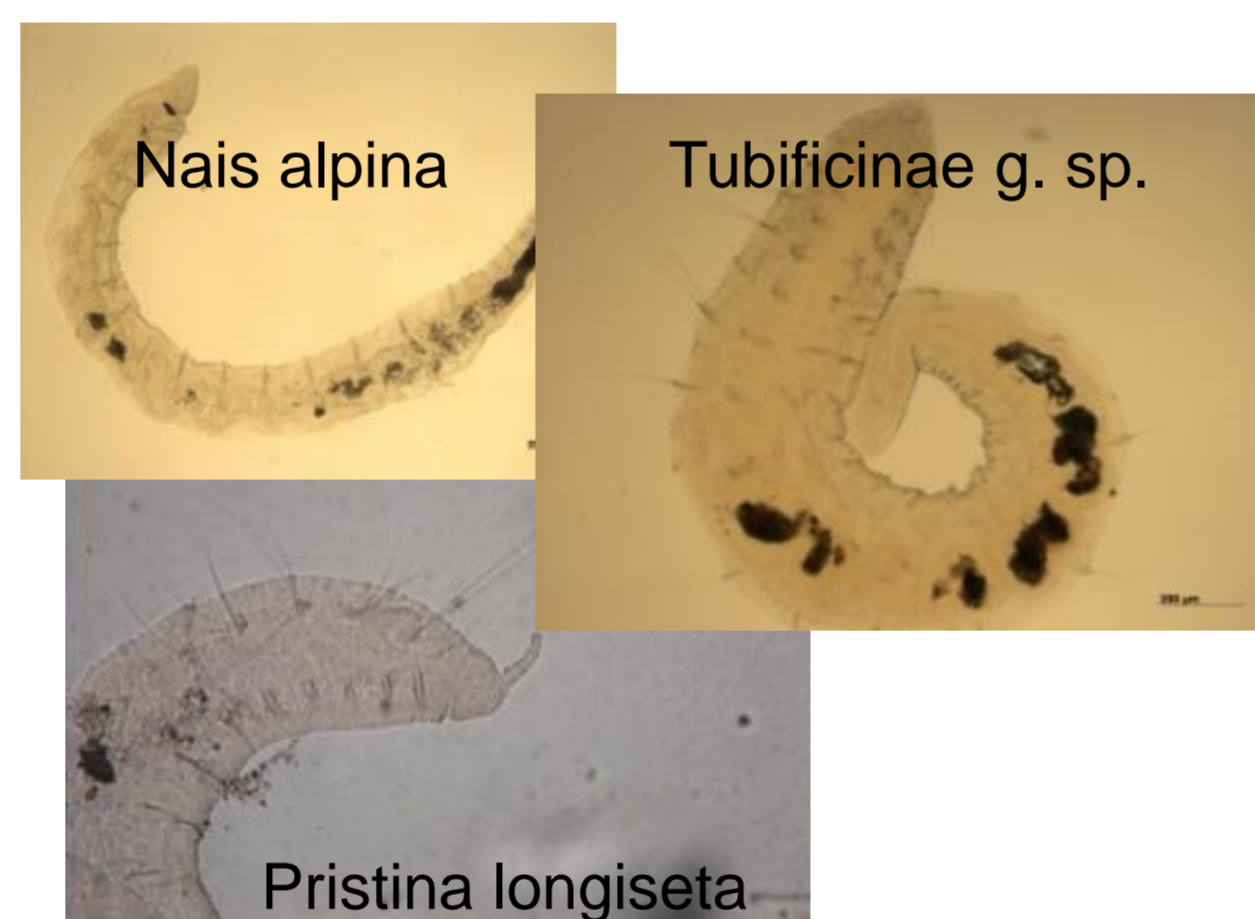
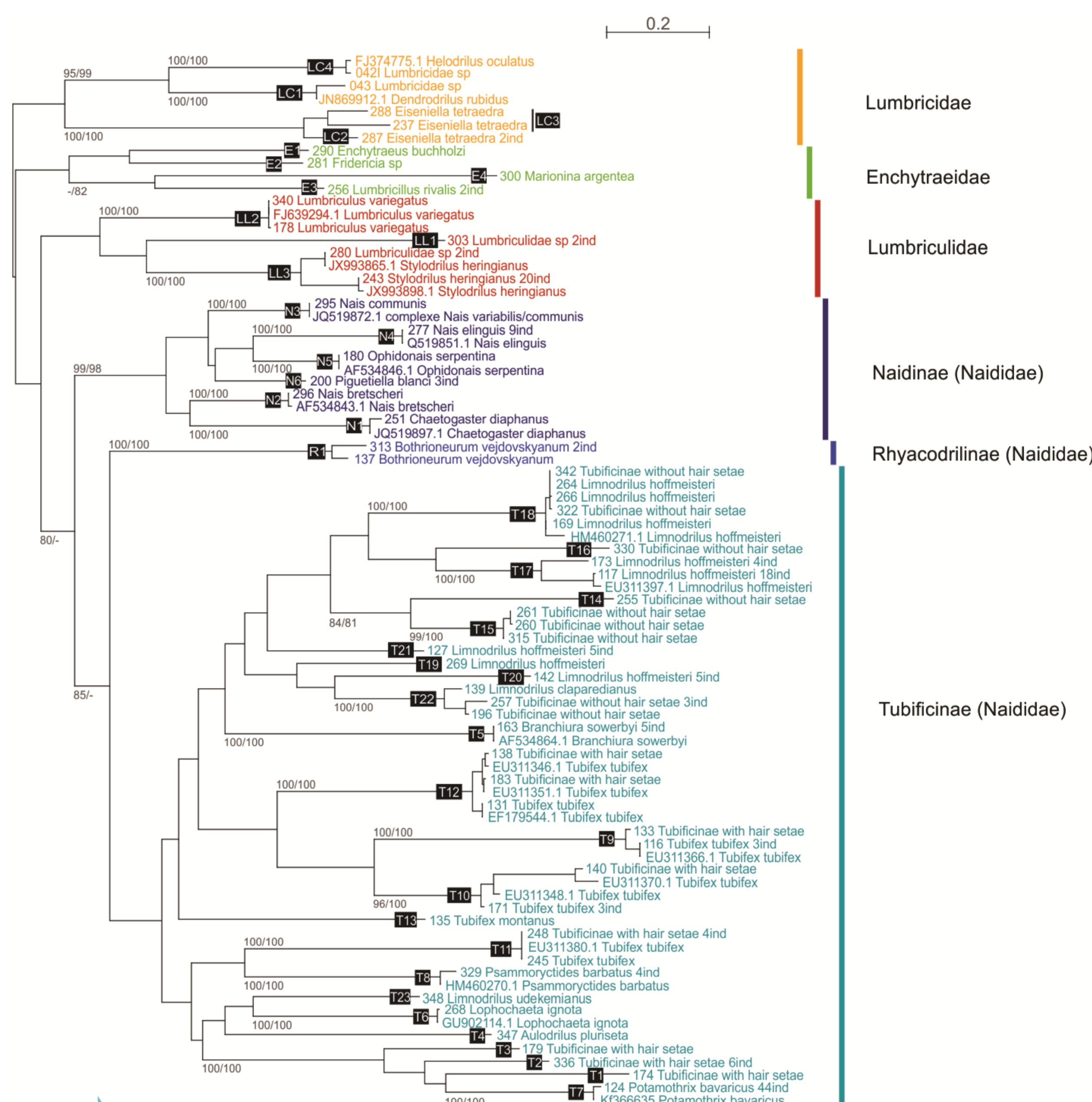


Fig. 1. Phylogenetic tree based on COI gene sequences : out of 185 sequenced specimens, 41 lineages were found, corresponding to 26 morphospecies, to unidentified species and to cryptic species within common morphospecies



2 Morphology / NGS analysis - Sediments from 10 sites were sieved and 100 specimens per sample were sorted for each analysis (morphology and NGS). For NGS analysis of a given sample, a small part of each specimen (similar sizes) was cut and all the parts 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.

- Per site, NGS allowed to detect more species or as many species as the morphological analysis (Tab. 2).
- 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.

Tab. 2. Number of taxa retrieved (tot=total)

Site	River	Morpho tot	Genetic tot	Morpho only	Genetic only	Morpho + Genetic
De Haller	Seymaz	7	17	2	12	5
Bourdigny	Avril	7	13	5	11	2
site 2	Venoge	7	10	2	5	5
site 1	Venoge	7	7	3	3	4
Abbaye	Talent	6	9	0	3	6
site 1	Cul-des-Prés	3	4	0	1	3
L'isle	Venoge	9	8	6	5	3
source	Tinière	9	7	5	3	4
downstre am	Boiron	10	10	5	5	5
EPFL	Sorge	16	17	9	10	7

3 Oligochaete Index - IOBS

Proof of concept: The oligochaete index (IOBS) was calculated using both, the morphological and genetic data according to [4] and [5].

Tab. 3. IOBS values (in blue : very good quality ; in green : good ; in yellow : medium ; in orange : poor ; in red : bad)

Site	De Haller	Bourdigny	Site 2	site 1	Abbaye	site 1	L'isle	source	downstream	EPFL
River	Seymaz	Avril	Venoge	Venoge	Talent	CDP	Venoge	Tinière	Boiron	Sorge
IOBS morpho	2	2.2	1	1	1.4	0.7	9.1	60	2.2	8.2
IOBS NGS	2.6	3.6	2.1	1.4	1.8	0.8	100	9.9	2.2	4.4

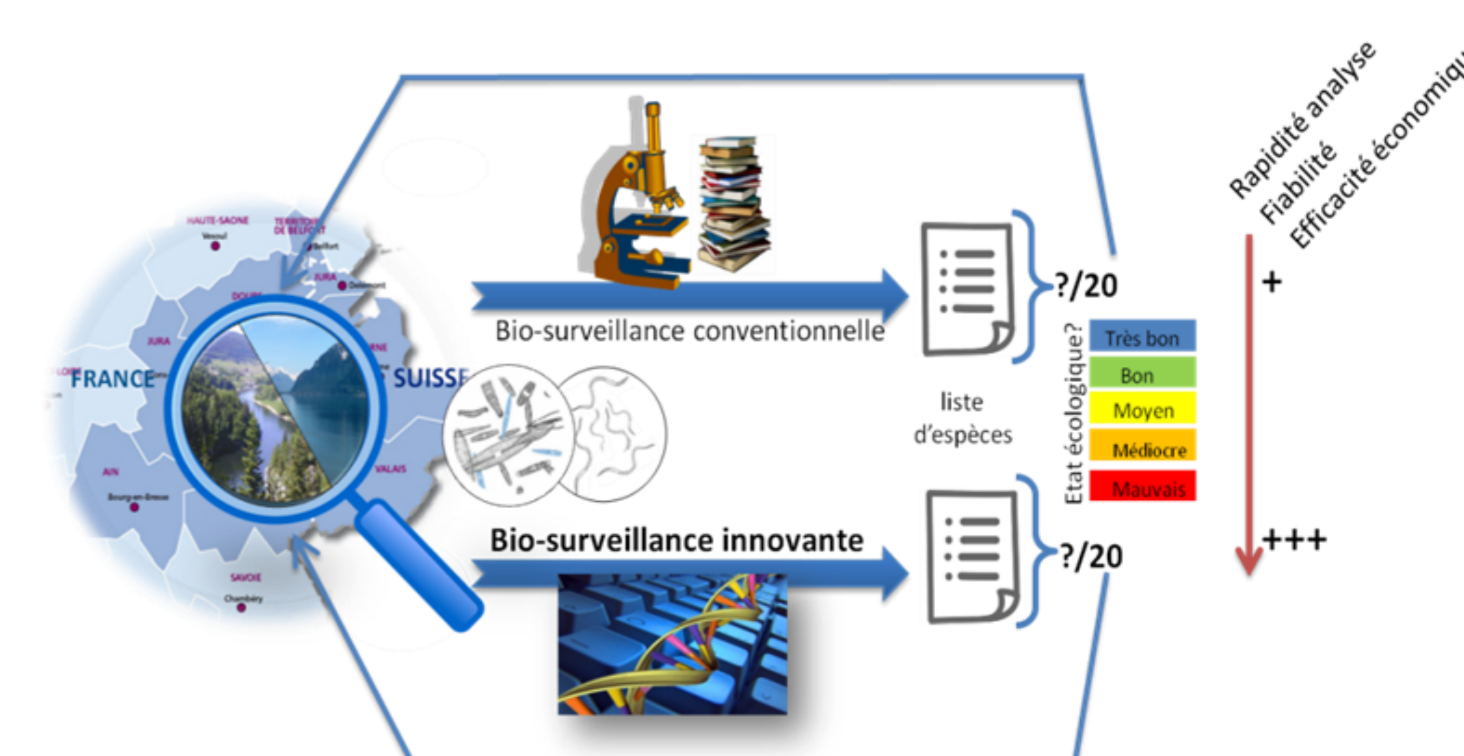
- For each sample, the ecological diagnostic established on the basis of the morphological and NGS data were generally in good agreement (Tab. 3).

Conclusions and Perspectives

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 expansion of the COI database and to develop a NGS-based oligochaete index based on the direct analysis of sediment samples.

Forthcoming...

Interreg project "SYNAQUA"
"SYNergie transfrontalière pour la bio-surveillance et la préservation des écosystèmes AQUatiques"



References:

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