

## Supplementary Materials for

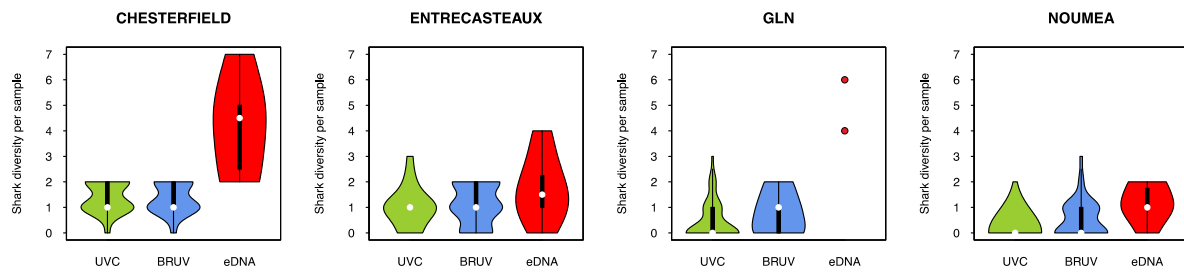
### **Environmental DNA illuminates the dark diversity of sharks**

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**fig. S1. Number of shark species per sample in overlapping collection sites.** Violin plot showing detected shark species richness by the different methods in Chesterfield, Entrecasteaux, GLN and Nouméa. Only two eDNA samples were collected in GLN (red dots). White dots are mean values; thick black bars correspond to interquartile ranges; thin black lines are 95% confidence intervals.

**table S1. Full sequences of the 24 tagged primer sets used.** The primer mix for each PCR included the reverse primer and an equimolar mixture of the two forward primers, all tagged with the same 8-bp tag (in lowercase in this table). A variable number of fully degenerate positions (Ns) was added at the beginning of each primer, to increase sequence diversity.

Reverse primers		Forward primers 1		Forward primers 2	
Shark-COI-MINIR_tag01	NNNNaacaagccAAGATTACAAAAGCGTGGGC	FishF1_tag01	NNaacaagccTCAACCAACCACAAAGACATTGGCAC	FishF2_tag01	NNaacaagccTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag02	NNNggaatgagAAGATTACAAAAGCGTGGGC	FishF1_tag02	NNNggaatgagTCAACCAACCACAAAGACATTGGCAC	FishF2_tag02	NNNggaatgagTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag03	NNNaattgccgAAGATTACAAAAGCGTGGGC	FishF1_tag03	NNNNaattgccgTCAACCAACCACAAAGACATTGGCAC	FishF2_tag03	NNNNaattgccgTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag04	NNNncgaccataAAGATTACAAAAGCGTGGGC	FishF1_tag04	NNcgaccataTCAACCAACCACAAAGACATTGGCAC	FishF2_tag04	NNcgaccataTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag05	NNNnatgetgacAAGATTACAAAAGCGTGGGC	FishF1_tag05	NNNnatgetgacTCAACCAACCACAAAGACATTGGCAC	FishF2_tag05	NNNnatgetgacTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag06	NNNgagacagAAGATTACAAAAGCGTGGGC	FishF1_tag06	NNNngagacagTCAACCAACCACAAAGACATTGGCAC	FishF2_tag06	NNNngagacagTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag07	NNNngagcttacAAGATTACAAAAGCGTGGGC	FishF1_tag07	NNngagcttacTCAACCAACCACAAAGACATTGGCAC	FishF2_tag07	NNngagcttacTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag08	NNNttaccaggAAGATTACAAAAGCGTGGGC	FishF1_tag08	NNNttaccaggTCAACCAACCACAAAGACATTGGCAC	FishF2_tag08	NNNttaccaggTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag09	NNNgagagctAAGATTACAAAAGCGTGGGC	FishF1_tag09	NNNngagagctTCAACCAACCACAAAGACATTGGCAC	FishF2_tag09	NNNngagagctTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag10	NNNnctgacctAAGATTACAAAAGCGTGGGC	FishF1_tag10	NNnctgacctTCAACCAACCACAAAGACATTGGCAC	FishF2_tag10	NNnctgacctTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag11	NNNnatgcttggAAGATTACAAAAGCGTGGGC	FishF1_tag11	NNNnatgcttggTCAACCAACCACAAAGACATTGGCAC	FishF2_tag11	NNNnatgcttggTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag12	NNNaaccctgAAGATTACAAAAGCGTGGGC	FishF1_tag12	NNNnaaccctgTCAACCAACCACAAAGACATTGGCAC	FishF2_tag12	NNNnaaccctgTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag13	NNNntaccgetAAGATTACAAAAGCGTGGGC	FishF1_tag13	NNntaccgetTCAACCAACCACAAAGACATTGGCAC	FishF2_tag13	NNntaccgetTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag14	NNNccagtatgAAGATTACAAAAGCGTGGGC	FishF1_tag14	NNNccagtatgTCAACCAACCACAAAGACATTGGCAC	FishF2_tag14	NNNccagtatgTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag15	NNNgagatgcAAGATTACAAAAGCGTGGGC	FishF1_tag15	NNNngagatgcTCAACCAACCACAAAGACATTGGCAC	FishF2_tag15	NNNngagatgcTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag16	NNNngtcaactAAGATTACAAAAGCGTGGGC	FishF1_tag16	NNngtcaactTCAACCAACCACAAAGACATTGGCAC	FishF2_tag16	NNngtcaactTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag17	NNNnacaaccgaAAGATTACAAAAGCGTGGGC	FishF1_tag17	NNnacaaccgaTCAACCAACCACAAAGACATTGGCAC	FishF2_tag17	NNnacaaccgaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag18	NNNngacctaAAGATTACAAAAGCGTGGGC	FishF1_tag18	NNNngacctaTCAACCAACCACAAAGACATTGGCAC	FishF2_tag18	NNNngacctaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag19	NNNnatggaggTAAAGATTACAAAAGCGTGGGC	FishF1_tag19	NNnatggaggTCAACCAACCACAAAGACATTGGCAC	FishF2_tag19	NNnatggaggTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag20	NNNtcatagcAAGATTACAAAAGCGTGGGC	FishF1_tag20	NNNtcatagcTCAACCAACCACAAAGACATTGGCAC	FishF2_tag20	NNNtcatagcTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag21	NNNctgagtctAAGATTACAAAAGCGTGGGC	FishF1_tag21	NNNctgagtctTCAACCAACCACAAAGACATTGGCAC	FishF2_tag21	NNNctgagtctTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag22	NNNngagtgaaAAGATTACAAAAGCGTGGGC	FishF1_tag22	NNngagtgaaTCAACCAACCACAAAGACATTGGCAC	FishF2_tag22	NNngagtgaaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag23	NNNggcatgtaAAGATTACAAAAGCGTGGGC	FishF1_tag23	NNNggcatgtaTCAACCAACCACAAAGACATTGGCAC	FishF2_tag23	NNNggcatgtaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag24	NNNgtgccataAAGATTACAAAAGCGTGGGC	FishF1_tag24	NNNgtgccataTCAACCAACCACAAAGACATTGGCAC	FishF2_tag24	NNNgtgccataTCGACTAATCATAAAGATATCGGCAC

**table S2. Metabarcoding pipeline for COI Elasmobranchii Fields *et al.* primers.**

<p><b>1. Paired-end alignment. Keep reads with quality &gt; 40. Demultiplexing.</b></p> <pre>illuminapairedend -r SHAK_S1_L001_R2_001.fastq SHAK_S1_L001_R1_001.fastq   obiannotate -S goodali:"Good_SHAK" if score&gt;40.00 else "Bad_SHAK"   obisplit -t goodali</pre> <pre>ngsfilter -t ngsfilter_SHAK_fields.tsv --fasta-output -u unidentified_SHAK.fasta Good_SHAK.fasta &gt; SHAK.filtered.fasta</pre>
<p><b>2. Filter sequences with lengths between 120 and 135 bp and with only 'ACGT'.</b></p> <pre>obigrep -p 'seq_length&gt;120' -p 'seq_length&lt;135' -s '^[ACGT]+\$' SHAK.filtered.fasta &gt; SHAK.filtered_length.fasta</pre>
<p><b>3. Group unique seqs.</b></p> <pre>obiuniq -m sample SHAK.filtered_length.fasta &gt; SHAK.unique.fasta</pre>
<p><b>4. Change ids to a short index. Change format to vsearch. Remove chimeras.</b></p> <pre>obiannotate --seq-rank SHAK.unique.fasta   obiannotate --set-identifier "'SHAK'%09d" % seq_rank' &gt; SHAK.new.fasta</pre> <pre>owi_obifasta2vsearch -i SHAK.new.fasta -o SHAK.vsearch.fasta</pre> <pre>vsearch --uchime_denovo SHAK.vsearch.fasta --sizeout --nonchimeras SHAK.nonchimeras.fasta --chimeras SHAK.chimeras.fasta --uchimeout SHAK.uchimeout.txt</pre>
<p><b>5. Cluster at 99% with sumacust. Get cluster centers.</b></p> <pre>sumacust -t 0.99 -s count -p 10 SHAK.nonchimeras.fasta &gt; SHAK.sumacust99.fasta</pre> <pre>obigrep -p 'cluster_center' SHAK.sumacust99.fasta &gt; SHAK.sumacust99.centers.fasta</pre>
<p><b>6. Taxonomic assignment using ecotag.</b></p> <pre>ecotag -d taxo_sharks -R db_Elasmobranchii_Bakker_et_al_2017.fasta SHAK.sumacust99.centers.fasta &gt; SHAK.ecotag.fasta</pre>
<p><b>7. Add taxa above order level.</b></p> <pre>owi_add_taxonomy -i SHAK.ecotag.fasta -o SHAK.ecotag.fasta.annotated.csv</pre>
<p><b>8. Recount abundances by sample.</b></p> <pre>obitab -o SHAK.sumacust99.fasta &gt; SHAK.sumacust99.tab</pre> <pre>owi_recount_sumacust -i SHAK.sumacust99.tab -o SHAK.sumacust99.counts.csv</pre>
<p><b>9. Combine ecotag and abundance files.</b></p> <pre>owi_combine -i SHAK.ecotag.fasta.annotated.csv -a SHAK.sumacust99.counts.csv -o SHAK_all_MOTUs.csv</pre>
<p><b>10. Collapse MOTUs.</b></p> <pre>owi_collapse -s 13 -e 88 -i SHAK_all_MOTUs.csv</pre>
<p><b>11. Curate the dataset manually.</b></p>
<p><b>12. Re-collapse MOTUs after curating.</b></p> <pre>owi_collapse -s 13 -e 88 -i SHAK_all_MOTUs_curated.csv</pre>