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Supplementary Materials for

Environmental DNA illuminates the dark diversity of sharks

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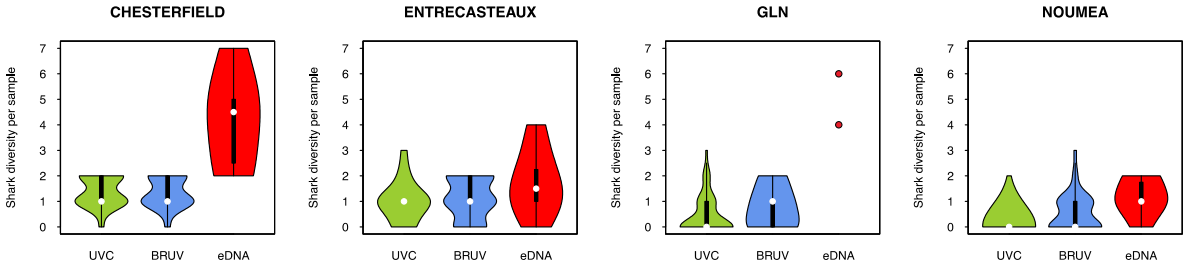

fig. S1. Number of shark species per sample in overlapping collection sites. Violin plot showing detected shark species richness by the different methods in Chesterfield, Entrecasteaux, GLN and Nouméa. Only two eDNA samples were collected in GLN (red dots). White dots are mean values; thick black bars 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	NNaattgccgAAGATTACAAAAGCGTGGGC	**FishF1_tag03**	NNNNaattgccgTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag03**	NNNNaattgccgTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag04	NNNNcgaccataAAGATTACAAAAGCGTGGGC	**FishF1_tag04**	NNcgaccataTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag04**	NNcgaccataTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag05	NNNatgctgacAAGATTACAAAAGCGTGGGC	**FishF1_tag05**	NNNatgctgacTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag05**	NNNatgctgacTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag06	NNtgagacagAAGATTACAAAAGCGTGGGC	**FishF1_tag06**	NNNNtgagacagTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag06**	NNNNtgagacagTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag07	NNNNgagcttacAAGATTACAAAAGCGTGGGC	**FishF1_tag07**	NNgagcttacTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag07**	NNgagcttacTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag08	NNNttaccaggAAGATTACAAAAGCGTGGGC	**FishF1_tag08**	NNNttaccaggTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag08**	NNNttaccaggTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag09	NNtgagagctAAGATTACAAAAGCGTGGGC	**FishF1_tag09**	NNNNtgagagctTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag09**	NNNNtgagagctTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag10	NNNNctgaccttAAGATTACAAAAGCGTGGGC	**FishF1_tag10**	NNctgaccttTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag10**	NNctgaccttTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag11	NNNatgcttggAAGATTACAAAAGCGTGGGC	**FishF1_tag11**	NNNatgcttggTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag11**	NNNatgcttggTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag12	NNaacaccgtAAGATTACAAAAGCGTGGGC	**FishF1_tag12**	NNNNaacaccgtTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag12**	NNNNaacaccgtTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag13	NNNNttaccgctAAGATTACAAAAGCGTGGGC	**FishF1_tag13**	NNttaccgctTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag13**	NNttaccgctTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag14	NNNccagtatgAAGATTACAAAAGCGTGGGC	**FishF1_tag14**	NNNccagtatgTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag14**	NNNccagtatgTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag15	NNtgagatgcAAGATTACAAAAGCGTGGGC	**FishF1_tag15**	NNNNtgagatgcTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag15**	NNNNtgagatgcTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag16	NNNNgtgcaactAAGATTACAAAAGCGTGGGC	**FishF1_tag16**	NNgtgcaactTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag16**	NNgtgcaactTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag17	NNNacaaccgaAAGATTACAAAAGCGTGGGC	**FishF1_tag17**	NNNacaaccgaTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag17**	NNNacaaccgaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag18	NNtgagcctaAAGATTACAAAAGCGTGGGC	**FishF1_tag18**	NNNNtgagcctaTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag18**	NNNNtgagcctaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag19	NNNNatggaggtAAGATTACAAAAGCGTGGGC	**FishF1_tag19**	NNatggaggtTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag19**	NNatggaggtTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag20	NNNtcatacgcAAGATTACAAAAGCGTGGGC	**FishF1_tag20**	NNNtcatacgcTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag20**	NNNtcatacgcTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag21	NNctgagtctAAGATTACAAAAGCGTGGGC	**FishF1_tag21**	NNNNctgagtctTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag21**	NNNNctgagtctTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag22	NNNNgaggtgaaAAGATTACAAAAGCGTGGGC	**FishF1_tag22**	NNgaggtgaaTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag22**	NNgaggtgaaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag23	NNNggcatgtaAAGATTACAAAAGCGTGGGC	**FishF1_tag23**	NNNggcatgtaTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag23**	NNNggcatgtaTCGACTAATCATAAAGATATCGGCAC
Shark-COI-MINIR_tag24	NNgtgccataAAGATTACAAAAGCGTGGGC	**FishF1_tag24**	NNNNgtgccataTCAACCAACCACAAAGACATTGGCAC	**FishF2_tag24**	NNNNgtgccataTCGACTAATCATAAAGATATCGGCAC

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

1. Paired-end alignment. Keep reads with quality > 40. Demultiplexing.

```
illuminapairedend -r SHAK_S1_L001_R2_001.fastq SHAK_S1_L001_R1_001.fastq | obiannotate -S
goodali:'"Good_SHAK" if score>40.00 else "Bad_SHAK"' | obisplit -t goodali

ngsfilter -t ngsfilter_SHAK_fields.tsv --fasta-output -u unidentified_SHAK.fasta
Good_SHAK.fasta > SHAK.filtered.fasta
```

2. Filter sequences with lengths between 120 and 135 bp and with only 'ACGT'.

```
obigrep -p 'seq_length>120' -p 'seq_length<135' -s '^[ACGT]+$' SHAK.filtered.fasta >
SHAK.filtered_length.fasta
```

3. Group unique seqs.

```
obiuniq -m sample SHAK.filtered_length.fasta >  SHAK.unique.fasta
```

4. Change ids to a short index. Change format to vsearch. Remove chimeras.

```
obiannotate --seq-rank SHAK.unique.fasta | obiannotate --set-identifier '"'SHAK'%09d" %
seq_rank' > SHAK.new.fasta
owi_obifasta2vsearch -i SHAK.new.fasta -o SHAK.vsearch.fasta
vsearch --uchime_denovo SHAK.vsearch.fasta --sizeout --nonchimeras SHAK.nonchimeras.fasta --
chimeras SHAK.chimeras.fasta --uchimeout SHAK.uchimeout.txt
```

5. Cluster at 99% with sumaclust. Get cluster centers.

```
sumaclust -t 0.99 -s count -p 10 SHAK.nonchimeras.fasta >  SHAK.sumaclust99.fasta

obigrep -p 'cluster_center' SHAK.sumaclust99.fasta >  SHAK.sumaclust99.centers.fasta
```

6. Taxonomic assignment using ecotag.

```
ecotag -d taxo_sharks -R db_Elasmobranchii_Bakker_et_al_2017.fasta
SHAK.sumaclust99.centers.fasta > SHAK.ecotag.fasta
```

7. Add taxa above order level.

```
owi_add_taxonomy -i SHAK.ecotag.fasta -o SHAK.ecotag.fasta.annotated.csv
```

8. Recount abundances by sample.

```
obitab -o SHAK.sumaclust99.fasta >  SHAK.sumaclust99.tab
owi_recount_sumaclust -i SHAK.sumaclust99.tab -o SHAK.sumaclust99.counts.csv
```

9. Combine ecotag and abundance files.

```
owi_combine -i SHAK.ecotag.fasta.annotated.csv -a SHAK.sumaclust99.counts.csv -o
SHAK_all_MOTUs.csv
```

10. Collapse MOTUs.

```
owi_collapse -s 13 -e 88 -i SHAK_all_MOTUs.csv
```

11. Curate the dataset manually.

12. Re-collapse MOTUs after curating.

```
owi_collapse -s 13 -e 88 -i SHAK_all_MOTUs_curated.csv
```