# Supplementary material

## Supplementary material 1: Sampling protocol

**Table S1.** Overview of eDNA samples collected along the Provence coast of the North-Western Mediterranean Sea. The sample names (XYa\_b) represent individual names for each filter according to the study region (X: I = Indian Ocean, M = Mediterranean Sea), sampling method (Y: A = Athena, N = Niskin, S = Submersible), individual stations (a), and the number of replicates within a station (b).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Provence coast | | | | | | | | |
| Name | **Replicate** | **Method** | **Estimated sampling volume [L]** | **Depth [m]**  **(Mean depth)** | **Category** | **Station** | **Date (yyyy/mm/dd)** | **Starting time of sampling**  **(hh:mm)** |
| MA1\_1 | 1 | Athena | ~ 30 | 0 | Surface | Beach Rock Cassis 5 | 2019/07/03 | 14:18 |
| MA1\_2 | 2 | Athena | ~ 30 | 0 | Surface | Beach Rock Cassis 5 | 2019/07/03 | 14:18 |
| MA1\_3 | 3 | Athena | ~ 30 | 0 | Surface | Beach Rock Cassis 5 | 2019/07/03 | 15:13 |
| MA2\_1 | 1 | Athena | ~ 30 | 0 | Surface | Cap Lardier 1 | 2019/07/08 | 08:45 |
| MA2\_2 | 2 | Athena | ~ 30 | 0 | Surface | Cap Lardier 1 | 2019/07/08 | 08:45 |
| MA2\_3 | 3 | Athena | ~ 30 | 0 | Surface | Cap Lardier 1 | 2019/07/08 | 09:37 |
| MA3\_1 | 1 | Athena | ~ 30 | 0 | Surface | Cap Negre 1 | 2019/07/19 | 08:51 |
| MA3\_2 | 2 | Athena | ~ 30 | 0 | Surface | Cap Negre 1 | 2019/07/19 | 08:51 |
| MA3\_3 | 3 | Athena | ~ 30 | 0 | Surface | Cap Negre 1 | 2019/07/19 | 09:45 |

**Table S1.** (Continued)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Name | Replicate | Method | Estimated sampling volume [L] | Depth [m]  (Mean depth) | Category | Station | Date (yyyy/mm/dd) | Starting time of sampling  (hh:mm) |
| MN1\_1 | 1 | Niskin | ~ 10 | 0 | Surface | Beach Rock Cassis 1 | 2019/07/02 | 15:00 |
| MN2\_1 | 1 | Niskin | ~ 10 | 20 | Mid-depth | Beach Rock Cassis 2 | 2019/07/02 | 15:20 |
| MN3\_1 | 1 | Niskin | ~ 10 | 40 | Mid-depth | Beach Rock Cassis 3 | 2019/07/02 | 15:34 |
| MN4\_1 | 1 | Niskin | ~ 10 | 60 | Mid-depth | Beach Rock Cassis 4 | 2019/07/02 | 16:00 |
| MN5\_1 | 1 | Niskin | ~ 10 | 0 | Surface | Cap Lardier 2 | 2019/07/08 | 14:09 |
| MN5\_2 | 2 | Niskin | ~ 10 | 50 | Mid-depth | Cap Lardier 2 | 2019/07/08 | 14:21 |
| MN5\_3 | 3 | Niskin | ~ 10 | 100 | Deep | Cap Lardier 2 | 2019/07/08 | 14:40 |
| MN5\_4 | 4 | Niskin | ~ 10 | 150 | Deep | Cap Lardier 2 | 2019/07/08 | 15:01 |
| MN5\_5 | 5 | Niskin | ~ 10 | 200 | Deep | Cap Lardier 2 | 2019/07/08 | 16:28 |
| MS1\_1 | 1 | Submersible | ~ 30 | 65-10 (38) | Mid-depth | Imperiaux 1 | 2019/07/01 | 12:06 |
| MS1\_2 | 2 | Submersible | ~ 30 | 65-10 (38) | Mid-depth | Imperiaux 1 | 2019/07/01 | 17:06 |
| MS2\_1 | 1 | Submersible | ~ 30 | 65 | Mid-depth | Beach Rock Cassis 6 | 2019/07/03 | 17:00 |
| MS2\_2 | 2 | Submersible | ~ 30 | 65 | Mid-depth | Beach Rock Cassis 6 | 2019/07/03 | 17:30 |
| MS2\_3 | 3 | Submersible | ~ 30 | 65 | Mid-depth | Beach Rock Cassis 6 | 2019/07/03 | 18:00 |

**Table S1.** (Continued)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Name | Replicate | Method | Estimated sampling volume [L] | Depth [m]  (Mean depth) | Category | Station | Date (yyyy/mm/dd) | Starting time of sampling  (hh:mm) |
| MS3\_1 | 1 | Submersible | ~ 30 | 70 | Mid-depth | Cap Lardier 3 | 2019/07/08 | 13:30 |
| MS3\_2 | 2 | Submersible | ~ 30 | 70 | Mid-depth | Cap Lardier 3 | 2019/07/08 | 14:00 |
| MS3\_3 | 3 | Submersible | ~ 30 | 70 | Mid-depth | Cap Lardier 3 | 2019/07/08 | 14:30 |
| MS4\_1 | 1 | Submersible | ~ 30 | 75 | Mid-depth | Banc de Magaud | 2019/07/06 | 19:00 |
| MS4\_2 | 2 | Submersible | ~ 30 | 75 | Mid-depth | Banc de Magaud | 2019/07/07 | 09:30 |
| MS4\_3 | 3 | Submersible | ~ 30 | 75 | Mid-depth | Banc de Magaud | 2019/07/07 | 10:00 |
| MS5\_1 | 1 | Submersible | ~ 30 | 120 | Deep | Tombant Americain | 2019/07/14 | 09:30 |
| MS5\_2 | 2 | Submersible | ~ 30 | 120 | Deep | Tombant Americain | 2019/07/14 | 10:00 |
| MS5\_3 | 3 | Submersible | ~ 30 | 120 | Deep | Tombant Americain | 2019/07/14 | 10:30 |
| MS6\_1 | 1 | Submersible | ~ 30 | 130 | Deep | Cap Negre 2 | 2019/07/19 | 10:00 |
| MS6\_2 | 2 | Submersible | ~ 30 | 130 | Deep | Cap Negre 2 | 2019/07/19 | 10:30 |
| MS6\_3 | 3 | Submersible | ~ 30 | 130 | Deep | Cap Negre 2 | 2019/07/19 | 11:00 |

**Table S2.** Overview of eDNA samples collected at the seamount La Pérouse in the Western Indian Ocean. The sample names (XYa\_b) represent individual names for each filter according to the study region (X: I = Indian Ocean, M = Mediterranean Sea), sampling method (Y: A= Athena, N = Niskin, S = Submersible), individual stations (a), and the number of replicates within a station (b).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| La Pérouse | | | | | | | | |
| Name | **Replicate** | **Sample method** | **Estimated sampling volume [L]** | **Depth [m]**  **(Mean depth)** | **Category** | **Station** | **Date (yyyy/mm/dd)** | **Time of sampling** |
| IS1\_1 | 1 | Submersible | ~ 30 | 60 | Mid-depth | - | 2019/10/27 | day |
| IS2\_1 | 1 | Submersible | ~ 30 | 62 | Mid-depth | - | 2019/10/31 | night |
| IS3\_1 | 1 | Submersible | ~ 30 | 63 | Mid-depth | - | 2019/11/01 | day |
| IS4\_1 | 1 | Submersible | ~ 30 | 65 | Mid-depth | - | 2019/10/28 | day |
| IS5\_1 | 1 | Submersible | ~ 30 | 65 | Mid-depth | - | 2019/10/29 | day |
| IS6\_1 | 1 | Submersible | ~ 30 | 85 – 135 (110) | Deep | - | 2019/11/05 | day |
| IS7\_1 | 1 | Submersible | ~ 30 | 100 - 125 (113) | Deep | - | 2019/10/31 | night |
| IS8\_1 | 1 | Submersible | ~ 30 | 100 - 140 (120) | Deep | - | 2019/10/30 | day |

## Supplementary material 2: Overview of the changed taxa

**Table S3**. Overview of the taxa in the data set from the Provence coast in the NW Mediterranean Sea (MS) that were adapted as their geographical distribution does not match the sampling area according to FishBase (Froese & Pauly, 2021). Taxa from both bioinformatic pipelines are shown (S = Species pipeline; M = MOTU pipeline).

|  |  |  |  |
| --- | --- | --- | --- |
| Provence coast | | | |
| Taxa in original data set (taxonomic level) | **Taxa in cleaned data set (taxonomic level)** | **Pipeline** | **Comment** |
| *Acanthurus nigrofuscus* (species) | Acanthuriformes (order) | S | Not in MS |
| *Cololabis saira* (species) | Belonidae (family) | S & M | Not in MS |
| *Istiophorus albicans* (species) | *Engraulis* (genus) | S | Erroneous NCBI identification |
| *Abudefduf* (genus) | Pomacentridae (family) | S & M | Unsuitable distributions |
| *Cololabis* (genus) | Belonidae (family) | S | No species available in the MS |
| *Foetorepus* (genus) | Callionymidae (family) | S | No species available in the MS |
| *Gobiusculus* (genus) | Gobiidae (family) | S & M | Unsuitable distributions |
| *Lutjanus* (genus) | Lutjaniformes (order) | S & M | Unsuitable distributions |
| *Speleogobius* (genus) | Gobiidae (family) | S | Unsuitable distributions |
| *Taeniura* (genus) | Myliobatiformes (order) | S | Unsuitable distributions |
| Cichlidae (family) | Ovalentaria (clade) | S & M | No species available in the MS |
| Embiotocidae (family) | Ovalentaria (clade) | S | No species available in the MS |
| Lutjanidae (family) | Lutjaniformes (order) | S | Unsuitable distributions |
| Osphronemidae (family) | [Percomorphaceae](https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Undef&id=1489872&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock) (clade) | S & M | No species available in the MS |
| Pomacentridae (family) | - | S | Wrong taxa assignment |
| Anabantiformes (order) | [Percomorphaceae](https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Undef&id=1489872&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock) (clade) | S & M | No species available in the MS |
| Cichliformes (order) | Ovalentaria (clade) | S | No species available in the MS |

**Table S4**.  Overview of the taxa in the data set from La Pérouse in the Western Indian Ocean that were adapted as their geographical distribution does not match the sampling area according to FishBase (Froese & Pauly, 2021). Taxa from both bioinformatic pipelines are shown (S = Species pipeline; M = MOTU pipeline).

|  |  |  |  |
| --- | --- | --- | --- |
| La Pérouse | | | |
| Taxa in original data set (taxonomic level) | **Taxa in cleaned data set (taxonomic level)** | **Pipeline** | **Comment** |
| *Agonostomus monticola* (species) | *Agonostomus* (genus) | S & M | Not in WIO |
| *Apolemichthys armitagei* (species) | *Apolemichthys trimaculatus* (species) | S & M | Hybrid |
| *Canthigaster coronate*  (species) | *Canthigaster cyanospilota*  (species) | S & M | Species divided into three species |
| *Chromis retrofasciata* (species) | *Chromis* (genus) | S | Not in WIO |
| *Derichthys serpentinus*  (species) | Anguilliformes (order) | S | Not in WIO |
| *Echidna xanthospilos* (species) | *Echidna* (genus) | S & M | Not in WIO |
| *Gymnothorax kidako* (species) | *Gymnothorax* (genus) | S & M | Not in WIO |
| *Lutjanus viridis* (species) | *Lutjanus* (genus) | S & M | Not in WIO |
| *Nannobrachium ritteri* (species) | *Nannobrachium* (genus) | S | Not in WIO |
| *Naso lopezi* (species) | *Naso* (genus) | S & M | Not in WIO |
| *Nemateleotris cf. magnifica NMBE 1066445* (species) | - | S & M | Used information from *N. magnifica* |
| *Scarus schlegeli* (species) | *Scarus* (genus) | S & M | Not in WIO |
| *Sphyrna sp. MV-2009* (species) | *Sphyrna* (genus) | S & M | Not found on FishBase |
| *Xenobalistes tumidipectoris* (species) | *Xenobalistes* (genus) | S | Unsuitable distributions |
| *Holacanthus* (genus) | Pomacanthidae (family) | S & M | No species available in WIO |
| *Stenobrachius* (genus) | Myctophidae (family) | S & M | No species available in WIO |
| Heterenchelyidae (family) | Anguilliformes (order) | S | No species available in WIO |
| Osphronemidae (family) | Percomorphaceae (clade) | S | No species available in WIO |
| Anabantiformes (order) | Percomorphaceae (clade) | S | No species available in WIO |
| Cichliformes (order) | [Ovalentaria](https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Undef&id=1489908&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock) (clade) | S & M | No species available in WIO |
| Salmoniformes (order) | [Euteleosteomorpha](https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Undef&id=1489388&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&lin=f&keep=1&srchmode=1&unlock) (cohort) | S | Unsuitable distributions |

## Supplementary material 3: Overview of the taxonomic findings

**Table S5.** Overview of the taxonomic findings from both bioinformatics pipelines for the North-Western Mediterranean Sea (Provence coast) and the Western Indian Ocean (La Pérouse) datasets.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | Provence coast | | La Pérouse | |
|  | | **MOTU**  **pipeline** | **Species pipeline** | **MOTU**  **pipeline** | **Species pipeline** |
| Nr. of reads | | 7’265’928 | 10’817’609 | 3’157’242 | 5’653’393 |
| Nr. of MOTU’s or sequences | | 113 MOTUs | 811 seq. | 213 MOTUs | 721 seq. |
| Number of taxa (total) | |  |  |  |  |
|  | Species | 30 | 41 | 40 | 57 |
|  | Genus | 46 | 57 | 76 | 102 |
|  | Family | 31 | 43 | 46 | 56 |
|  | Order | 21 | 33 | 30 | 33 |
|  | Higher taxa | 4 | 10 | 8 | 11 |
| Number of taxa (lowest taxonomic rank) | |  |  |  |  |
|  | Species | 30 | 41 | 40 | 57 |
|  | Genus | 25 | 48 | 52 | 83 |
|  | Family | 12 | 25 | 21 | 32 |
|  | Order | 5 | 17 | 13 | 17 |
|  | Higher taxa | 4 | 10 | 8 | 12 |

## Supplementary material 4: Statistical analyses – depth differences

**Table S6.** Welch two-sample t-test to test whether the mean number of reads within a species’ known depth range (x) is equal to the mean number of reads outside a species’ known depth range (y). The alternative hypothesis was that the true difference in means is not equal to 0. The statistical significance criterion was a p-value of 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Welch Two-Sample t-test | | | | | | |
| t | **df** | **p - value** | **Lower**  **95% - CI** | **Upper**  **95% - CI** | **Sample estimates**  **mean of x** | **Sample estimates**  **mean of y** |
| Provence coast | | | | | | |
| 1.654 | 108.120 | 0.101 | - 1,203.264 | 13,341.577 | 12,358.607 | 6,289.451 |
| La Pérouse | | | | | | |
| 0.206 | 141.650 | 0.837 | - 4,130.344 | 5,090.117 | 6,108.077 | 5,628.190 |

**Table S7.** Kendall's rank correlation test to test whether the number of reads correlates with the difference between the species’ known depth range and the sampling depth at which a species was collected. The alternative hypothesis was that true tau is not equal to 0. The statistical significance criterion was a p-value of 0.05.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Kendall's rank correlation tau | | | | | |
| z | | **p - value** | | **tau** |
| Provence coast | | | | | |
| - 0.793 | | 0.428 | | - 0.067 | |
| La Pérouse | | | | | |
| - 1.184 | | 0.237 | | - 0.089 | |