**Environmental DNA metabarcoding for monitoring metazoan biodiversity in Antarctic nearshore ecosystems – Supplemental Information**

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***In silico* PCR**

We used *in silico* PCR to explore taxonomic coverage, resolving power and suitability of potential benthic arthropod metabarcodes, focussing on those targeting mitochondrial COI and 16S, and nuclear 18S rRNA genes. Target taxa were arthropods known to occur in East Antarctic benthic communities, molluscs and annelids (Table S2). To ensure primer-binding sites were present, we targeted complete or near-complete mitochondrial genomes, as well as complete 18S rRNA genes.

We searched the NCBI nucleotide database for mitochondrial genomes for each taxon in Table S1 by combining the search terms: “complete OR partial”, “mitochond\*” and restricting the output to sequences between 10,000 and 20,000 bp. We searched the NCBI nucleotide database for complete 18S sequences for each taxon in Table S2 by combining the search terms: “complete AND 18S”, NOT “internal” (to remove internal transcribed spacer, or ITS, sequences) and restricting the output to sequences between 1,000 and 5,000 bp.

We used the program ECOPCR (Ficetola et al. 2010) to compare taxonomic coverage and resolution for each metabarcode in Table 1, S3 and S4. ECOPCR uses a pattern-matching algorithm to identify sequences within a database that can be amplified with a given primer pair by constraining the relative orientation of and maximum distance between primer-binding sites, as well as the number of mismatches between primer and target sequences (Ficetola et al. 2010).

Each sequence database (mitochondrial and complete 18S) was converted into a format compatible with ECOPCR using the ecoPCRFormat.py script. Inosine residues in the COI reverse primer (Table 1, S3 and S4) were changed to ‘N’. The ECOPCR parameters used were amplicon length between 50 and 1000 bp, with a maximum of three mismatches in each primer, with no restriction on mismatches in base pairs at the 3’ end. These settings are relaxed compared to *in vitro* PCR where mismatches in the last two base pairs at the 3’ end would prevent amplification, as the main question we were addressing was species-level resolution of the amplified sequence. The suitability of primer sequences for benthic arthropods should be checked and changed as necessary prior to their use in metabarcoding studies.

A caveat for *in silico* PCR with ECOPCR is that amplicons that differ by a single base are regarded as ‘resolved’. Greater sequence divergence would typically be desired to confidently resolve distinct taxa.

**Table S2.** DNA sequence data used for *in silico* PCR. Data downloaded from GenBank 10th August 2021.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Phylum** | **Superorder/Class** | **Order** | **Mitochondrial genomes**  | **Complete 18S** |
| Arthropoda | Peracarida | Amphipoda | 181 | 120 |
|  |  | Isopoda | 37 | 88 |
|  |  | Tanaidacea | 1 | 1 |
|  |  | Cumacea | 1 | 1 |
|  | Ostracoda |  | 17 | 3 |
|  | Pycnogonida |  | 10 | 4 |
|  | *Total* |  | *247* | *217* |
|  |  |  |  |  |
| Annelida |  |  | 285 | 200 |
| Mollusca |   |   | 1907 | 407 |

**Table S3.** Primers, taxonomic coverage and resolution (species-level) of metabarcodes estimated by *in silico* PCR against a database of annelid mitochondrial genomes or complete 18S rRNA gene sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Name** | **Locus** | **Primer sequence (5'-3')** | **Mean ± SD****length (bp)****(min.–max.)** | **Species-level coverage** | **Species-level resolution** | **Reference** |
| Leray COI | COI | GGWACWGGWTGAACWGTWTAYCCYCC | 313 ± 1 | 112/119 (94%) | 112/112 (100%) | Leray et al. (2013) |
|  |  | TAIACYTCIGGRTGICCRAARAAYCA | (310-316) |  |  |  |
| Sauron COI | COI | GGDRCWGGWTGAACWGTWTAYCCNCC | 313 ± 1 | 115/119 (97%) | 113/115 (98%) | Rennstam Rubbmark et al. (2018) |
|  |  | TAIACYTCIGGRTGICCRAARAAYCA | (310-316) |  |  |  |
| Leray-XT | COI | GGWACWRGWTGRACWITITAYCCYCC | 313 ± 1 | 117/119 (98%) | 113/117 (97%) | Wangensteen et al. (2018) |
|  |  | TAIACYTCIGGRTGICCRAARAAYCA | (310-316) |  |  |  |
| V4\_18S | 18S, V4 | CCAGCASCYGCGGTAATTCC | 379 ± 11  | 178/180 (99%) | 39/178 (22%) | Stoeck et al. (2010),  |
|  |  | ACTTTCGTTCTTGATYRATGA | (376-508) |  |  | Piredda et al. (2017) |
| Euka03 | 18S, V9 | CCCTTTGTACACACCGCC | 138 ± 2  | 37/180 (21%) | 14/37 (38%) | Taberlet et al. (2018) |
|  |  | CTTCYGCAGGTTCACCTAC | (136-144) |  |  |  |
| Poly01 | 16C | CCGGTYTGAACTCAGMTCA | 63 ± 1  | 116/119 (97%) | 86/116 (74%) | Taberlet et al. (2018) |
|   |   | TGGCACCTCGATGTTGGCT | (61-66) |   |   |   |

**Table S4.** Primers, taxonomic coverage and resolution (species-level) of metabarcodes estimated by *in silico* PCR against a database of mollusc mitochondrial genomes or complete 18S rRNA gene sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Name** | **Locus** | **Primer sequence (5'-3')** | **Mean ± SD****length (bp)****(min.–max.)** | **Species-level coverage** | **Species-level resolution** | **Reference** |
| Leray COI | COI | GGWACWGGWTGAACWGTWTAYCCYCC | 313 ± 1 | 532/622 (86%) | 492/532 (92%) | Leray et al. (2013) |
|  |  | TAIACYTCIGGRTGICCRAARAAYCA | (307-313) |  |  |  |
| Sauron COI | COI | GGDRCWGGWTGAACWGTWTAYCCNCC | 312 ± 2 | 590/622 (95%) | 545/590 (92%) | Rennstam Rubbmark et al. (2018) |
|  |  | TAIACYTCIGGRTGICCRAARAAYCA | (304-319) |  |  |  |
| Leray-XT | COI | GGWACWRGWTGRACWITITAYCCYCC | 312 ± 2 | 604/622 (97%) | 559/604 (93%) | Wangensteen et al. (2018) |
|  |  | TAIACYTCIGGRTGICCRAARAAYCA | (304-319) |  |  |  |
| V4\_18S | 18S, V4 | CCAGCASCYGCGGTAATTCC | 443 ± 114  | 321/326 (98%) | 231/321 (72%) | Stoeck et al. (2010),  |
|  |  | ACTTTCGTTCTTGATYRATGA | (378-925) |  |  | Piredda et al. (2017) |
| Euka03 | 18S, V9 | CCCTTTGTACACACCGCC | 137 ± 9  | 82/326 (25%) | 53/82 (65%) | Taberlet et al. (2018) |
|  |  | CTTCYGCAGGTTCACCTAC | (120-192) |  |  |  |
| Gast01 | 16S | CCGGTCTGAACTCAGATCA | 64 ± 2  | 586/622 (94%) | 286/586 (49%) | Taberlet et al. (2018) |
|   |   | TTTGTGACCTCGATGTTGGA | (60-76) |   |   |   |

**Supplementary References**

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