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Abstract

Continuing the international efforts of the ARMS Marine Biodiversity Observation Network (ARMS-MBON), we present data from the second sampling campaign, coming from 56 Autonomous Reef Monitoring Structures (ARMS) deployed in 2020 and 2021 along European coasts under the European Marine Omics Biodiversity Observation Network (EMO BON). The data set includes information on sampling locations and conditions, sample archiving, and quality reports of collected samples. Data and metadata are openly accessible and can be downloaded from the associated GitHub repository. Sequence data can be accessed via the European Nucleotide Archive (ENA) through the corresponding accession numbers. Images of ARMS plates are stored in PlutoF and can be downloaded through links provided in this paper. Sequence data was processed and

explored with the PEMA pipeline, resulting in 17,194, 7,235 and 5,261 unique ASVs/ OTUs for COI, 18S and ITS, respectively. In this data set, ARMS revealed the presence of over 61 eukaryotic phyla, aligning with our previous sampling campaign. Among these phyla, 35 had ASVs/OTUs identified to the species level. With this data set and its associated paper, we provide a standardised resource for marine biodiversity monitoring and scientific analyses of benthic biodiversity. The presented data product supports future studies on the status and changes in species composition, distribution, and genetic diversity.

Introduction

In an era of significant global changes, the study and conservation of biodiversity has become a major focus for scientists worldwide. Only recently have the intricate connections between the health of these ecosystems and human well-being started to gain attention in public health discourse and decision-making, in the light of the One Health concept (Horton et al. 2014). Oceans, historically overlooked in conservation efforts, cover over 90% of the biosphere and are critical habitats teeming with wildlife (Cowan 1997). To this date, more than 240,000 marine species have been described (Ahyong et al. 2025), and an average of 2,332 new species are discovered every year (Bouchet et al. 2023). However, the distribution and ecology of most known species in the global ocean is still, to a large extent, unknown (Guidi et al. 2020), while data describing the population size and distribution range is lacking for the majority of marine species (Abreu et al. 2022, Mora et al. 2011). Investigating species distribution, range, abundance, and genetic diversity is therefore a critical field of action for the conservation of marine systems.

However, most marine habitats face increasing threats such as biodiversity loss and degradation (Luypaert et al. 2020, Mazaris et al. 2019, O'Hara et al. 2021), while processes such as climate change can significantly and abruptly alter the community composition and food web structure. Coastal areas are particularly vulnerable due to their proximity to human activities, for instance tourism and industrial development (González Hernández et al. 2023, Green et al. 2022, Harris et al. 2022), and their sensitivity to climate change (Burkett et al. 2009). Understanding the dynamics between human activities and ecosystem response is more important than ever. To this end, the frameworks of Essential Biodiversity Variables (EBVs; Geijzendorffer et al. 2016, Kissling et al. 2018a, Kissling et al. 2018b, Proença et al. 2017), Essential Ocean Variables (EOVs; Miloslavich et al. 2018, Muller-Karger et al. 2018), Essential Climate Variables (ECVs; Bojinski et al. 2014, Zeng et al. 2019) and Essential Ecosystem Service Variables (EESVs; Balvanera et al. 2022) have emerged to provide key metrics for monitoring biodiversity and oceanic changes at regional and global scale. EBVs aim to capture critical aspects of biodiversity change, such as species populations and genetic diversity, while EOVs focus on oceanic processes and ecosystem health. Given the complexity of marine environments and the often subtle nature of their ecological shifts, these frameworks highlight the necessity for innovative monitoring techniques and widely

adhered protocols capable of delivering high-resolution insights into biodiversity patterns and changes. These techniques must operate on large scales and deliver high-resolution data to effectively capture the rapid loss of biodiversity and support conservation efforts, research and policy. Genetic methods, such as DNA/eDNA metabarcoding, have been developed and are increasingly used as they become cheaper and more efficient, while providing high taxonomic resolution (Taberlet et al. 2012, Ruppert et al. 2019). Moreover, consensus standardised and public protocols have to be applied so results can be comparable across time and space.

The Autonomous Reef Monitoring Structures Marine Biodiversity Observation Network (ARMS-MBON), initiated in 2018, carries out continuous genetic monitoring of hard-bottom communities across Europe (Obst et al. 2020) using standardized protocols. Following the initial conceptual outlines by Obst et al. (2020) and Santi et al. (2023), the data from the first sampling campaign (2018-2020) were published by Daraghmeh et al. (2025). These first years offered valuable perspectives on the pan-European genetic diversity of hard-bottom benthic ecosystems, demonstrated the effectiveness of DNA metabarcoding in improving traditional monitoring techniques, and highlighted the obstacles faced in establishing a standardised marine monitoring network.

ARMS-MBON began under the ASSEMBLE Plus project (2017-2022) and is now coordinated by the European Marine Biological Resources Center (EMBRC) under the European Marine Omics Biodiversity Observation Network (EMO BON) program, led by EMBRC (Santi et al. 2023). EMO BON collects samples from the water column (*Wa*), soft substrates (*So*), and hard substrates using ARMS (*Ha*), aiming to allow researchers to explore marine diversity across different habitats. Thereby, EMO BON actively contributes to the UN Decade of Ocean Science for Sustainable Development and the global Ocean Biomolecular Observing Network initiative, which is one of the actions endorsed by the Decade (OBON; Meyer et al. 2023).

Building on the foundations laid by this initial ARMS-MBON sampling campaign, the current data paper presents the second ARMS-MBON data release, focusing on 56 ARMS deployed along European coasts from 2020 to 2021. The data set includes material samples, metadata, images, sequence data, derived taxonomic observations, and documentation, all adhering to FAIR principles (Tanhua et al. 2019).

Value of the dataset

The data set presented in this paper holds significant value for both marine ecology research and marine conservation programs. It provides standardised, high-resolution genetic data on benthic species across European coasts. The data reported here are comparable with the previous and subsequent data sets from EMO BON observatories and thereby allow for the monitoring of the status and changes of benthic biodiversity. This data set enhances biodiversity monitoring by contributing to Essential Biodiversity Variables (EBVs) and providing data on species occurrence, alpha/beta diversity, and genetic diversity, addressing knowledge gaps in marine species distribution and diversity through 56 ARMS deployments. It supports scientific research, conservation planning,

and global interoperability by integrating with initiatives like the Ocean Biodiversity Information System (OBIS), the Global Biodiversity Information Facility (GBIF), the European Marine Observation and Data Network (EMODnet), and the European Digital Twin of the Ocean (EU DTO).

Ultimately, this data set provides a critical resource for understanding and protecting marine biodiversity in an era of rapid environmental change, offering long-term value for biodiversity research, ecosystem management, and global conservation efforts.

Methods

Sampling, ARMS processing and image data

The original observatory design, field work methodologies, and sample processing procedures as well as instructions for biobanking and data management have been described in Obst et al. (2020). During this second ARMS MBON campaign, field work, image data collection and sample collection followed the same guidelines as for the first sampling campaign (Daraghmeh et al. 2025), mainly guided by the ARMS MBON Handbook v2.0 and the EMO BON Handbook v2.0 that can be accessed on the ARMS-MBON GitHub repository, the EMO BON website and the Ocean Best Practices platform (see Suppl. material 1 for links).

General description of the sampling campaign

The presented data are derived from 56 ARMS units, corresponding to 56 individual sampling events. These include 55 unique Unit IDs, as two sampling events occurred at the same ARMS location (BelgiumCoast_AJJCD78, one deployment in 2020 and one in 2021, see Suppl. material 2).

Geographic coverage

The data set's geographical range includes 13 observatories, across 10 countries, covering 6 ecoregions (Table 1, Fig. 1). For more information about the observatories and sites, see Github links in Suppl. material 1.

Temporal coverage

The data set is composed of data from ARMS units deployed in 2020 and others in 2021, and retrieved during both years. These ARMS units were deployed during periods ranging between 66 days in Plymouth, UK, and 412 days in Piran, Slovenia (Fig. 2). In the dataset presented by Daraghmeh et al., (2025), no significant linear association between deployment duration and the number of species identified or the ASV/OTU richness was detected. Deployment duration is, however, an important measure to track depending on which studies are performed with ARMS data.

General information on the observatories and ARMS deployments (e.g., coordinates, habitat type, deployment depth) and on the sampling events and their resulting material samples (e.g., date of deployment and retrieval, material sample IDs, preservative used) can be found on the GitHub repository (see Suppl. material 1 for respective links) and in Suppl. material 2.

Laboratory protocols for amplicon sequencing

Until 2021, material samples have been sent to the Hellenic Centre for Marine Research (HCMR), Crete, Greece, for sequencing. This data set is therefore composed of the three final sequencing batches processed in this facility, in September 2020, April 2021 and August 2023. The HCMR institute conducted amplicon sequencing for 162 material samples following the ARMS-MBON Molecular Standard Operational Procedure (see Suppl. material 1 for link). DNA metabarcoding was performed on the eukaryotic mitochondrial and nuclear marker genes cytochrome *c* oxidase subunit I (COI), 18S rRNA (18S), and for 68 samples, on the internal transcribed spacer (ITS) region. The ITS marker was used exclusively during the project's early stages and is currently no longer in use; this data paper therefore presents the last results for this marker.

For the COI marker gene, the *m1COlintF* and *jgHCO2198* primers (Leray et al. 2013, Geller et al. 2013) were used and for 18S rRNA, *All18SF* and *All18SR* (Hardy et al. 2010) were chosen. Sequencing was performed with Illumina MiSeq.

All sequences from successful sequencing events are now publicly accessible in the European Nucleotide Archive (ENA) (see Data resources section below). Negative control sample sequences are also available. Additional details on how the sequence data was demultiplexed, including whether the reads include primer sequences, are documented in Suppl. material 3.

Data management

Data management followed the same steps and logic as for our first data release, where the whole procedure has been described and documented (Daraghmeh et al. 2025). In short, event metadata (observatory, event, and sample metadata, ENA accession numbers for sequencing data) were recorded on the ARMS-MBON project Googlesheet, and ARMS plate images and associated spreadsheets were uploaded to the PlutoF platform (https://www.plutof.ut.ee). After quality control (i.e., checking for inconsistent formats, missing entries or incomplete ones), all metadata and image data entered on PlutoF, and all spreadsheet data were harvested and uploaded to the ARMS-MBON GitHub space (see Suppl. material 1 for links). When organising the data on GitHub, we created new repositories, specific to this second data release, but following the same structure as for the first (Daraghmeh et al. 2025). Each repository of the second data release was then packaged as a Research Object Crate (RO-Crate; https://www.researchobject.org/ro-crate/) (Soiland-Reyes et al. 2022) to produce machine-accessible and interoperable data sets.

Data processing and exploration

Bioinformatics processing

A brief exploration of the sequencing outputs was performed to assess the quality and taxonomic coverage of the data. To this end, sequence data was processed using the Pipeline for Environmental DNA Metabarcoding Analysis, PEMA v.2.1.4 (Zafeiropoulos et al. 2020), as for the first data release. For details on data processing, see Suppl. material 4, extracted from Daraghmeh et al. (2025), which describes the methods used.

The novelty of this second data release is that we ran PEMA using workflows developed by the European infrastructure LifeWatch ERIC (Arvanitidis, 2024). All PEMA runs were performed on the platform MyLifeWatch (https://my.lifewatch.eu/). On this platform, users can build workflows by connecting different wrappers (see Suppl. material 5 for additional information). We built our own workflow using: 1) the wrapper "PEMA Sequence Retriever" to obtain the raw data from ENA using the accession numbers for each sample (see Suppl. material 3); 2) the wrapper "Import File" to upload a parameter file and 3) the wrapper "PEMA Runner" to run PEMA using the outputs from the two previous wrappers. The workflows run in a Docker-based orchestrator that has a multi-server backend infrastructure currently established between datacenters from the Picasso Supercomputer (University of Malaga), the Scientific Computing Center of Andalusia (CICA, Seville), eBRIC (Huelva) and the University of Granada (Benítez-Hidalgo et al. 2021).

Data from the same sequencing batches were processed in the same workflow run, to allow the use of specific parameters for samples sequenced in similar conditions. All parameter files used for each PEMA run are available in the *analysis_release002* repository on the ARMS MBON GitHub (see Suppl. material 1 for respective link).

Blank correction and merge of the runs

We first performed a blank correction to discard any potential contaminant sequences, using the R package *decontam* v1.20.0 (Davis et al. 2018), and the *prevalence* method. Results from *decontam* were double-checked prior removal of ASVs/OTUs (i.e., exact number of reads found in samples and in negative control, taxonomic classification of these potential contaminants). Sequences identified as contaminants by *decontam* but that had many more reads in true samples than in negative controls, and were classified in a marine taxa known in the study area, were kept in the data set. Sequences identified as contaminants by *decontam* that had many more reads in the negative control or that were being classified as a contaminant taxa (e.g., Insecta, *Homo sapiens*, etc.), were removed from the data set. A list of the ASVs/OTUs that have been removed can be found in Suppl. material 6 .

We then combined data from individual PEMA runs for each marker gene and curated the merged data sets to create a single data set for visualisation and further inspection of the data set. Since PEMA does not automatically apply a confidence threshold for taxonomic assignments of COI ASVs, we excluded all taxonomic assignments with confidence values below 0.8 for the rest of our analysis. Sequences with one of the following classifications were discarded as potential contaminants: *Homo sapiens*, *Canis lupus*, or part of the following genera *Bos, Felis*, *Sus*, *Gynaikothrips*, *Dorypteryx*, *Fannia*, *Bactrocera*, *Aleochara*, *Larus*, *Entomobrya*, *Hypogastrura*, *Psylla*, *Tanytarsus*, *Ptinus*, *Paratanytarsus*.

Taxonomic assessment

We analysed the data to determine the number and abundance of unique phyla, identified species, and ASVs/OTUs classified to the species level. When reference databases failed to provide accurate phylum-level classification, we manually corrected these classifications by identifying the correct taxonomy through literature. We then used custom code to update the classifications in the dataset, ensuring consistency and accuracy across all samples. We also checked for unique species found exclusively in any of the three markers data sets, as well as unique species detected by all of the three markers.

Results

Overall description

Genetic data set

Out of the 56 retrieved ARMS, 162 material samples were obtained. In most cases, three size fractions (one sessile and two motile) were processed for each ARMS unit, but for various reasons some of the sampling events did not result in 3 fractions (*e.g.* not enough material, technical constraints, etc.). Additionally, out of these 162 sequenced samples, 155 were successfully sequenced (i.e., samples containing sequences that are deposited on ENA) both using the COI and 18S marker genes. For the ITS marker, 68 samples (out of 69 samples processed for this marker) were successfully sequenced (Table 2).

We assessed the sequencing depth of each sequencing run. The distribution of total sequencing reads across different sequencing dates (20-Sep, 21-Apr, and 23-Aug) and across markers (COI, 18S, ITS) showed notable variation (Fig. 3). We also compared the mean sequencing depth per sample of this data set and of the previous one (Daraghmeh et al. 2025) for each marker (Suppl. material 7).

Image data set

During this campaign, 8,362 photographs of ARMS plates were taken and stored on PlutoF. This comprises images of both sides of the settlement plates as well as close-ups of individual specimens or colonies.

Exploration of the genetic data set

The sequenced data published on ENA and processed with PEMA, resulted in 17,194, 7,235 and 5,261 unique ASVs/OTUs for COI, 18S and ITS, respectively (Table 3). After further curation and filtering (i.e., negative-control-correction and removal of unclassified sequences), 153 samples with 17,175 ASVs remained for the COI data set and 148 samples with 6,258 OTUs remained for the 18S data set. For ITS, 50 samples with 645 ASVs remained (here, ASVs were inferred from only two sequencing runs, i.e., September 2020 and April 2021). This corresponded to 1,436,988 sequence reads for COI, 1,198,635 sequence reads for 18S and 34,105 sequence reads for ITS (Table 3). We also compared the average number of ASVs/OTUs per sample of this data set and of the previous one (Daraghmeh et al. 2025) for each marker (Suppl. material 8) .

Combining the results from the 3 marker genes, 61 phyla have been recovered, including 31, 58 and 10 coming from the COI, 18S and ITS data sets, respectively. Among these 61 phyla, 35 have species-level identifications (Table 3), including 28, 27 and 15 from the COI, 18S and ITS data sets, respectively.

When assessing the phyla's relative read abundances for each marker, we found that the COI data set is dominated by Arthropoda (16%), Cnidaria (7.5%), Annelida (5.5%) and Mollusca (2.5%), but also that more than half of the reads (61%) come from sequences that have not been classified at the phylum level (Fig. 4A). For 18S, the main phyla were Arthropoda (20%), Mollusca (15%), Chordata (15%) and Myzozoa (9%). Only around 7% of the reads could not be assigned to a phylum (Fig. 4C). In the case of ITS, the data set was largely dominated by the phylum Ascomycota (55%), followed by Basidiomycota (10%). Here, 32% of the reads did not get assigned to a phylum (Fig. 4E).

As expected, the three marker genes are complementary as they identify different groups of taxa (Fig. 4). This is supported by Fig. 5, which shows that 682 (out of 697), 94 (out of 108) and 127 (out of 128) identified species were unique to the COI, 18S and ITS data sets, respectively. Fourteen species were found in both the COI and 18S data sets, while only one was found across the ITS and COI data sets. No species were found in all three data sets.

At the genus level (Suppl. material 9), we observe a similar pattern: 491 (out of 523), 80 (out of 102) and 87 (out of 97) identified genera were unique to the COI, 18S and ITS data sets, respectively. Twenty-two genera were found in both the COI and 18S data sets, while ten were found across the ITS and COI data sets. No genera were found in all three data sets.

Discussion

This paper presents the set of raw and processed amplicon sequencing data from the second sampling campaign of ARMS-MBON, from deployments performed in 2020 and 2021 and continues the genetic monitoring program initiated in 2018. The raw sequencing, image data and metadata are open-access (CC BY), allowing all interested users to use and reprocess them according to their specific needs. Additionally, we processed the sequencing data using PEMA v.2.1.4 (Zafeiropoulos et al. 2020), and all taxonomic occurrences will be published on GitHub.

Raw data and the subsequent PEMA outputs presented here are comparable to our first data release (Daraghmeh et al. 2025) and to other similar studies (Leray and Knowlton 2015, Gielings et al. 2021, Coker et al. 2023). More specifically, for COI and ITS, the mean sequencing depth per sample was in a similar range to that reported in Daraghmeh et al. (2025) (Suppl. material 7). However, it was lower for 18S, which could be due to lower sequencing quality. These patterns likely reflect differences in sample quality, sequencing conditions, or library preparation across the dates and suggest potential batch effects that should be accounted for when performing further statistical analyses. Additionally, the average number of ASVs/OTUs per sample was 60% lower in the second release for COI, 17% lower for 18S and the same for ITS (Suppl. material 8). Differences between data sets can be due to different sequencing quality or differences in sample preparation protocols, sequencing platforms, or bioinformatics processing pipelines (Smith and Peay 2014, Sims et al. 2014), which however has not been the case in this study. Variability in sequencing performance across markers may also reflect inherent differences in primer efficiency, target gene diversity, or amplification success, all of which can influence the recovery of ASVs/OTUs. Multi-marker strategies have been recommended to improve taxonomic coverage (Portas et al. 2022) and provide more accurate community composition estimates (Günther et al. 2018, Stefanni et al. 2018).

The ARMS-MBON monitoring network has undergone significant growth and development since its initial years, marked by the addition of new observatories and an increasingly structured approach to sample collection, data management, and analysis. This expansion has greatly enriched our data set, enhancing our capacity for long-term monitoring of marine ecosystems. The integration into the EMO BON network in 2021, which conducts extensive water and sediment sampling across Europe, represents a major advancement.

Moreover, our ongoing efforts to refine methodologies are supported by the collective expertise and insights from the partners' fieldwork and data analysis experiences. This continuous improvement cycle not only strengthens the monitoring capabilities of ARMS-MBON but also ensures that our methods remain at the forefront of marine biodiversity research. The synergy between ARMS-MBON and EMO BON, alongside our commitment to methodological advancements, allow us to make substantial contributions to the understanding and preservation of marine ecosystems.

The ARMS-MBON network is well-equipped to provide comprehensive data for studying biodiversity patterns in marine ecosystems over the long term. For instance, it has already been shown that ARMS-BON network data is useful for the identification of NIS (Daraghmeh et al. 2025, Pagnier et al. 2025). Using a variety of methodologies, ARMS-MBON can generate detailed insights into species distribution, community structure, and genetic diversity. The network's extensive array of observatories facilitates the collection of high-resolution, spatially-diverse data, which is critical for understanding the complex dynamics of marine biodiversity. Additionally, the data generated align closely with Essential Biodiversity Variables (EBVs), ensuring compatibility with global biodiversity monitoring frameworks and enhancing the utility of the data set for large-scale ecological assessments. This robust and expanding data set enables rigorous statistical analyses, providing valuable information on both short-term patterns and long-term trends.

In addition to the extensive genetic data provided in this second data release, we also include a comprehensive set of high-resolution images from the deployed ARMS units. While these images remain underutilised in current analyses, they offer significant potential for complementary research. In fact, studies showed that ARMS photo-analyses can be used to compare marine benthic communities (David et al. 2019). Visual data can provide crucial context for the genetic findings by documenting the physical appearance of the communities, offering insights into species behavior, habitat structures, and possible environmental changes over time. Furthermore, the images could be used for cross-referencing with genetic data to improve species identification and validate the presence of non-indigenous species (NIS) detected via metabarcoding (Blair et al. 2024). This visual documentation also holds value for the development of more integrative approaches to biodiversity monitoring that combine genetic, ecological, and visual data. We encourage the broader scientific community to explore the rich potential of this image data set in future research.

Benefits sharing statement

ARMS-MBON and EMO BON represent a large-scale research collaboration with scientists from across Europe and beyond. All network partners of the observatories mentioned in this manuscript provided genetic samples and are included as co-authors. All continuously generated raw and processed data from this network is shared with the public and scientific community (see above). Our research addresses the urgent need for large-scale and long-term monitoring of marine biotic communities through extensive collaborative efforts.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Resources

Data and metadata are accessible through the following:

- Resource 1: Genetic data

see below

- Resource 2: Sample metadata

https://github.com/arms-mbon/data_release_002

- Resource 3: Bioinformatics data and metadata (inputs, outputs, parameters)

https://github.com/arms-mbon/analysis_release_002

- Resource 4: Code used for the post-PEMA analyses

https://github.com/arms-mbon/code_release_002

- Resource 5: Sample access (For physical samples, requests can be directed to the corresponding author or the institutions responsible for sample archiving, as detailed in the data set documentation)

https://www.embrc.eu

- Resource 6: Image data (Images of ARMS plates from this data set are stored on PlutoF and can be downloaded using the links provided in the dedicated CSV file below)

https://github.com/arms-mbon/data_release_002/blob/main/ImageData_release002.csv

Resource 1

Download URL

All the raw sequence files and negative controls of this study were submitted to the European Nucleotide Archive (ENA) with the umbrella study accession number PRJEB72316 (available at http://www.ebi.ac.uk/ena/data/view/PRJEB72316)

Resource identifier

The accession numbers of the component projects under the umbrella study are PRJEB37740, PRJEB37757, PRJEB33796, PRJEB37754, PRJEB37756, PRJEB37757, PRJEB72186, PRJEB37740, PRJEB37741, PRJEB37753, PRJEB72187, PRJEB37756, PRJEB37754, PRJEB37744

Data format

FASTA

Conclusion

In conclusion, the second data release of ARMS-MBON provides an enriched and continued data set for benthic biodiversity in Europe, including both raw and processed amplicon sequencing data alongside high-resolution images. This release highlights the network's commitment to advancing marine biodiversity monitoring through rigorous methodologies and integration with global frameworks such as EBVs. By fostering accessibility, interoperability and sustainability, ARMS-MBON continues to make significant contributions to understanding and preserving marine ecosystems.

Usage Rights

Data accessibility statement

All data presented in this manuscript are publicly available with a CC BY licence (see main text and Supplementary Material for detailed descriptions). Standard operating procedures and protocols are available on the dedicated ARMS-MBON GitHub repository (https://github.com/arms-mbon/documentation). All metadata and access to all image data generated during this sampling campaign of ARMS-MBON to date can be found on GitHub (https://github.com/arms-mbon/data_release_002). All genetic raw data generated by ARMS-MBON to date can be accessed on the European Nucleotide Archive (ENA) through the accession numbers provided via the GitHub repository (https://github.com/arms-mbon/data_release_002) and under the umbrella study PRJEB72316 (https://www.ebi.ac.uk/ena/browser/view/PRJEB72316). Metadata, access to image data and accession numbers for genetic data specifically for the data set presented in this manuscript are provided in the Supplementary Files and are also available on the respective GitHub repository (https://github.com/arms-mbon/data_release_002).

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Conflicts of interest

The authors have declared that no competing interests exist.

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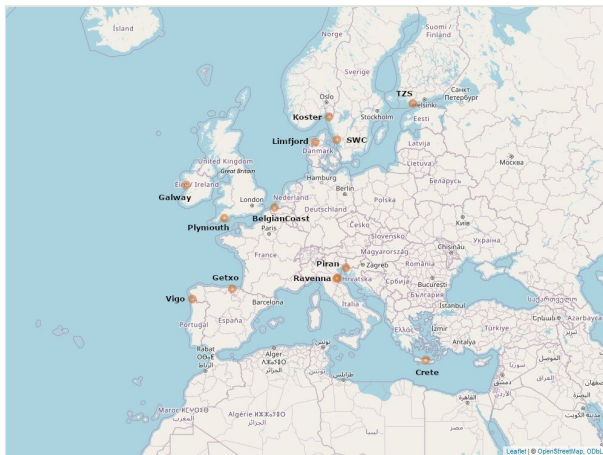Remote Sensing 11 (10). https://doi.org/10.3390/rs11101186

Figure 1.

Locations of observatories that deployed ARMS units during the 2020–2021 ARMS-MBON sampling campaign. The two Ravenna (Italy) observatories are presented as a single entity here due to their proximity. TZS - Tvärminne Zoological Station. SWC - Swedish West Coast.
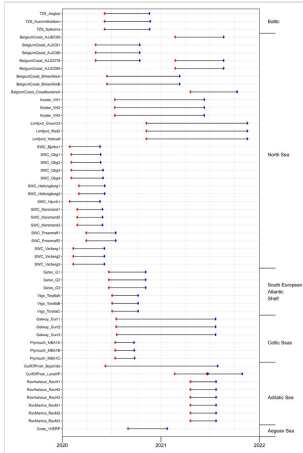
Figure 2.

Sampling events of the second ARMS-MBON sampling campaign. Axis on the left shows ObservatoryID_UnitID combinations, axis on the right shows groupings of observatories into larger regions. Red semicircle: time of deployment. Blue semicircle: time of retrieval. Where red and blue semi circles meet, a new ARMS unit was deployed for a consecutive period at the same spot upon retrieval of the first unit. Where lines contain more than two semicircles (see GulfOfPiran_LukaKP), multiple units were deployed at the exact same spot at the same time but were retrieved at different time points.
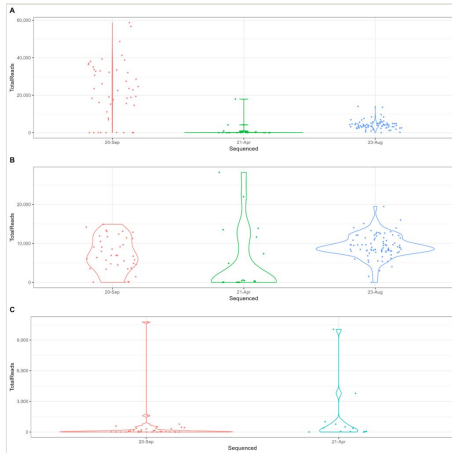
Figure 3.

Sequencing depth of each sequencing batch (20-Sep, 21-Apr, and 23-Aug), for COI (**A**), 18S (**B**) and ITS (**C**). The violin plots display the distribution of total reads for each sequencing batch, with the width of the plot indicating the density of reads at different depths.
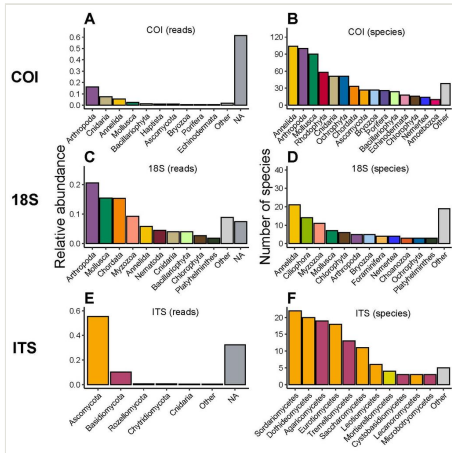
Figure 4.

Relative read abundance of the ten most abundant phyla in the COI **(A)** and 18S **(C)** and five most abundant phyla in the ITS data sets **(E)**. Less abundant phyla are grouped as *Other*, while relative abundance of sequence reads unclassified at phylum level are grouped as *NA*. Number of identified species within each phylum for COI **(B)** and 18S **(D)** and within each class for ITS **(F)**. Phyla / classes with less than ten (i.e., COI) or three (i.e., 18S and ITS) identified species are grouped as *Other*. Colours correspond to the same unique phyla across all plots. Class level representation was chosen in **(F)** for better taxonomic resolution and colours correspond to the fungal phylum each class belongs to.
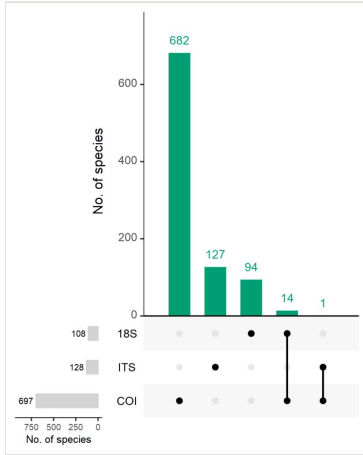
Figure 5.

UpSet plot showing the number of species identified using the three marker genes: COI, 18S, and ITS. Green bars represent the number of species identified (based on the applied confidence threshold) that are shared across the various combinations of marker gene data sets. The matrix below the bar plot indicates which combinations of marker genes correspond to each bar. Bars on the left display the total number of species identified within each marker gene data set. Notably, no species were found to be common across all three data sets.

Table 1.

Table 1. Locality and geographical coordinates of the observatories collecting ARMS samples during the second sampling campaign. Ecoregions are according to Spalding et al. (2007).

Locality	Country	Coordinates	Ecoregion
RavennaM	Italy	44.492426 N; 12.287623 E	Adriatic Sea
RavennaH	Italy	44.421488 N; 12.209497 E	Adriatic Sea
Vigo	Spain	42.2284 N; -8.7787 E	South European Atlantic Shelf
Crete	Crete	35.343153 N; 25.136605 E	Aegean Sea
Plymouth	United Kingdom	50.3673 N; -4.1554 E	Celtic Seas
PiEGetxo	Spain	43.33858 N; -3.014639 E	South European Atlantic Shelf
BelgiumCoast	Belgium	51.43333 N; 2.808331 E	North Sea
Galway	Ireland	53.315443 N; -9.671564 E	Celtic Seas
Piran	Slovenia	45.54875 N; 13.5507 E	Adriatic Sea
TZS	Finland	59.841505 N; 23.248879 E	Baltic Sea
Koster	Sweden	58.875155 N; 11.103194 E	North Sea
SWC	Sweden	57.1107004 N; 12.2439775 E	North Sea
Limfjord	Denmark	56.89985 N; 9.05663333 E	North Sea

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Table 2.

Table 2. Overview of the processed samples from the second ARMS-MBON sampling campaign. *
ITS amplicon sequencing has since been discontinued, these are the last sequencing batches for
this marker.

Sample collection	Overview		
Number of ARMS units retrieved	56		
Number of derived material samples (i.e., biological samples and technical replicates)	162		
Photographic images obtained from ARMS units	8,362		
Marker gene sequencing	COI	18S	ITS
Sequencing batches	3	3	2
Number of samples sequenced successfully (i.e., number of deposited ENA accessions)	155	155	68
Number of negative controls available	3	3	2

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Table 3.

Table 3. Overview of results from the sequence data processing using PEMA

	COI	18S	ITS
Overall number of unique ASVs/OTUs prior to any curation	17,194	7,235	5,261
Number of PEMA-processed samples with classified ASVs/OTUs remaining after negative control correction (excl. negative controls)	153	148	50
Number of unique, classified, blank-corrected ASVs/OTUs	17,175	6,258	645
Number of ASVs/OTUs after curation and filtering	17,161	6,251	642
Sequencing depth (total read number of unique, classified, negative-control-corrected ASVs/OTUs)	1,436,988	1,198,635	34,105
Number of phyla recovered	30	52	9
ASV/OTUs with Linnean species name classification (with the confidence thresholds applied here)	1,502	193	138
Number of unique species identified with Linnean name	697	108	128
Number of phyla represented in species identifications	28	27	3 (15 classes)
	Combined		
Number of phyla recovered	61		
Number of phyla represented in species identifications	35		

Supplementary materials

Suppl. material 1: Supplementary Table S1

Authors: Pagnier et al.
Data type: table
Brief description: Overview of ARMS-MBON and EMO BON project web pages, GitHub repositories and IMIS metadata records for taxonomic occurrences of data release 002 submitted to EurOBIS.
Download file (15.93 kb)

Suppl. material 2: Sample metadata

Authors: Pagnier et al.
Data type: csv
Download file (35.08 kb)

Suppl. material 3: List of ENA numbers

Authors: Pagnier et al.
Data type: csv
Download file (35.08 kb)

Suppl. material 4: Supplementary Text S1

Authors: Pagnier et al.
Data type: text
Brief description: Bioinformatics processing of raw sequence data (extracted from Daraghmeh et al. 2024, first data release)
Download file (191.35 kb)

Suppl. material 5: Supplementary Text S2

Authors: Pagnier et al.
Data type: text
Brief description: Description of the MyLifeWatch platform and Workflow Studio
Download file (15.15 kb)

Suppl. material 6: Details of the blank correction

Authors: Pagnier et al.
Data type: xlsx
Download file (191.35 kb)

Suppl. material 7: Supplementary Figure S1

Authors: Pagnier et al.
Data type: graph

Brief description: Bar plot showing the mean sequencing depth per sample for each marker in both Data Paper 1 (Daraghmeh et al., 2024) and Data Paper 2 (this present data release).
Download file (34.23 kb)

Suppl. material 8: Supplementary Figure S2

Authors: Pagnier et al.
Data type: graph
Brief description: Bar plot showing the mean number of ASVs/OTUs per sample for each marker in both Data Paper 1 (Daraghmeh et al., 2024) and Data Paper 2 (this present data release).
Download file (76.73 kb)

Suppl. material 9: Supplementary Figure S3

Authors: Pagnier et al.
Data type: graph
Brief description: UpSet plot showing the number of genera identified using the three marker genes: COI, 18S, and ITS.
Download file (34.23 kb)