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ORIGINAL ARTICLE

Deep-sea sponge derived environmental DNA analysis reveals demersal fish biodiversity of a remote Arctic ecosystem

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Abstract

The deep-sea is vast, remote, and largely underexplored. However, methodological advances in environmental DNA (eDNA) surveys could aid in the exploration efforts, such as using sponges as natural eDNA filters for studying fish biodiversity. In this study, we analyzed the eDNA from 116 sponge tissue samples and compared these to 18 water eDNA samples and visual surveys obtained on an Arctic seamount. Across survey methods, we revealed approximately 30% of the species presumed to inhabit this area and 11 fish species were detected via sponge derived eDNA alone. These included commercially important fish such as the Greenland halibut and Atlantic mackerel. Fish eDNA detection was highly variable across sponge samples. Highest detection rates were found in sponges with low microbial activity such as those from the class Hexactinellida. The different survey methods also detected alternate fish communities, highlighted by only one species overlap between the visual surveys and the sponge eDNA samples. Therefore, we conclude that sponge eDNA can be a useful tool for surveying deep-sea demersal fish communities and it synergises with visual surveys improving overall biodiversity assessments. Datasets such as this can form comprehensive baselines on fish biodiversity across seamounts, which in turn can inform marine management and conservation practices in the regions where such surveys are undertaken.

KEYWORDS
12S, Arctic Mid-Ocean Ridge, baseline, biodiversity, eDNA, metabarcoding, monitoring, sponge grounds, vulnerable ecosystems

1 | INTRODUCTION

Environmental DNA (eDNA) is genetic material obtained directly from environmental samples without any obvious signs of biological source material (Thomsen & Willerslev, 2015). Biodiversity assessment by the analysis of eDNA is now commonly used throughout baseline data collection and conservation projects (Beng & Corlett, 2020; Mauvisseau et al., 2020; Miya, 2022; Shen et al., 2022; West et al., 2020). Over the past decade, projects assessing species diversity and presence/absence through the use of eDNA has increased exponentially (Beng & Corlett, 2020). The wide applicability of how detection of eDNA can be used for assessing biodiversity, and to some extent abundance, of many species across a range of habitats makes it an attractive approach (Grant et al., 2021; Jeunen et al., 2019; Sard et al., 2019; Stat et al., 2017). The success of biodiversity assessments through eDNA is arguably down to its non-invasive nature, cost effectiveness, and scope for generating large, repeatable data sets (Mauvisseau et al., 2019; Stoeckle et al., 2021).

However, there is a large uncertainty around eDNA sampling efficiency and accuracy, a result which often returns alternate community diversity profiles compared to profiles obtained from more traditional survey methods (Mathieu et al., 2020; Polanco Fernández et al., 2021; Valdivia-Carrillo et al., 2021). Further uncertainties can arise from variation in sampling effort (e.g., filter type, number of replicates), and the risk of sample contamination (Burian et al., 2021; Klepke et al., 2022; Lynggaard et al., 2022). Researchers therefore attempt to improve various steps in the protocol of how species can be detected through eDNA. For example, an 'Environmental Sample Processor' has been suggested to speed up sample collection and appears able to monitor marine species in situ (Hansen et al., 2020). However, passive filtration methods are thought to offer an alternative to direct physical sampling of the water source (Bessey et al., 2021; Jeunen et al., 2022; Kirtane et al., 2020; Verdier et al., 2022).

A different filtering approach, aimed at tackling some of these challenges associated with eDNA sampling could be the proposed utilization of naturally filtering organisms such as sponges (phylum Porifera) or bivalves (phylum Mollusca; Jeunen et al., 2021; Mariani et al., 2019; Turon et al., 2020). In a pioneering study, Mariani et al. (2019) used nine marine sponge samples to assess their potential as natural eDNA samplers. Turon et al. (2020) further validated this concept, by metabarcoding 16 different sponge species collected on shallow water tropical reefs in marine protected areas in Vietnam, and in doing so identified 90 tropical fish species. This corresponded to one third of the diversity reported in the area. They also documented environmental variables driving differential eDNA capture and found significant differences in the detection of eDNA from fish when comparing eutrophic and well-preserved environments. However, no differences were linked to sponge taxonomy or to morphology. This latter result is interesting as different sponge species are known to filter water at different rates (Weisz et al., 2008), and sponge morphology has been shown to influence the ability to capture or retain particles within their tissues (Kahn et al., 2015; Morganti et al., 2019). The latest study on sponge eDNA conducted a controlled experimental study in aquaria to test exactly this (Cai et al., 2022). In contrast to Turon et al., they did identify differences in fish eDNA detection across sponge species and this was argued to be due to the filtering efficiency and/or microbial activity of the sponges (Cai et al., 2022; Turon et al., 2020). Sponge species can be classified as high microbial abundance (HMA) or low microbial abundance (LMA) species (Moitinho-Silva et al., 2017). HMA sponges have lower pumping rates than LMA sponges, in some cases 52%–94% lower filtration (Weisz et al., 2008), suggesting that LMA sponges might be more ideal for eDNA filtration and subsequent detection. The degradation rates of eDNA in sponges have also been shown to be variable between species (Cai et al., 2022). Some species of sponges mirror the degradation rates of eDNA in the water column, while others retain eDNA traces for longer periods (up to 72 h; Cai et al., 2022). The use of sponges as natural filters therefore seems promising; however, the extent to which it can be applied to more extreme and remote ecosystems necessitates further investigations.

The deep-sea, i.e., the water column and seafloor below 200 m, is the largest biome and likely the least explored on our planet (Ramirez-Llodra et al., 2010). Its limited accessibility compounded by the high costs of survey time and exploration tools has led to less than 0.0001% of the benthic surface of the deep-sea having been surveyed (Danovaro et al., 2020; Howell et al., 2021). Furthermore, much of the Arctic deep-sea has never been explored and the communities residing there are understudied due to the particularly challenging conditions that make sampling difficult in this region. Despite our limited understanding of these realms, the ecosystems and many of the inhabiting species are under increasing pressure from human impacts and the effects of climate change (Danovaro et al., 2020; Levin & Le Bris, 2015; Morato et al., 2020; Pham et al., 2019).

The Schulz Bank, located on the Arctic Mid-Ocean Ridge (AMOR), is an Arctic seamount selected as a case study site in scope of the EU-funded Horizon 2020 SponGES project. Having been surveyed annually from 2016 to 2019 using several observational and physical sampling tools, this seamount has now been characterized in a significant amount of detail. In summary, we know about its biological communities (Meyer et al., 2019, 2022), their recovery from trawling impacts (Morrison et al., 2020), the microbial diversity (Busch et al., 2022; Busch, Hanz, et al., 2020), oceanographic conditions and dynamics (Hanz et al., 2021, 2022; Roberts et al., 2018), as well as an understanding of ecological functioning in the region (Maldonado et al., 2021). These surveys revealed the seamount summit and slope, hosts an array of vulnerable marine ecosystems (VMEs)—most notably the impressive sponge grounds. These are dominated by various species of habitat-forming sponges (classes Hexactinellida and Demospongiae). However, while these sponge communities are relatively well-known (Meyer et al., 2022), the fish diversity is only known from limited observations using Autonomous Underwater and Remotely Operated Vehicles (AUVs/ROVs). Despite this, the Schulz's sponge grounds have been hypothesized to serve as habitat and potential nursery for many fish (Meyer et al., 2019), therefore providing

important ecosystem functions as also observed in sponge VMEs in other areas (Hawkes et al., 2019; Kenchington et al., 2013).

Understanding fish communities in this region is important on several levels. Most notably, one of economic importance. The global fishing fleet has been increasing their fishing depth and reach poleward in the pursuit of new fishing grounds and stock—especially catching Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum, 1792)) and Beaked redfish (*Sebastes mentella* Travin, 1951; Christiansen et al., 2014; Jørgensen et al., 2020; Victorero et al., 2018; Villasante et al., 2012). The most northern commercial fisheries in the Barents Sea have an estimated annual landed value of 15–20 billion NOK (Jørgensen et al., 2020), a number which underlines the economic importance of the fish residing in these Arctic waters. Furthermore, redfish have been observed to utilize dense epifauna habitats as breeding grounds (Auster, 2005), emphasizing that this ecosystem could be of importance for recruitment of valuable fish stock. Therefore, it is important to assess what diversity of commercially important fish in particular are present at these sponge grounds, enabling or facilitating monitoring and protection of this habitat as nursing and feeding grounds.

We therefore aimed to (i) investigate if eDNA extracted from sponges collected from the Schulz sponge grounds could be used to assess the fish diversity in the area, (ii) test if detection probability was driven by particular sponge traits (growth form, systematics, and HMA/LMA condition), and (iii) compare the results obtained with different observation methods (sponge eDNA, water eDNA, and in-situ observations made with a ROV).

2 | METHODS

2.1 | Study area

The Schulz Bank (73°52′ N 7°30′ E) lies at the transitional point between the Mohn and Knipovich Ridges and at the eastern edge of the Greenlandic basin at the interface between the Greenland, Iceland, and Norwegian Seas (Hopkins, 1991; Figure 1). Schulz Bank is influenced by three water masses that occur in these seas: the Norwegian Atlantic Water above the seamount, the Norwegian Arctic Intermediate Water at the summit and slopes, and the Norwegian Deep Water on the slopes and base (Hopkins, 1991; Jeansson et al., 2017; Roberts et al., 2018). The summit sits at 580 m below the surface and hosts a dense sponge ground. A geodiid-dominated community covers the seamount's steeply sloping bedrock walls until reaching the seafloor at around 2700 m depth (Meyer et al., 2022).

2.2 | Sample collection

We sampled 116 individual sponges from 16 different species ($n = 2$–17) using the remotely operated vehicle (ROV) *Ægir 6000* (University of Bergen), across two cruises conducted between 2017 and 2018 on board the Norwegian research vessel *G. O. Sars* (GS2017110 and GS2018108). These included representatives of three sponge classes (Hexactinellida, Demospongiae, and Calcarea) and 12 families with

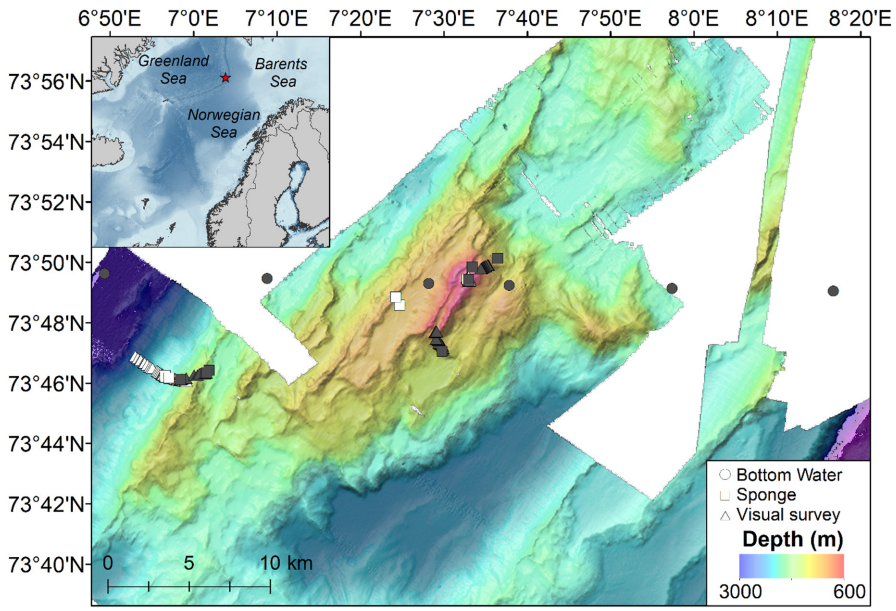

FIGURE 1 Topographic map of the sampling locations on Schulz Bank on the Arctic Mid-Ocean Ridge with an inset showing the seamount location (red star) relative to Norway, Greenland, and Iceland. Water samples collected above the seafloor are denoted with circles, sponge samples collected by remotely operated vehicle (ROV) are denoted with squares, and fish observations made from ROV videos are denoted with triangles. Colors represent sampling years: white (2017) and gray (2018). The digital bathymetry for this figure was extracted from EMODnet Bathymetry Consortium (2020) and has a resolution of 1/16 × 1/16 arc min. The bathymetry of Schulz Bank was provided by the Centre for Deep Sea Research, University of Bergen, Norway.

various morphologies, and species that are described as LMA and HMA sponges (Table 1). The sponges were collected from the summit (580 m) and the bedrock walls to a depth of 2184 m. Taxonomic identifications based on the analysis of external and internal morphological characters were performed both on board and at the University of Bergen by sponge taxonomists, and using taxonomic literature for the overall boreo-Arctic region (Cárdenas et al., 2013; Hestetun et al., 2017). Voucher samples were deposited in the collection of the University Museum of Bergen, after fixation in 99% ethanol, and small fragments sent to the Marine Ecology Research Division at GEOMAR Helmholtz Centre for Ocean Research.

All 18 seawater samples were collected in 2018, using Rosette-mounted Niskin bottles, combined with a conductivity-temperature-depth (CTD) sensor system (SBE 9, Sea-Bird Electronics Inc.). Bottom water samples were taken in three biological replicates between 978 and 2966 m water depth and approximately 10 m above the seafloor. For each sample, 2 L of seawater was filtered onto polyvinylidene fluoride filter membranes (Merck Millipore) with a pore size of 0.22 µm and a diameter of 47 mm. The collection data for all sponge and water samples can be found at Zenodo (https://zenodo.org) with DOI 10.5281/zenodo.7326708.

2.3 | Video observations

Fish observations were made during six ROV dives in 2017 and 2018 (GS2017110-23-ROV12, GS2017110-33-ROV-14, GS2017110-41-ROV-19, GS2018108-19-ROV-12, GS2018108-25-ROV-17, and GS2018108-34-ROV-22; Meyer et al., 2022). The videos selected for image analysis were dives that contained dedicated transects traversing over large areas (average transect length = 2979 m) with limited biological sampling besides occasional periods of opportunistic fauna collection. Videos were watched in the VLC media player, and images containing fish were extracted from the videos. A total of 151 images from 2017 ($n = 107$) and 2018 ($n = 44$) were extracted from the three video transects. The identification of fish from the images was checked and confirmed by experts using taxonomic literature for the region as a guide (Whitehead et al., 1987).

To ensure comparability with the water eDNA samples, only fish observed in 2018 were included in the comparable analysis. However, fish observations from 2017 were used to confirm the observations made in 2018 and note additional fish species not observed in 2018 to add to the overall number of species detected for this ecosystem.

2.4 | Molecular processing of samples

All samples (seawater filters and sponge tissue samples) were stored at −80°C. DNA was extracted from approximately 0.25 g of sponge tissue, or from half a seawater filter, using the DNeasy PowerSoil Kit (Qiagen), following manufacturer's instructions. DNA extractions from filters and sponge tissue were performed days after sampling upon arrival at GEOMAR Kiel (i.e., in 2017 and 2018). The quality and yield of the extracted DNA was evaluated by NanoDrop spectrophotometry. Aliquots of DNA from 134 selected sponge (sponge species

TABLE 1 Overview of the 116 sponge samples analyzed in this study.

Species	Class	Growth form	Microbial abundance	Depth range (m)	Replicates (*n*)
Amphidiscella monai	Hexactinellida	Stalked	LMA	1729–2184	8
Aphrocallistidae indet.	Hexactinellida	Vase	LMA	2113	4
Caulophacus arcticus	Hexactinellida	Stalked	LMA	1656–2113	2
Cladorhiza sp.	Demospongiae	Arborescent	LMA	1678	3
Clathrina pellucida	Calcarea	Tubular	LMA	2184	4
Craniella infrequens	Demospongiae	Globular (small)	HMA	587–897	17
Craniella zetlandica	Demospongiae	Globular (small)	HMA	587	4
Geodia hentscheli	Demospongiae	Globular (small)	HMA	587–2184	15
Geodia parva	Demospongiae	Globular (massive)	HMA	587–2184	14
Geodia sp.	Demospongiae	Globular (massive)	HMA	587	6
Hexadella dedritifera	Demospongiae	Encrusting	LMA	580–600	5
Lissodendoryx complicata	Demospongiae	Arborescent	LMA	1297	4
Schaudinnia rosea	Hexactinellida	Vase	LMA	580–1316	16
Stelletta rhaphidiophora	Demospongiae	Globular (massive)	HMA	580–2184	4
Stylocordyla borealis	Demospongiae	Stalked	LMA	897–1034	6
Thenea valdiviae	Demospongiae	Globular (small)	LMA	587	4

Note: For each sponge species, class, growth form, microbial abundance (high: HMA, and low: LMA), the depth range (m) of collection and number of replicate specimens are listed. For additional collection metadata, see the deposited metadata file at the repository https://zenodo.org with DOI: 10.5281/zenodo.7326708 (Brodnicke et al., 2022).

with two or more specimens collected) and water samples were analyzed for fish DNA in laboratories designed for work with low concentration DNA and with strict cleaning procedures. The presence of fish DNA in the extracts from sponges and water samples was confirmed by amplification of a fragment of the mitochondrial 12S rRNA gene in a quantitative-PCR (qPCR) setup, using the vertebrate primer sets MiFish-U targeting bony fish and MiFish-E targeting elasmobranchs (Miya et al., 2015). The qPCR setup was prepared as 25 μL total reaction volumes comprising 1 μL forward and reverse primer (each in concentrations of 10 μM), 2 μL dNTP (25 mM), 0.2 μL AmpliTaq gold DNA polymerase (5 u/μL) (Applied Biosystems), 2.5 μL PCR Gold buffer (×10) (Applied Biosystems), 0.25 μL BSA (25 mg/mL) (Fisher Biotec), 0.5 μL GC enhancer (Applied Biosystems), 2 μL $MgCl_2$ (25 mM), 1 μL SYBR-green mix (DMSO, SYBR and ROX), 2 μL template DNA and 12.55 μL ddH_2O. Thermocycler settings were setup for the qPCR on an MxPro3005 (Agilent Technologies), with 10 min at 95°C, 50 cycles of 30 s at 95°C, 30 s at 50°C and 1 min at 72°C, followed by 5 min at 72°C. The 134 extracts were sent to the sequencing facility at the Genome Centre (Barts and the London School of Medicine and Dentistry). Here amplicon amplification was performed in triplicates of each sample with negative controls. The PCR reactions were set up as for the tested qPCR and the same thermocyler settings were used. The Amplicons were sequenced to a total of 15 M 250 paired-end read sequence depth on illumina MiSeq technology.

2.5 | Data analysis

The 250 paired-end demultiplexed sequences were truncated using 'DADA2', which is based on the DADA algorithm (Rosen et al., 2012), by adopting previously published bioinformatic code (Reinholdt Jensen et al., 2021). The code was executed in R v4.0.2 using 'Sickle' v1.33 in which process chimeras were removed (Callahan et al., 2016; Joshi & Sickle, 2011; R Core Team, 2022). For 'sickle', we set the 'fastqPairedFilter function' and maxEE value to 100 and 2, respectively, and the length and quality to 100 and 28, respectively. The remaining sequences were assigned taxonomical identification by adopting previously published code and using Basic Local Alignment Search Tool (BLAST) in 'blastn' v.2.8.1 on the National Center for Biotechnology Information (NCBI) genbank nucleotide database on 2022-Jan-25 (Altschul et al., 1990; Benson, 2004; Frøslev et al., 2017). We requested a maximum of 2000 identified sequences per sequence submitted with >90% query coverage and >80% sequence similarity.

Each of the unique taxonomically assigned sequences (2718) was further filtered and manually checked for misidentification occurring in the pipeline and the GenBank BLAST function (Ashelford et al., 2005). We only kept sequences matching with 100% sequence overlap and >98% sequence similarity with the query sequence, similar to previous studies with species level taxonomic assignment (Sigsgaard et al., 2019). The DNA sequences found in the negative controls, non-target sequences which comprised non-chordates, domestic animals (common contaminants of eDNA samples both in field and during handling; Cai et al., 2022; Klepke et al., 2022; Sigsgaard et al., 2019). Fish not known from this geographic region were excluded from both water and sponge eDNA data (Appendix S1: Table S1). Manual curation that beforehand excludes non-native species for the assessed area is commonly done in eDNA studies including marine sponge eDNA studies (Cai et al., 2022; Turon et al., 2020). Sequence reads equally similar (same % sequence similarity) to several taxa, the taxa matching the geographic and deep-sea region was assigned to the sequence read.

The resulting species occurrence data from sponge tissue samples, filtered water samples and video were processed in R v4.1.2 (R Core Team, 2022). The presence/absence data was modeled as the presence of a fish given the species of sponge with a generalized linear model (GLM) with a binomial distribution fitting presence/absence data. This was done using the 'glm' function that includes the interaction effect between fish and sponge species. The detection probability of a fish given the fish occurrence and sponge species were extracted on the response scale of the model (0-1). Difference in fish detection by sponge species and grouped by: microbial activity (HMA or LMA), sponge growth form, sponge taxonomic ranks, expedition year and observation method (water eDNA, sponge eDNA and video observations) were also calculated by estimated marginal means from the fitted models including those variables. We grouped the sponges based on their microbial abundance (high and low), which is linked to ecological relevance (Morganti et al., 2020; Weisz et al., 2008). However, although there was relatively even distribution of HMA and LMA sponges in our sample set, the opportunistic nature of the sampling meant this was not designed or planned with any such accuracy and interpretation of the results should be taken with this in mind. The classification of microbial abundance are based on Busch et al. (2022) and Busch, Beazley, et al. (2020). The significant differences between sponge species, sampling years and observation methods were assessed by pairwise comparisons. Figures were prepared with the ggplot2 package (Wickham, 2016), the map in 'ArcGIS' v10.8.1 and all statistical output can be found in Appendix S1: Supplementary Material S1.

3 | RESULTS

3.1 | Fish diversity detected by analysis of sponge derived eDNA

Eleven fish species were found by eDNA analysis across the 16 sponge species (Figures 2 and 3). The highest diversity was detected from the sponge *Schaudinnia rosea* (Fristedt, 1887) (seven fish species), followed by *Geodia parva* (Hansen, 1885) (five fish species) and *Amphidiscella monai* (Tabachnick and Lévi, 1997) (four fish species). Pairwise comparison (between the sponge species) found no significant difference in fish diversity detected ($p > 0.05$). However, *A. monai* had the highest single fish detection probability (0.375), i.e., 37% *A. monai* sampled were positive for American plaice (*Hippoglossoides platessoides* (Fabricius, 1780)) eDNA. 43.8% of the sponge species assessed in this study (or seven of 16) revealed no fish eDNA. There was also no significant difference in fish detection between sponges sampled in different years ($p = 0.98$).

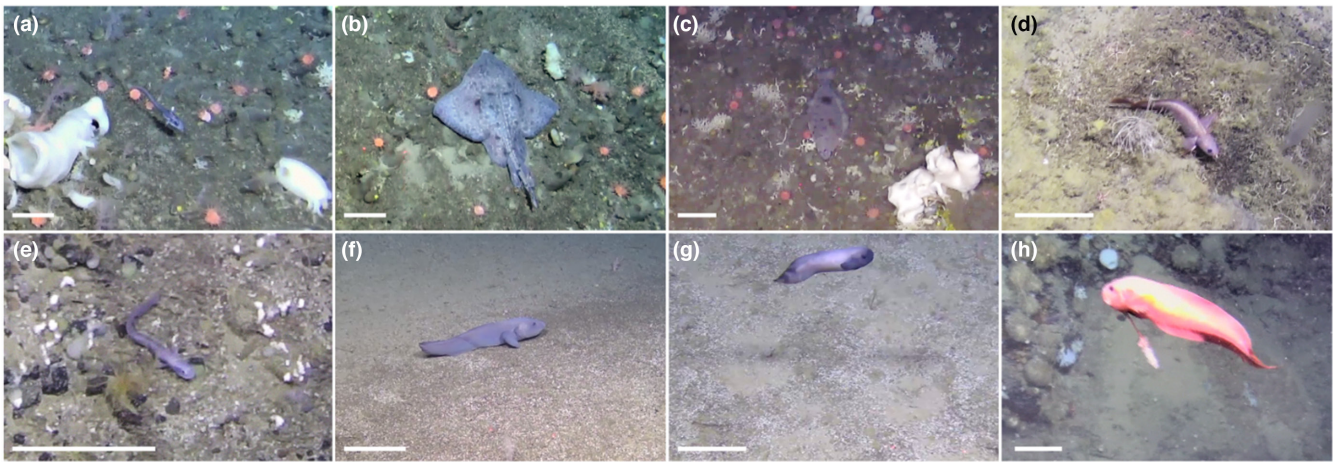

FIGURE 2 Representative images of eight of the fish species observed across the two cruises in the ROV video footage. (a) *Macrourus berglax*, (b) *Amblyraja hyperborea*, (c) *Reinhardtius hippoglossoides*, (d) *Gaidropsarus argentatus*, (e) *Lycenchelys platyrhina*, (f) *Lycodes frigidus*, (g) *Paraliparis bathybius*, and (h) *Rhodichthys regina*. Scale bars in each panel represents the laser distance of 16 cm.

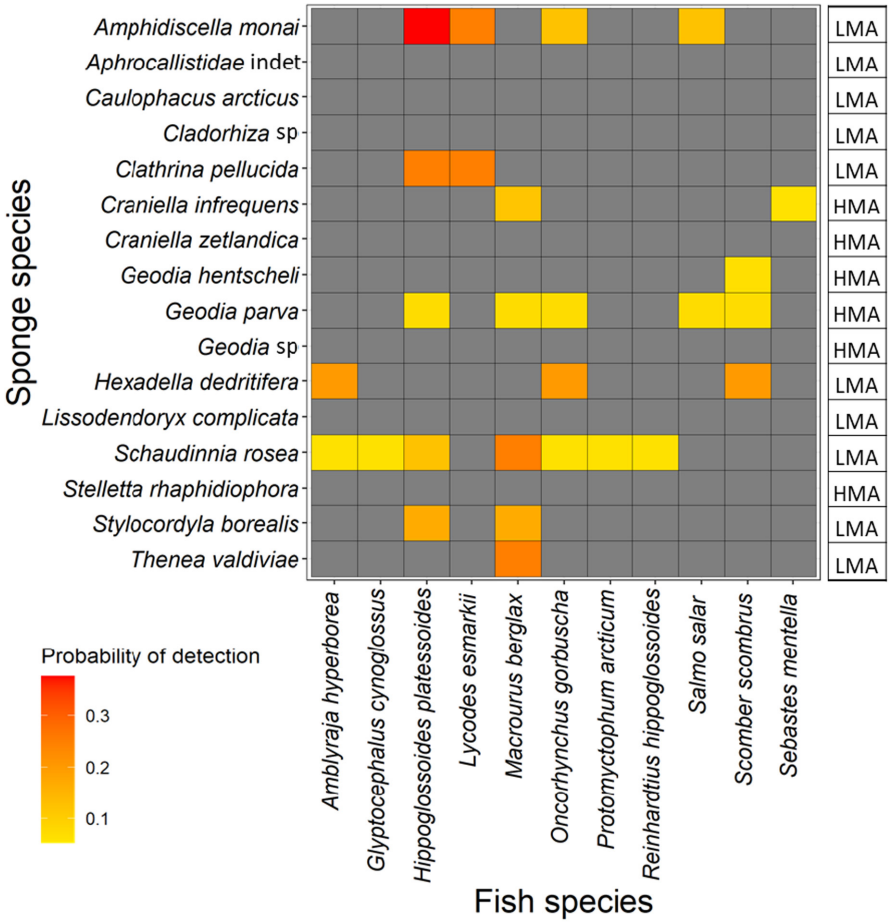

FIGURE 3 Detection probability (0-1) of fish eDNA in 16 species of deep-sea sponges across the sampling years 2017 and 2018. Gray: 0 detection probability. Right bar indicates the high or low microbial abundance (HMA/LMA) status of the sponge species.

3.2 | Sponge traits impacting fish eDNA detection

When exploring the impact of microbial abundance in sponges, there is a significant difference ($p = 0.01$) between the eDNA extracted from HMA and LMA sponges. LMA sponges have the highest number of fish species detected and have a higher overall detection probability (mean: 0.038 ± 0.039 SD, compared to a mean: 0.014 ± 0.017 SD) when compared to those sponges classified as HMA (Figure 4a). This was corroborated by the species accumulation curves for all 16 species of sponges and revealed a clear improvement of sampling efforts targeting LMA sponges (Figure 4b). However, the asymptote was not reached for any sponge group suggesting that more fish species are likely to be detected with increasing sampling and sequencing efforts. Furthermore, even within the LMA sponge group,

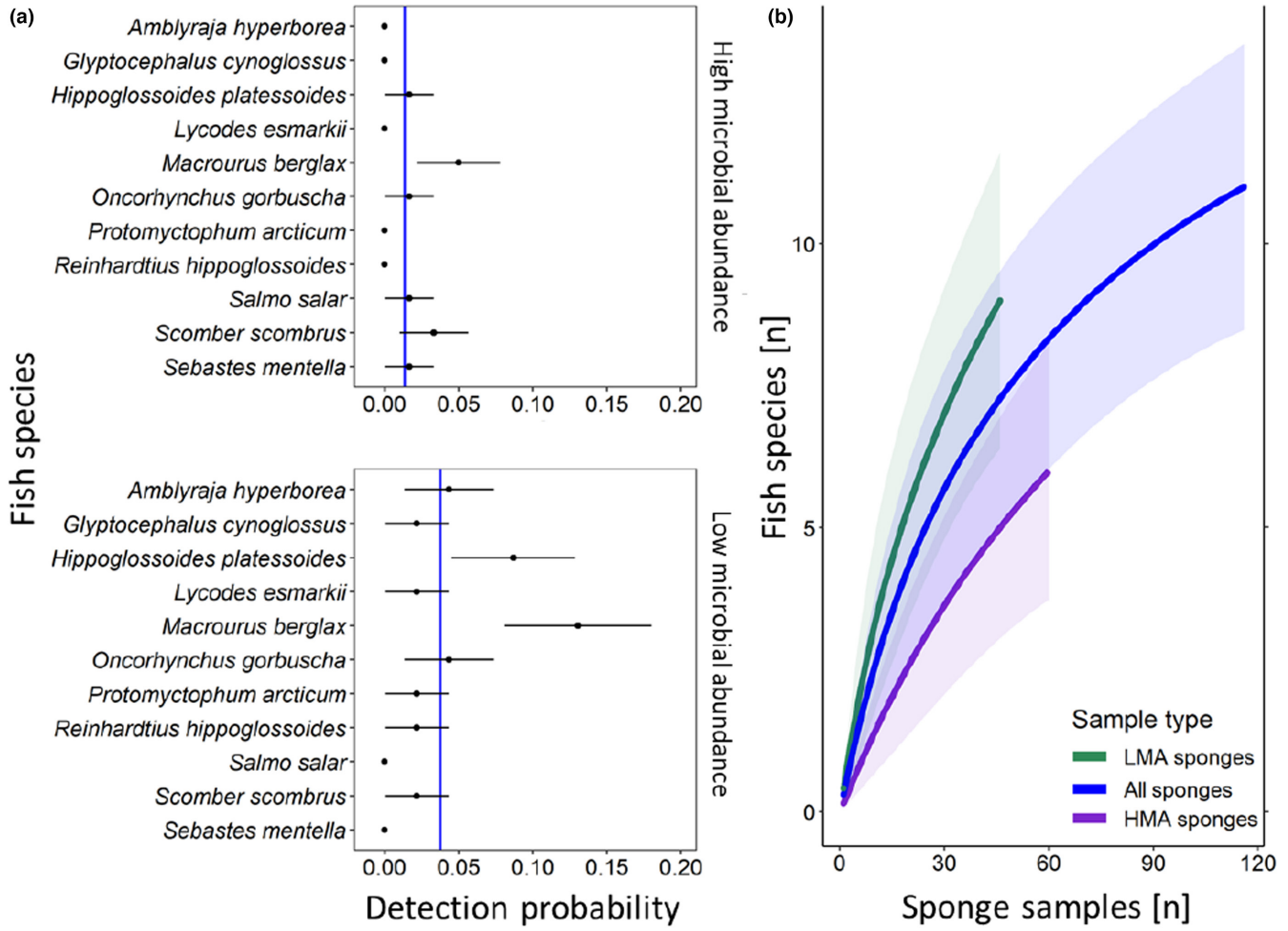

FIGURE 4 (a) The detection probability of fish species in sponges across the surveyed area grouped by microbial abundance; high and low. Horizontal bars are 95% confidence intervals, a single dot is no detection, and the blue vertical lines are mean probability for each group. (b) Fish eDNA detection curves based on LMA (low microbial abundance) sponges (green, top), all sponges (blue, middle), HMA (high microbial abundance) sponges (purple, bottom).

the detection probability of fish eDNA from sponge tissue was rather low (<0.15). Across all 16 sponge species, American plaice and Roughhead grenadier (*Macrourus berglax* (Lacepède, 1801)) were the most commonly detected fish and Witch flounder (*Glyptocephalus cynoglossus* (Linnaeus, 1758)), Greater eelpout (*Lycodes esmarkii* Collett, 1875), Lanternfish (*Protomyctophum arcticum* (Lütken, 1892)) and Greenland halibut the least. Of note, we were also able to detect the Minke whale (*Balaenoptera acutorostrata* (Lacépède, 1804)) in eDNA of the sponge, *Lissodendoryx* (*Lissodendoryx*) *complicata* (Hansen, 1885) (data not shown).

On class level, Hexactinellida sponges had the highest detection probability and was significantly different from Demospongiae, which showed the lowest ($p = 0.0014$) (Material S1: Figure S1a). No differences were found on family or order level ($p > 0.05$). Furthermore, sponge growth form did not influence fish detection probability ($p > 0.05$). Nevertheless, encrusting, stalked, tubular, and vase forms had similarly high detection, while globular growth forms had the lowest detection (Material S1: Figure S1b). Arborescent species always failed to reveal any fish detection.

3.3 | Comparing fish diversity assessment approaches

We detected 17 fish species across all three detection approaches and sampling years (2017 and 2018). Comparing detection methods in year 2018, we detect a significant difference ($p < 0.0001$) between the number of fish observed in videos and those detected by eDNA in filtered water. Surprisingly, water eDNA only detected a maximum of one species per sample (Figure 5a). To summarize, nine species were detected in the sponge tissue (several species detected in some samples), six with ROV and three in water eDNA (Figure 5b). There was little overlap between survey approaches and were no species which were detectable across all three approaches. Only American plaice was found in both the water eDNA samples and the sponge eDNA samples and Roughhead grenadier was found in both the sponge eDNA samples and observed on video. Greenland halibut have been observed in video footage from 2017 and was likely present in 2018 but not filmed in that year. The ROV from 2018 detected: Arctic skate (*Amblyraja hyperborea* (Collett, 1879)),

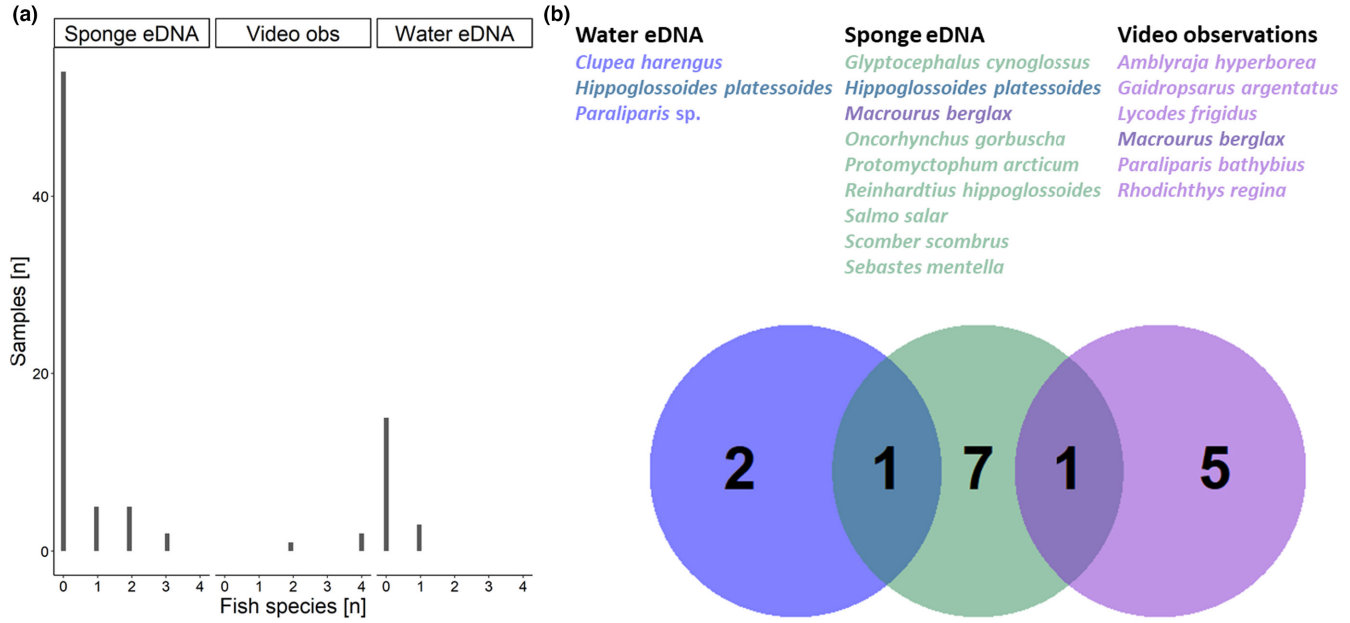

FIGURE 5 (a) Frequency of fish observation in the three observation approaches compared in 2018. Video observations correspond to remotely operated vehicle transects. (b) Venn diagram of fish species detected in each of three observation approaches used during 2018. Colors of species names represent the approach color (water eDNA (blue), sponge eDNA (green), or video observations (purple)) or shared color with neighboring observation approach.

Arctic rockling *Gaidropsarus argentatus* (Reinhardt, 1837), Glacial eelpout (*Lycodes frigidus* Collett, 1879), Roughhead grenadier, Black seasnail (*Paraliparis bathybius* (Collett, 1879)) and Threadfin seasnail (*Rhodichthys regina* Collett, 1879). *Lycenchelys platyrhina* (Jensen, 1902) and Greenland halibut were instead noted in the ROV dives from 2017.

4 | DISCUSSION

eDNA is a cost-effective way to detect species and shows promise for conservation practices and to inform marine protection measures (Grant et al., 2021; Mauvisseau et al., 2020; Shen et al., 2022; Thomsen & Willerslev, 2015). The eDNA approach has already been applied widely in the marine realm, including hard to reach areas such as the deep (Brandt et al., 2021; Miya, 2022). More recently, the concept of extracting eDNA from 'natural filters' such as sponges has been proposed (Cai et al., 2022; Mariani et al., 2019; Turon et al., 2020) and warrants further investigation to understand its practicality.

Here, we assessed the potential of using eDNA extracted from deep-sea Arctic marine sponges to assess demersal fish communities, in the context of a remote deep-sea ecosystem, the Schulz Bank. This was successful, and we identified the presence of 11 fish species through sponge derived eDNA. We believe, this observed number of species corresponds approximately to 19% of the fish species known to inhibit this part of the Arctic (i.e., the 57 species previously described; Christiansen & Reist, 2013; Møller et al., 2010). This is probably a cautious statistic, as the fish diversity at Schulz Bank specifically would likely be lower—yet this remains unknown. Indeed, only three species have previously been recorded at this location using AUV/ROVs imagery (Meyer et al., 2019). If we take this value, this current study has therefore almost quadrupled the known number of fish species for this site. Interestingly, our percentage species detection in the Arctic deep-sea is comparable with the percentage of species detection from sponge eDNA from shallow tropical coral reefs (compared to visual surveys; Turon et al., 2020). This points to the fact that eDNA extracted from deep-sea sponges will be a useful approach for marine biodiversity assessment of the deep moving forward.

We further show that sponges with low microbial abundance (LMA) have (on average), higher fish detection probability than sponges with high microbial abundance (HMA). Out of the 16 sponge species sampled, *Amphidiscella monai* revealed the highest detection probability for a single fish species. This could be attributed to *A. monai* LMA status which may translate into slower DNA degradation (Busch et al., 2022; Moitinho-Silva et al., 2017; Zulkefli et al., 2019). In support of this, we also find that when focusing only on LMA sponge species, detection per sponge sampled is greatly improved. Additionally, LMA sponge species detect a wider array of fish species. This could also be a result of the higher than average pumping rates, or better particle retention—both often reported in LMA sponge species (Kahn et al., 2015; Morganti et al., 2019; Weisz et al., 2008). Although, *A. monai* gave the highest fish detection probability, *Schaudinnia rosea*, displayed the widest diversity of fish in its eDNA. This may point to some sponge species being more optimal for eDNA sampling, i.e., a species that retains more eDNA from their surroundings than others. Indeed, this was supported by our results showing sponges from the class Hexactinellida, by and large, retained the most eDNA. This may mean certain taxonomic groups should be targeted for this type of research. However,

all the Hexactinellida sponges investigated in this study were also LMAs—so we cannot detangle these two factors *re* optimum eDNA retention. The finding that certain species outperform others has been shown before (Cai et al., 2022; Turon et al., 2020). In this regard, we also show an inability to retrieve eDNA from seven sponge species assessed. This result importantly suggests which sponges not to target for future sponge eDNA studies.

In total, we were able to identify 17 fish species at Schulz Bank—this was across the 2 years and the different methods. This equates to almost 30% of the estimated diversity of the region (Christiansen & Reist, 2013; Møller et al., 2010). However, there was low overlap in species detection between ROV observations and the eDNA samples. This was not completely unexpected, as Turon et al. presented similar results between sponge eDNA and visual surveys of the fish communities inhabiting coral reefs (Turon et al., 2020). Indeed, sponge eDNA has already been shown to more commonly detect vagrant fish species (Turon et al., 2020). In our study, more pelagic fish such as Atlantic mackerel (*Scomber scombrus* Linnaeus, 1758), Atlantic salmon (*Salmo salar* Linnaeus, 1758) and Lanternfish were found in sponge eDNA than in the water samples or the ROV videos. Another explanation to this lack of overlap between survey methods and the greater number of fish being detected by the sponge eDNA samples may be due to the sponges ability to retain filtered eDNA for periods of days or weeks (Cai et al., 2022).

However, despite performing better, the same lack of overlap between sponge eDNA and the visual survey means that one cannot rely on the use of eDNA alone. Even the two types of eDNA samples (sponge and water) shared only one species between them. This may be explained due to a methodological aspect of the sampling. Sponges are likely to filter the water in the benthic boundary layer, while the water eDNA was collected from 10 m above the seafloor. That said, they both detected pelagic and demersal fish species, so this suggest that any effect like this would be minimal. An alternate hypothesis explaining this lack of similarity between sample types could be due to the nature of the survey site itself and the spatial distances between the water and sponge eDNA sampling (Mathieu et al., 2020). Indeed, hydrological investigations at the survey site suggests vertical density stratification occurs (Hanz et al., 2021). This could result in reduced water mixing and the effective trapping of shed eDNA on micro- or local-scales (Jeunen et al., 2020). Furthermore, stochasticity in eDNA in any water body has also commonly been shown to lead to incongruence between samples and survey methods and can even be temporally variable (Agersnap et al., 2022; Jensen et al., 2022). The later could have larger impacts when you consider diurnal vertical migrations of certain fish species such as the Lanternfish (Saunders et al., 2015).

Interestingly, within the sponge eDNA samples we observed a relatively high probability of finding certain fish species such as the American plaice and Roughhead grenadier. Such a result may be indicative of particular high abundances or biomass of these species at the survey site (Meyer et al., 2019; Salter et al., 2019). Furthermore, we were able to detect several commercially important fish species using this approach, which may offer an important economic use for this survey method. For example, the Greenland halibut, Atlantic mackerel and Beaked redfish. Detection of these species was certainly not unexpected as redfish are known to use structures like sponges and corals for their breeding grounds (Auster, 2005). However, Greenland halibut are often documented with high abundances at deep-sea sponge grounds, likely attracted due to the prey availability or suitable nursery/hatchery grounds (Hogg et al., 2010; Kenchington et al., 2013; Klitgaard & Tendal, 2004). In contrast, the detection of Arctic skate by sponge eDNA warrants special note. This species is of particular interest, as the current status and life-history of this deep-sea skate is largely unknown (Climent, 2021). Our study supports the idea that Schulz Bank may be a key nursery ground for this species Arctic skate (Meyer et al., 2019), and further use of sponge eDNA may shed even more light on this deep-sea dwelling elasmobranch.

Despite, the interesting findings shown here, eDNA retention across all sponge samples was relatively low or highly variable at best. We therefore acknowledge that this may point to a possible limit regarding the applicability of said approach. That said, there are several improvements to the sampling and methodology which could be implemented into future studies likely improving reliability. For example, the selection of sponges from certain classes, those with LMA, higher pumping efficiency, better particle retention, and/or lower metabolism. Furthermore, sponges have internal environmental and chemical gradients (Hughes et al., 2022). eDNA might be better preserved in certain compartments of the sponge. Specifically targeting different tissue types could therefore yield more eDNA per sample. One final interesting finding from this study was that despite the relatively large sample size ($n = 116$), we did not reach the asymptote of the species accumulation curve. This suggests a larger number of sponge samples would be necessary to give a full representative assessment of the diversity present within this region. To reach this sample number, without decimating a vulnerable ecosystem, sponge bycatch from fisheries could/should be utilized instead (Klitgaard & Tendal, 2004).

Our study provides new ecological insights into the fish community of a vulnerable Arctic deep-sea ecosystem (Danovaro et al., 2020; Hogg et al., 2010). This proof-of-concept study also provided a robust baseline fish diversity assessment of the sponge ground compiling several survey approaches ensuring robust and reliable data. Such integrative use of sponge and water eDNA in combination with more traditional survey methods such as ROVs will be increasingly important tools to inform marine spatial planning, conservation, and protection of remote, hard to reach areas, such as the Schulz Bank (Danovaro et al., 2020). Continued monitoring of such sites may well identify northerly range expansions or crashes in populations of key or commercial fish species as the impacts of climate change continue (Christiansen et al., 2014; Danovaro et al., 2020).

AUTHOR CONTRIBUTIONS

All the authors meet all the criteria for authorship defined in the Scientific Reports author guidelines. OBB, MJS, and UHH

conceived the study idea. Study design and sample selection was done by OBB, MJS, KB, and UHH. KB and UHH provided DNA extracts, metadata and sponge ID which was complemented by JRX. JRX and HKM provided remotely operated vehicle imagery and data. HKM annotated video footage and made the site map and fish figure (Figures 1 and 2, respectively). DNA assessment and bioinformatics was done by OBB and SWK. Statistics and figures of genetic results were performed by OBB. PRM performed fish ID and taxonomical assignment and curation of fish sequences. All authors contributed to discussion and interpretation of results. OBB and MJS lead writing of the manuscript and all authors critically contributed to the manuscript drafts and approved the final version for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare there are no competing interests.

DATA AVAILABILITY STATEMENT

Metadata can be downloaded at Zenodo (https://zenodo.org) with DOI: 10.5281/zenodo.7326708. Sequence data is available from the corresponding author on request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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