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Exploring Mesophotic Coral Reef Boundaries Using Environmental DNA Metabarcoding

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ABSTRACT

Mesophotic coral reefs are deep tropical reefs (40–160 m) characterized by light attenuation that are renowned for their unique biodiversity and ecological significance. They have long been proposed to supplement shallow reefs and serve as refugia for shallow-water species in the face of ocean warming. Nevertheless, these deep reefs are still severely understudied compared to shallow reefs, mainly due to the technical challenges inherent to deep water environments. Here, we utilized environmental DNA (eDNA) metabarcoding to provide high-resolution spatial biodiversity profiling and evaluate changes in the community structure across the steep depth gradient in the Gulf of Aqaba, Red Sea. eDNA was extracted from water samples spanning from shallow reefs to mesophotic depths and beyond (10 to 200 m), utilizing a metabarcoding pipeline focusing on metazoan assemblages. We show that community structure differs significantly across depth, particularly between the shallow reef and lower mesophotic. Specifically, we show that depth proved to be more influential than site in shaping the respective community structures, regardless of the statutory status and protection level of the sites. We thus provide quantitative evidence to support the current mesophotic classification as defined by abiotic factors. We further provide support for effective species overlap between the upper mesophotic and shallow depth communities, asserting the potential for effective bathymetric range shifts in response to warming. This study highlights the potential of eDNA metabarcoding as a practical tool for mesophotic reefs and deep-sea monitoring.

1 | Introduction

Coral reefs are known as the "rainforests of the ocean" as they contain the highest levels of biodiversity per unit area, translating to up to 25% of all marine species, despite covering only 1% of the marine environment (Spalding 2001). However, the world's coral reefs are facing major threats, and to date, coral reef cover around the world has been reduced by over 50% since the 1950s (Eddy et al. 2021). This decline is mainly attributed to rising sea temperatures and subsequent coral bleaching (Eakin et al. 2019; McLachlan et al. 2020), but is also associated with coastal development and overexpansion (Carlson et al. 2019), mass mortalities (Roth et al. 2024), ocean acidification (Mollica et al. 2018), light pollution (Levy et al. 2020), and predator outbreaks, such as the crown-of-thorns seastar (Uthicke et al. 2024).

Apart from the unique characteristics and composition of coral reefs across their wide geographical distribution, coral reefs can be broadly categorized based on depth and light attenuation into shallow and mesophotic reefs. Light is the fundamental abiotic factor governing the distribution of zooxanthellate corals (Lesser et al. 2009). Mesophotic reefs are thus defined as coral reef communities that occupy habitats that are characterized by lowlight penetration. Mesophotic reefs can be further divided into roughly two segments: the upper and lower mesophotic (Kahng et al. 2019; Tamir

et al. 2019). While the boundaries between the two are mainly set based on depth and light penetration, these areas can also be differentiated by their species assemblages and community structure (Lesser et al. 2009). Further delineation between the upper (i.e., shallower) and lower (i.e., deeper) parts of mesophotic reefs has been described roughly at the 60 m depth range (Lesser et al. 2019), demonstrating that the upper mesophotic fauna is more related to the shallow reef, while the lower mesophotic is characterized by the dominance of deep species (Bongaerts et al. 2017; Rocha et al. 2018; Loiseau et al. 2023). The rariphotic zone defines the habitat just under mesophotic reefs (~150 to ~310 m), forming a transition zone between the coral reef community and the deep-sea community (Baldwin et al. 2018; Jacquemont et al. 2024).

The most commonly used boundary between shallow and mesophotic reef is the 30 m depth mark (Pyle and Copus 2019). However, considering the attenuation of light as the crucial factor in determining this boundary, absolute mesophotic depth thresholds differ significantly across the world oceans. In clear oligotrophic water habitats, the mesophotic boundary is often set at a greater depth (e.g., 40 m, Kahng et al. 2019). In contrast, habitats found in more turbid waters, such as Singapore's or Australian nearshore reefs, experience significantly reduced light penetration (Morgan et al. 2016; Chow et al. 2019). Consequently, the mesophotic boundary in such regions may manifest at depths as shallow as 20 m (Morgan et al. 2020). Specifically, a fundamental caveat when examining the boundary between shallow and mesophotic reefs is the common 30 m depth limit of scientific SCUBA diving, reflecting a technical limitation rather than a real biological boundary (Pyle and Copus 2019). The lower boundary of the mesophotic reef is often set to a depth of 150 m, although zooxanthellate corals can occasionally be found at even greater depths and have been recorded down to a depth of 172 m (Kahng et al. 2019; Rouzé et al. 2021). Consequently, a more accurate definition of the lower mesophotic boundary is the deepest depth occupied by photosynthetic zooxanthellate corals (Kahng et al. 2010).

One of the best studied reef systems to date is the coral reefs of the northern Red Sea and Gulf of Aqaba (GOA), characterized by exceptionally clear and oligotrophic water, subsequently demonstrating a deeper boundary between the shallow and mesophotic reefs, set at around 40 m (Tamir et al. 2017, 2019). This region is known for its high levels of endemism (DiBattista et al. 2016) as well as being home to one of the northernmost coral reefs in the world. Particularly, corals of the GOA are often acknowledged as "super corals" due to their high heat resistance, and as such, have been proposed as a refuge for coral species in the wake of intensifying global ocean warming (Fine et al. 2013). Furthermore, mesophotic reefs are sometimes considered as potential refugia for shallow corals, although the latter require species overlap between the upper mesophotic and shallow reefs (Slattery et al. 2011; Laverick et al. 2018; Tavakoli-Kolour et al. 2024; Velasco-Lozano et al. 2024) and mounting evidence shows that deep reefs are not immune to disturbances (e.g., Rocha et al. 2018). These two factors emphasize the importance of mesophotic reefs in the GOA, making them one of the best studied coral reef systems in the world (Berumen et al. 2013; Turner et al. 2017; Eyal et al. 2019). Furthermore, the unique bathymetric structure of the GOA, and particularly the steep bathymetric slope (Tibor et al. 2010), places the mesophotic and shallow reefs at remarkably high proximity due to the short distances between depths (Eyal et al. 2019), sometimes only a few meters apart, and with a short transition zone, which makes them an appealing case study to examine mesophotic boundaries. However, although mesophotic reefs make up about 80% of coral reefs (Loya et al. 2019), these reefs are still severely understudied compared to the shallow, more accessible reefs, with much of the biodiversity and community structures of mesophotic communities still undescribed (Menza et al. 2007; Slattery et al. 2024).

Over the past decade, environmental DNA (eDNA) metabarcoding has emerged as a reliable method for biodiversity monitoring and a fast and cost-effective alternative to traditional visual censusing and capture-based surveys (Aglieri et al. 2021; Fediajevaite et al. 2021; Roblet et al. 2024). eDNA metabarcoding has several advantages compared to traditional survey methods: (1) it is highly effective in identifying rare, cryptic, and elusive species, (2) it is noninvasive, causing no damage to organisms or the environment (Beng and Corlett 2020; Yao et al. 2022), (3) it requires little taxonomic expertise and is less prone to biases that are derived from the observer, and (4) it can be used for biodiversity monitoring in habitats that are hard to reach by traditional surveys, most notably, deep-sea regions (Laroche et al. 2020; Govindarajan et al. 2021). Still, eDNA metabarcoding is not without limitations, particularly as it heavily relies on accurate barcode reference databases (Weigand et al. 2019), is prone to DNA contamination, persistence and transport (Goldberg et al. 2016; Harrison et al. 2019), and data are mostly limited to biodiversity assessment and species distribution (Minamoto 2022). In recent years, eDNA-based studies for monitoring the marine environment have increased exponentially (Takahashi et al. 2023; Liu et al. 2025), becoming an important tool for biodiversity conservation across diverse environments (Deiner et al. 2017). Nevertheless, little work has been done using eDNA metabarcoding to explore mesophotic coral reefs, and most coral reef eDNA studies still largely focus on surface or shallow water (< 30 m) environments, with only a few studies focusing on the mesophotic (Hoban et al. 2023; Muff et al. 2023; Nishitsuji et al. 2024).

Here we aim to explore the boundaries of mesophotic coral reefs in the GOA, asking whether expected depth boundaries—36 m for the boundary between the shallow reef and the upper mesophotic, 76 m for the boundary between the upper and lower mesophotic, and 120 m for the boundary between the lower mesophotic and the rariphotic zone (following Tamir et al. 2019) are also reflected in shifts in the metazoan community structure. We employed high-resolution eDNA sampling across the depth gradient, spanning from shallow reefs (10 m) to the rariphotic zone (200 m) – beyond the range of coral reefs. Leveraging the exceptionally dense spatial arrangement of mesophotic reefs in the GOA, we further assessed the sensitivity of contemporary eDNA metabarcoding pipelines to resolve changes in species distributions and community composition across spatially adjacent localities. Together, our results highlight eDNA metabarcoding as a powerful and scalable tool for monitoring biodiversity patterns in mesophotic reefs and other remote or logistically challenging marine environments where conventional survey approaches are limited or impractical.

2 | Materials and Methods

2.1 | Study Sites

eDNA was obtained from water samples collected at three sites in the GOA: Kissoski Beach (KB), Eilat's Coral Nature Reserve (ECNR), and Princess Beach (PB) (Figure 1). Sites formed a north to south gradient with growing distance from the main urban centers near the cities of Eilat (Israel) and Aqaba (Jordan), with the KB site being closest to the urban centers, the ECNR 5.1 km to the south of KB, and the PB site being the furthest south 2.5 km from the ECNR. Apart from its geographic position between KB and PB sites, the ECNR represents a restricted, well-protected area, with tight regulations on human activities and disturbance. Sites also differ in their structural complexity; while the corals at the KB and the PB are largely arranged in scattered knolls formations (freestanding coral structures), the reefs of the ECNR consist of a continuous reef flat. The GOA exhibits a pronounced seasonal hydrodynamic cycle characterized by deep convective mixing during winter (November–March), when the water column can mix to several hundred meters in depth, and strong thermal stratification during summer (June–September), when mixing is restricted to the upper ~30 m of the water column (Genin et al. 1995; Labiosa et al. 2003). Water samples were collected in the first week of July 2023, during midsummer time in the GOA and the peak in water column stratification. Environmental measurements were obtained from the National Monitoring Program of Israel in the Gulf of Eilat (https://iui-eilat.huji.ac.il/en/about-monitoring-program) and are available in Table S1.

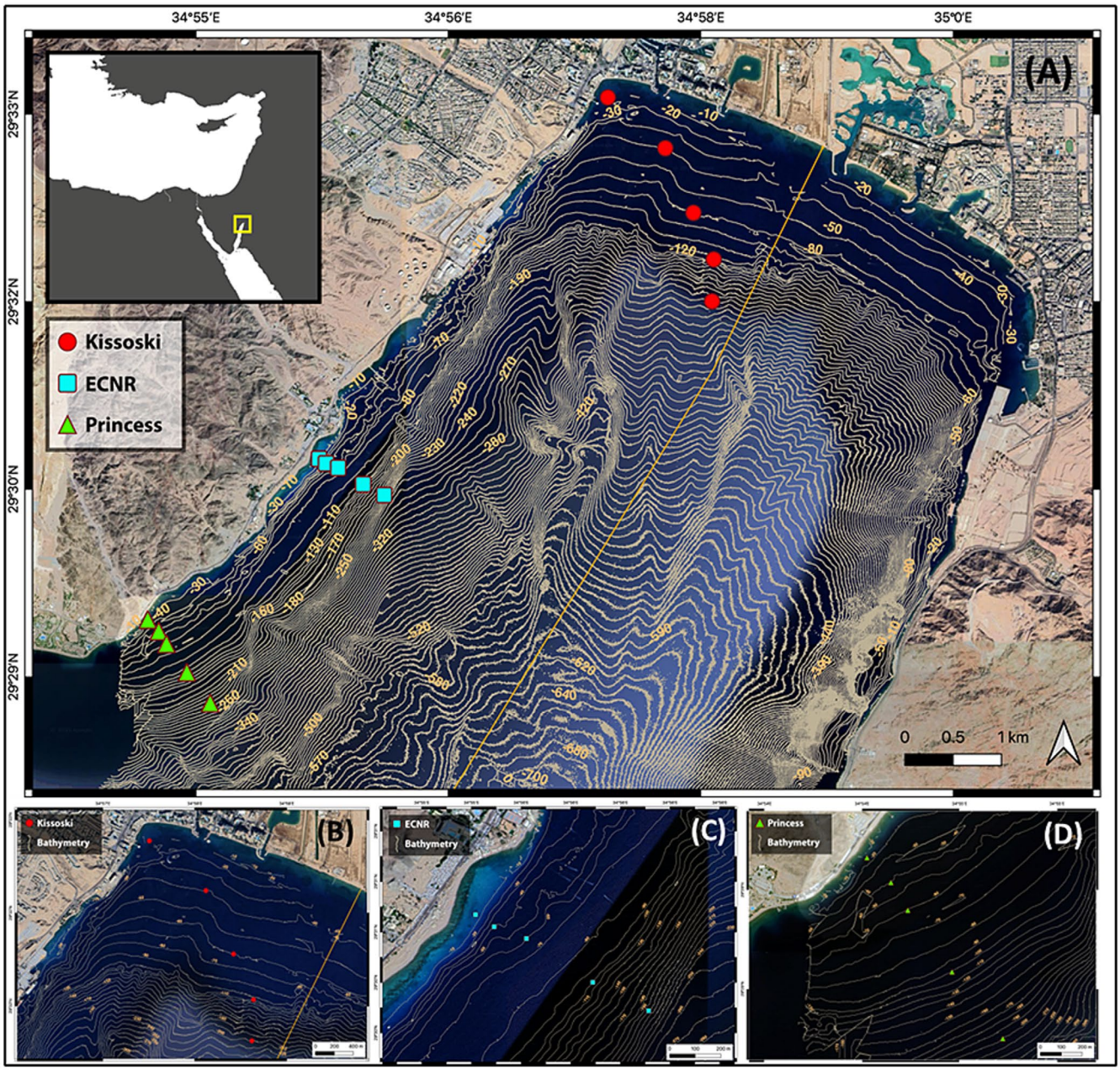

FIGURE 1 | (A) Map of the northern GOA showing bathymetry and sampling locations. Site specific maps: (B) Kissoski Beach (KB), (C) Eilat Coral Nature Reserve (ECNR), (D) Princess Beach (PB). Colored symbols represent sampling points. Sampling coordinates can be found in Table S2.

2.2 | eDNA Sampling

At each site, samples were collected at five different depths: 10, 40, 60, 100, and 200 m, at a distance of approximately one meter from the bottom. Depth was measured by a pre-marked rope and cross-validated by a single-beam echo sounder operated from the sampling vessel. All samples were collected during daytime, between 9 a.m. and 12 p.m. Sampling depths were selected to represent the shallow reef (10 m), the border between the shallow and upper mesophotic reef (40 m), the upper mesophotic reef (60 m), the lower mesophotic reef (100 m), and the rariphotic depth (200 m), following the classification of Tamir et al. (2019). Water samples were collected using Go-Flow 8 L Niskin bottles (General Oceanics) that were deployed from a small boat. While eDNA sampling from larger water volumes is largely regarded as beneficial—capable of detecting rare and low-represented taxa (Juhel et al. 2020; Stauffer et al. 2021), the strict regulations for sampling in the northern GOA restricted current sampling to small vessels operating hand-deployed Niskin samplers. At each site, sampling was conducted from the shallowest to the deepest point in order to eliminate potential carryover genetic material from the deep; however, carryover in the opposite direction cannot be ruled out. While it is inevitable that the sampling device must cross shallow depths on the way to the deepest point (risking potential eDNA carryover to the deep), deep to shallow carryover can be avoided by deploying an ascending sampling design. Following retrieval of the Niskin bottles, 5.5 L of seawater were collected into sterile containers and kept cool until retrieval to the laboratory. To further reduce the risk of cross contaminations, strict sterilization protocols were maintained throughout, including the use of single-use gloves at all stages and application of bleach sterilization of all containers, operating surfaces, and filtration elements. Field ready kits for each sampling point (including tubing, gloves, and accessories) were sterilized by UV cross linking and prepared in sealed bags for field deployment. Gloves and draining pipes were replaced between samples. The full 5.5 L containers were kept in a cooling tank and transported to a 4°C fridge immediately upon return to shore. All equipment was sterilized with a 10% bleach solution and washed in deionized water (DW) at the end of each sampling day. Sterilized control containers containing DW were taken on the boat during each sampling day to account for potential contamination during sampling.

2.3 | Laboratory Procedures

2.3.1 | eDNA Filtering

All samples were filtered within 8 h of sampling. Filtration was conducted at a designated laboratory at the Interuniversity Institute of Marine Sciences (IUI) in Eilat. Each water sample container was filtered in triplicate technical repetitions, with a water volume of 1.8 L filtered on each filter. We used 47 mm sterile cellulose filters with a pore size of 0.45 μm (Millipore). Field controls were filtered under the same conditions as regular samples. Technical replicates were established as a contamination control procedure and to alleviate the risk of filter clogging during filtration. Following filtration, filters were kept at −80°C until use. All filtering equipment was purified with 10% bleach solution and UV irradiated for 10 min between samples.

2.3.2 | eDNA Extraction

DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen) one week after filtration, employing a modified extraction protocol. Briefly, samples were incubated overnight in 900 μL lysis buffer containing 40 μL Proteinase K and 860 μL Buffer ATL at 56°C and 550 rpm. The next morning, 900 μL of Buffer AL was added to each sample, and the tubes were incubated for an additional 30 min under the same conditions. At this point, the filters were removed, and the solution was divided into two 1.5 mL tubes, each containing 400 μL of molecular-grade 100% ethanol. The solution was transferred to the spin columns (600 μL of the solution in each of four loading rounds) and centrifuged at 8000 rpm for 1 min, discarding the flowthrough between centrifugations. The remaining steps followed the manufacturer's manual. Final DNA elutions (using 50 μL EA buffer) were stored at −80°C and transported on ice to Tel-Aviv for further processing. Samples from different sites were extracted on different days, and on each extraction day, a negative control comprising only reagents was included. Upon completion of the extractions, final elutions were measured using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific) to determine DNA concentrations. As in the field procedures, working spaces and tweezers were sterilized with a 10% bleach solution between samples, and tubes were UV-irradiated prior to use.

2.3.3 | PCR Amplification

Samples were PCR amplified using the mlCOIintF and JgHCO2198 primer set (Leray et al. 2013), which generates a 313-base pair (bp) amplicon of the COI gene that is widely used to differentiate between metazoan species (Table 1). The present 'COI-first' approach was selected given the limited and highly biased taxonomic knowledge from the region (strongly skewed towards fishes and echinoderms), aiming to capture the broadest breadth of metazoans that are represented in public databases. The SP1 and SP2 linkers were added to the forward and reverse primers, respectively, to facilitate the library preparation stage (Table 1). The samples were amplified in a final volume of 20 μL using 10 μL Amplitaq Gold 360 Master Mix (Thermo Fisher Scientific), 0.8 μL of each primer, 6.4 μL DDW, and 2 μL of template DNA. Thermocycler conditions were as follows: initial denaturation at 94°C for 10 min followed by 35 cycles of 95°C for 30 s, 46°C for 30 s, 72°C for 1 min, and a final elongation stage at 72°C for 10 min (Turanov et al. 2024). A single PCR amplification was performed for each sample, including controls. Amplification success was evaluated by gel electrophoresis. DNA concentrations in the amplified samples were checked on a Qubit 4.0 fluorometer (Invitrogen) using the Qubit dsDNA Broad Range Assay Kit.

Library construction and next-generation sequencing were performed by Syntezza Inc. (Jerusalem, Israel). Samples were sequenced on an MGI DNBSEQ-G400 platform using DNA nanoball technology (Anslan et al. 2021), and the PE300 kit, which provides a read length of 300 bp from each direction. The samples were sequenced in three runs, with a total of 30 million reads and an average of 666 K reads per sample.

TABLE 1 | Primers (in blue) and linkers (in black) used in the current study, target gene, primer name, sequence, amplicon length, and source.

Target gene	Primer name	Primer sequence (5′-3′)	Amplicon length (bp)	References
COI	mlCOIintF	ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGWACWGGWTGAACWGTTAYCCYCC	313	Leray et al. (2013)
	JgHCO2198	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT TAIACYTCIGGRTGICCRAARAAYCA		Geller et al. (2013)

2.4 | Bioinformatic Processing

2.4.1 | Data Cleaning and Clustering

Sequence reads were processed in R v.4.2.2 (R Core Team 2024) on an Ubuntu (22.04) Linux platform. Primers and linkers were removed from the demultiplexed FASTQ files using Cutadapt v.3.5 (Martin 2011). Reads were then processed using the "DADA2" v.1.32 package (Callahan et al. 2016). Lowquality reads were filtered out, and read ends were trimmed using the "filterAndTrim" function. Identical reads were subsequently merged into unique sequences using the "derepFastq" function. Sequence data were denoised and resolved into amplicon sequence variant (ASV) using the "dada" function with the pool setting set to "pseudo" to expedite computational speed while still recovering rare ASVs (Callahan et al. 2017). Paired reads were then merged using the "mergePairs" function. Finally, chimeras were removed from the data set using the "removeBimeraDenovo" function. Post-clustering quality control of the ASVs was done using the "lulu" v.0.1 package with the default settings in R (Frøslev et al. 2017).

2.4.2 | Taxonomic Assignments

Three databases were used as reference for the taxonomic assignments: the BOLD system for COI barcoding of metazoans (release 4.24, downloaded on May 16, 2025) (Ratnasingham and Hebert 2007), the midori2 reference database for eukaryotic mitochondrial genes (release December 17, 2023, downloaded on June 9, 2025) (Machida et al. 2017), and our in-house database that mainly comprises taxonomically verified echinoderms from the GOA. All reference databases were pooled together using the "cat" command in the Ubuntu shell. The assignment was run locally using the BLASTn algorithm, setting a minimal query cover of 95% and 80% identity. We used the following cutoffs for assessing different taxonomic levels: 80% for Phylum, 85% for Class, 90% for Order, 95% for Family, 97% for Genus, and 98% for Species (Dugal et al. 2022). The algorithm returned the best three matches to check for conflicting assignments. The data were then cleaned manually and set to a uniform format with eight taxonomic levels (kingdom to species). The top match was selected based on the e-value score first and then on identity percentage. In case of conflicting matches, all candidate matches were checked for synonymy using the WoRMS database (World Register of Marine Species [WoRMS] 2025), and all fitted taxonomic assignments were made accordingly. If conflicting assignments remained, we checked the taxon distribution in WoRMS, FishBase (Froese and Pauly 2025), and the "Coral Trait Database" (Madin et al. 2016). In the sole instance where conflicting assignments could still not be resolved, the sequence was assigned to a higher taxonomic rank. The detected taxa were visualized using an interactive sunburst chart, created with the "plotly" package in R (Data S1).

2.5 | Statistics and Ecological Analysis

All statistical and ecological analyses were conducted in R v.4.2.2 (R Core Team 2024). We analyzed the data at two levels, initially at the ASV level (i.e., without taxonomic assignment), and subsequently at the taxonomically assigned genus or species level. While the ASV analysis is "blind" to taxonomy, it includes a far larger database and is not limited by the extent and completeness of taxonomic assignment in reference databases (Tapolczai et al. 2019; Marques et al. 2020; Mächler et al. 2021).

The "beta. pair" function from the "betapart" package (Baselga and Orme 2012) was used to calculate beta diversity based on the Jaccard Index. At each level, values were checked for both site and depth. Dissimilarity between sites and depths was compared to assess significant differences. Dissimilarity was checked between samples at the ASV and genus levels. The Jaccard dissimilarity index was calculated for both levels using the "vegdist" function from the "vegan" package (Oksanen et al. 2020). Significant effects of site and depth were checked using the "ADONIS" PERMANOVA function with 999 permutations. Results were visualized using nonmetric multidimensional scaling (NMDS) and plotted using "ggplot2". Finally, when significant differences between sites or depths were observed, a Tukey post hoc test was conducted to check which sites and depths were significantly different.

Fish and echinoderm species were also compared between the depths where they were detected in eDNA samples, and their known depth ranges from the literature (Table S3). Fish depth ranges were obtained from FishBase using the rfishbase package (Boettiger et al. 2012), and echinoderm depth ranges were obtained from OBIS (2025) and Schultz (2010). Fish and echinoderm species were also checked for their known geographic ranges to detect any ASVs that were assigned to unexpected species. Unexpected species were excluded from the depth analysis.

3 | Results

3.1 | Sequencing Data

After filtering, trimming, removal of chimeras, and post-clustering quality control, we obtained 13,616,964 reads from the 45 samples. The maximum number of reads per sample was

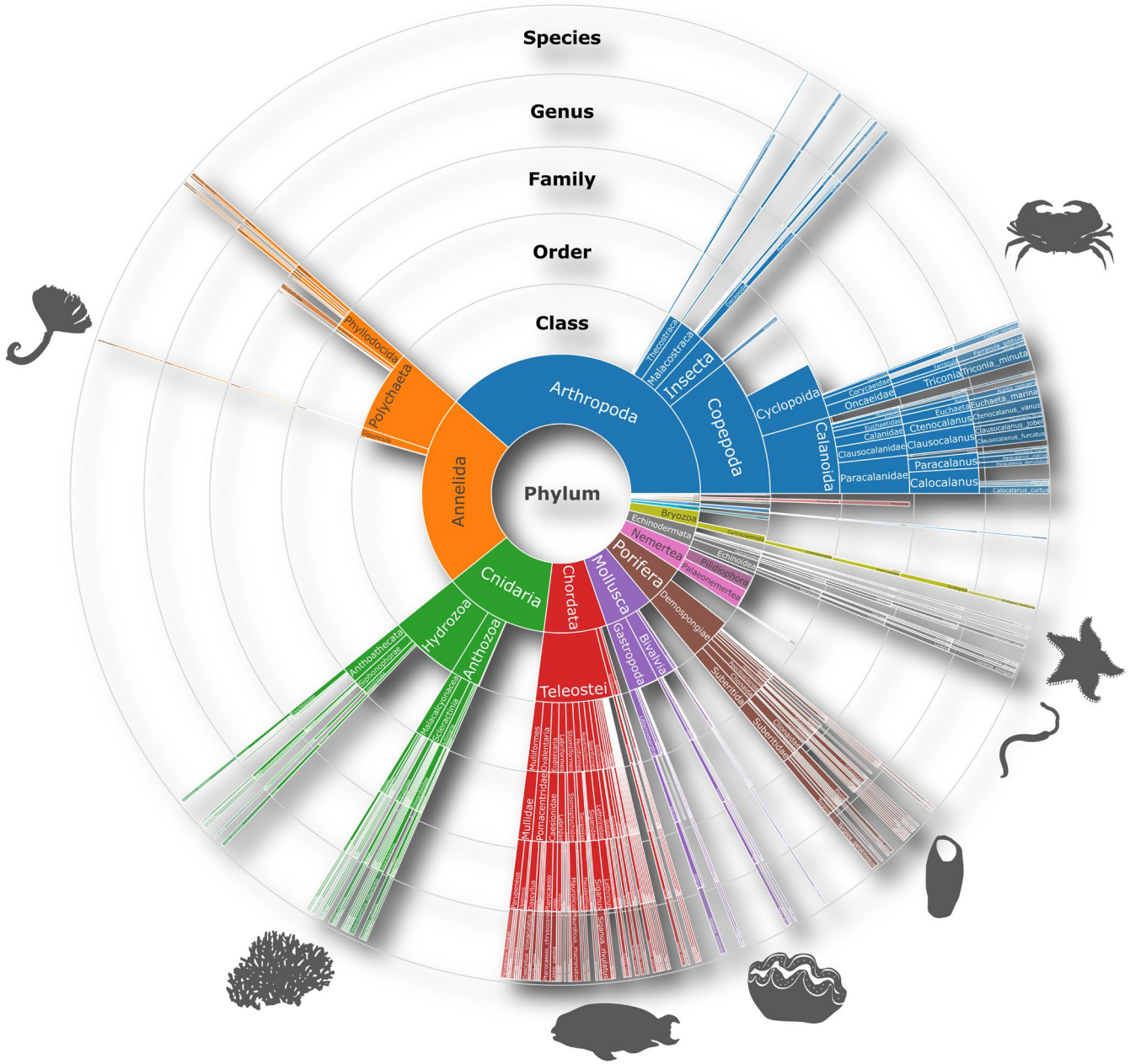

FIGURE 2 | Sunburst chart of metazoan taxa detected using eDNA metabarcoding, colored by phyla. Taxonomic level, from phylum to species, is represented by the taxonomy assignment by taxonomy levels. Phyla image Illustration created using PhyloPic (an interactive HTML version is available as Data S1).

434,851, while the minimum was 145,622, with an average of 302,599 reads per sample. These reads were clustered into 6782 ASVs, of which 3626 (~53.47%) were taxonomically assigned. Of these, 2074 (~57.2%) were from nontarget groups, primarily protists (1951 ASVs), with a smaller portion of algal and fungal sequences (90 and 33 ASVs, respectively). Additionally, three ASVs were identified as belonging to human DNA, and 11 ASVs were identified as terrestrial arthropods and were subsequently excluded from the analysis. A total of 1538 ASVs were identified as metazoan sequences belonging to 18 phyla. Of these, 793 ASVs were identified to the class level, 415 to the order level, 323 to the family level, 277 to the genus level, and 226 to the species level (Figure 2, Full assignment rates table of metazoan ASVs across phyla and taxonomic levels is available in Table S4). In total, we detected six unexpected species that are not known to occur in the GOA, of which two species of ray and four are teleost fish (list and known ranges are summarized in Table S5).

3.2 | Community Structure by Depth

Significant community-level differences were found between depths at both ASV and genus levels (PERMANOVA: $p < 0.001$ for both). At the ASV level, the most significant differences were found between the rariphotic depth (200 m) and the lower mesophotic depth (100 m) and the other, shallower, depths (Figure 3A). The shallow reef (10 m) did not differ significantly

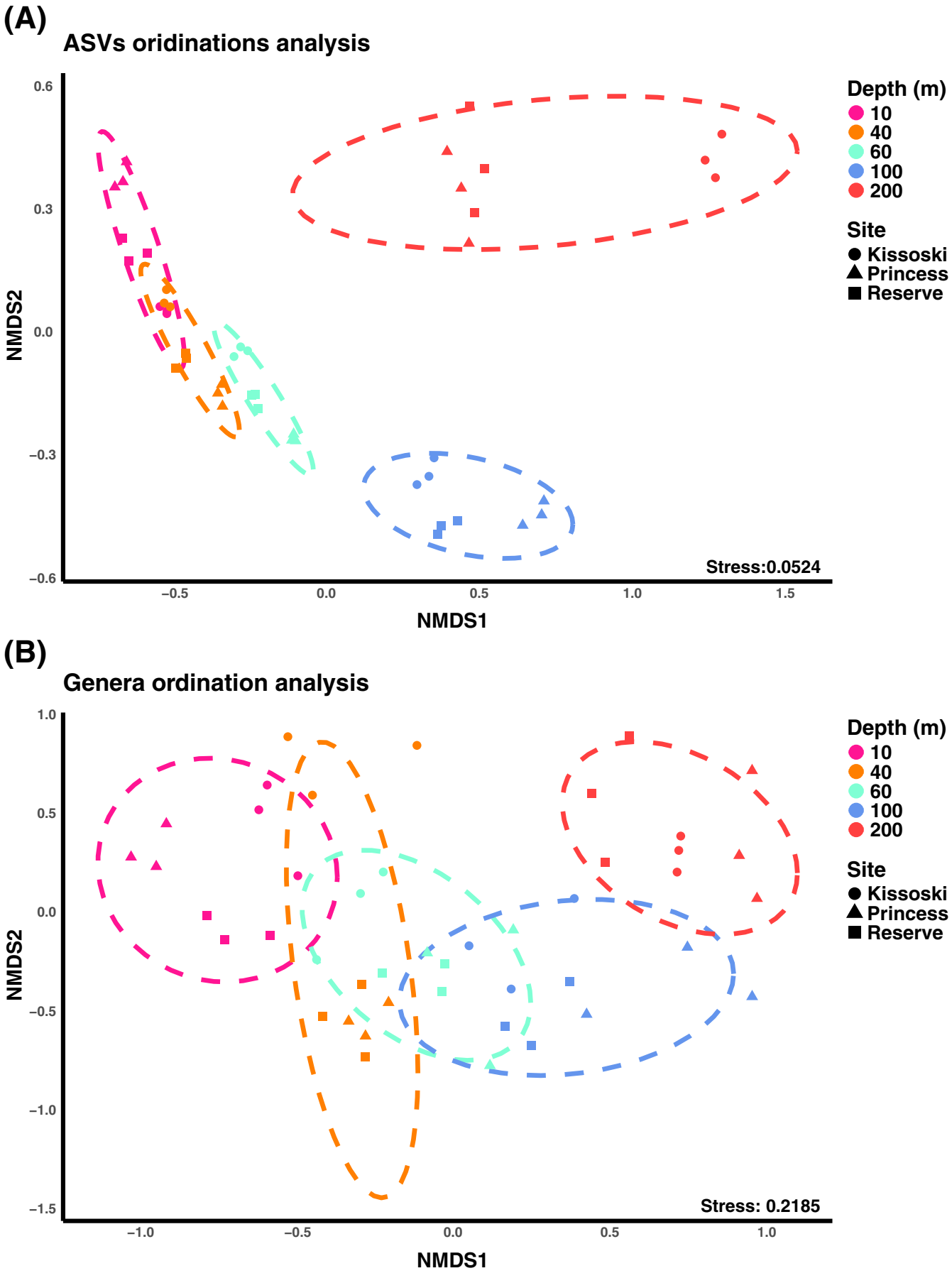

FIGURE 3 | Legend on next page.

FIGURE 3 | Nonmetric Multidimensional Scaling (NMDS) ordination analysis of the community structure by depth and site using Jaccard distance, 85% confidence of the ellipse of the depth centroids. (A) Analyses by ASVs dissimilarity. (B) Analyses by assigned genera dissimilarity. ECNR—Eilat Coral Nature Reserve; Kissoski—Kissoski Beach; Princess—Princess Beach.

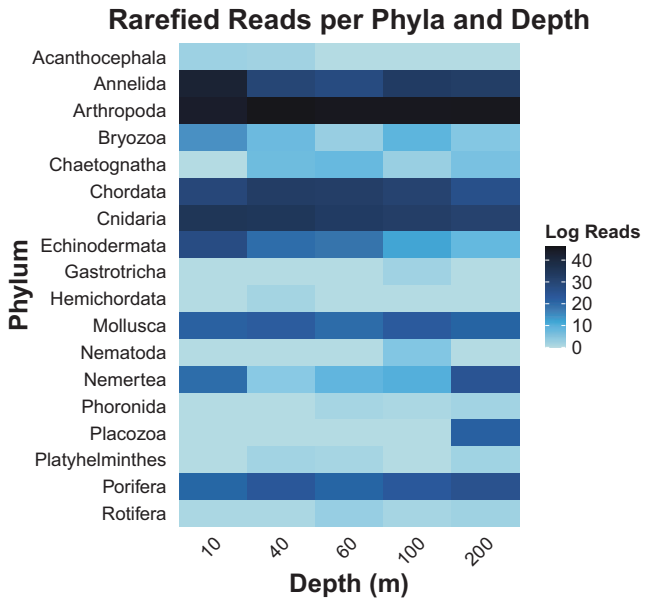

FIGURE 4 | Heatmap of log-transformed numbers of rarefied ASV read abundance across the sampled depth gradient and detected phyla. The color gradient represents the read count, with dark blue representing higher read counts and light blue representing lower counts. A raw reads version is given in Figure S2.

from the upper mesophotic depths (40 m and 60 m), with no significant difference detected between the latter. At the genus level, significant differences between the rariphotic depth (200 m) and the two shallow depths (10 and 40 m), as well as between the shallow reef (10 m) and the lower mesophotic depth (100 m) were detected (Figure S1).

While some environmental parameters remained consistent across sites (e.g., salinity, pH, alkalinity), others showed higher values at the northern sites (most proximate to the urban centers at the tip of the gulf) compared to the southernmost sites (e.g., Chl-*a*, Ammonia, Nitrate, Phosphate) (Table S1A). With respect to the depth gradient, water temperature decreased from about 26°C at the surface to 22°C at 200 m depth. Chl-*a* concentrations, on the other hand, increased from the surface down to a depth of 100 m and steadily decreased thereafter towards 200 m (Table S1B). Other variables such as salinity, pH, and alkalinity did not vary significantly across the depth.

3.3 | Community Structure by Site

Community composition did not significantly differ between sites at the ASV level (ASV PERMANOVA: $p = 0.131$), apart from one notable exception: the KB site at 200 m depth, which differed from all other sites, while the ECNR and PB sites appeared more similar to one another (KB-PB: $p < 0.03$, KB–ECNR: $p < 0.001$, ECNR–PB: $p > 0.6$).

3.4 | Phylum and Species Depths Diversity

Several patterns emerge when examining species-specific eDNA profiles across phyla. Some phyla, such as echinoderms and cnidarians, show peaks at shallow depths (10 m), with eDNA concentrations declining along the depth gradient (Figure 4). Others, such as Annelida and Nemertea, show peaks at both shallow and deep depths with lower values at mesophotic depths. Chordates showed an opposite trend, with the highest ASV reads at the mesophotic depths (40 m) and a reduced representation in deep water (100 m). Phyla such as Porifera and Placozoa peaked at deep water (200 m), with the number of ASV reads decreasing towards shallower depths. In contrast to the abovementioned phyla, arthropods showed consistent amounts of eDNA across the entire depth range, from 10 to 200 m. These patterns were consistent across both rarefied reads (Figure 4) and raw reads (Figure S2).

All echinoderm species were detected within their known depth range, except for *Ophiomastix elegans* (Peters, 1851), which was detected down to 200 m, below its known depth range of 60 m (Table S3). We excluded one fish species (*Maurolicus mucronatus* Klunzinger, 1871) that didn't have a reported depth range in FishBase (nor validated depths reported on GBIF). A total of 15 fish species were detected at greater depth than their known depth (Table S6); seven of them were detected both in and below their known depths, and eight species were detected exclusively in depths greater than their known depth range. One species, *Amblyglyphidodon flavilatus* Allen & Randall, 1981, was detected 2 m shallower than its known range (Figure 5).

4 | Discussion

4.1 | Defining Mesophotic Reefs

Just like shallow reefs, mesophotic coral reefs experience growing pressure and threats from both global climate-driven stressors (e.g., rising temperatures, acidification, etc.) and direct local anthropogenic sources (Rocha et al. 2018). Still, despite the growing attention to mesophotic reefs over the past decade (Lesser et al. 2009; Kahng et al. 2016; Loya et al. 2019; Pyle and Copus 2019; Slattery et al. 2024), they remain understudied, mainly due to their inaccessibility, requiring the use of remotely operated vehicles (ROVs) or technical diving. A fundamental step in guiding informed management and conservation efforts of this unique region is obtaining a better understanding of community structure and the boundaries of mesophotic reefs. However, most studies to date have defined these boundaries based on abiotic factors (e.g., depth and light attenuation) and community structure partitioning, typically focusing on a single taxon. In contrast, eDNA metabarcoding can simultaneously capture biodiversity and community structure across a wide range of taxa. Here, we show that community structure along the mesophotic depth range in the GOA (Figure 3) perfectly

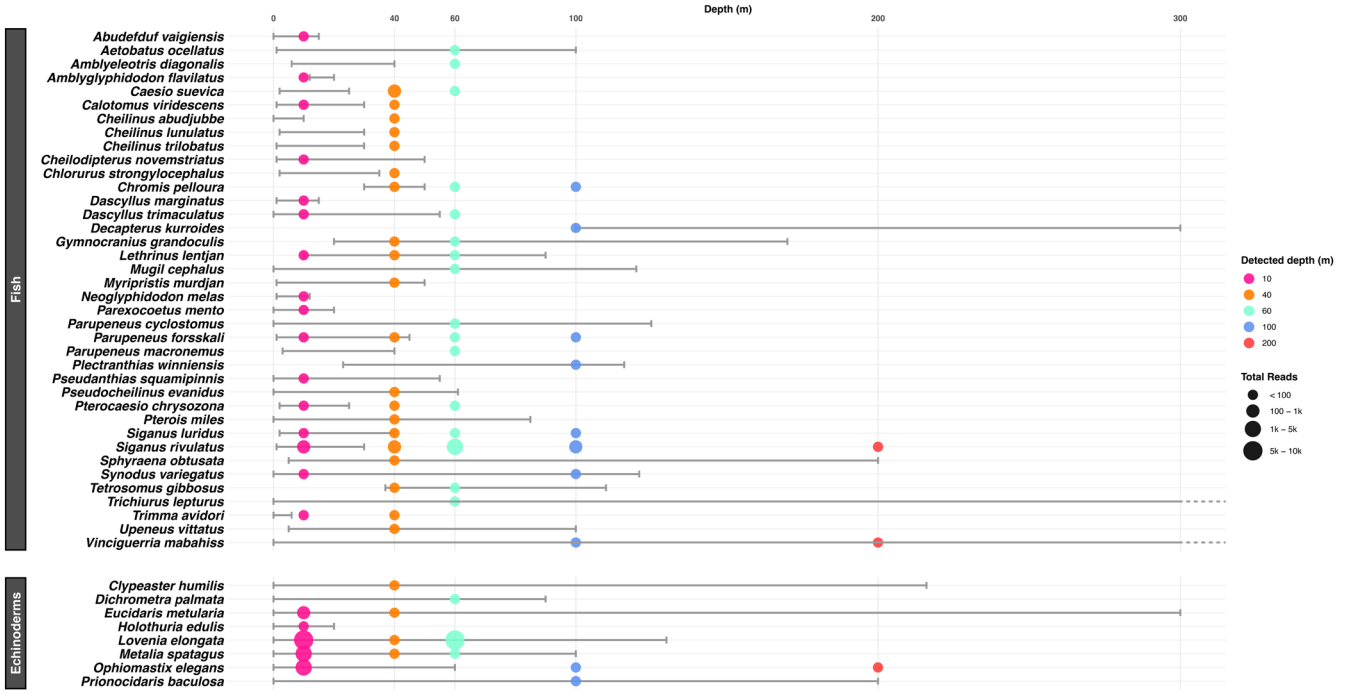

FIGURE 5 | Depth comparison between species detected depth using eDNA metabarcoding and known depth from literature, the point represents the depths species detected, and the gray line represents the known depth, the size of the bubble represents the relative ASVs abundance of each species. On the lower part, the detected fish species, the maximum depth was trimmed at 300 m for visualization. On the top part echinoderm species.

aligns with abiotic boundary definitions of mesophotic reefs. Our results show that in the GOA, the 40 m depth mark serves as the transition zone between shallow and mesophotic reefs, as it shows overlapping community compositions between the shallow reef (10 m) and the upper mesophotic (60 m). This notion holds true for both ASV and taxon-dependent compositions. These results align closely with previous studies in the GOA that placed the mesophotic boundary at approximately 40 m depth based on light attenuation (Tamir et al. 2019), further supporting the use of light availability as a reliable proxy for community structure.

Nevertheless, defining mesophotic reefs solely based on community structure can be challenging. We show a slow shift with significant overlap between the shallow and upper mesophotic, at depths of 10, 40 and 60 m (Figure 3). Indeed, some species that were expected to be confined to the shallow reef were found at mesophotic depths (Figure 5). For example, we detected 10 fish species at upper mesophotic depths (40 and 60 m) that, by reference, are known to inhabit only shallow reefs (up to 30 m). An outstanding example is *Siganus rivulatus* Forsskål & Niebuhr, 1775, which was detected across all sampled depths, down to 200 m, even though the known maximum depth of the species is set at only 30 m. While eDNA carryover from shallow water remains a possibility, these results align with other eDNA metabarcoding studies that detected fish species at greater depths than their previously known ranges (Hoban et al. 2023; Muff et al. 2023; Sanchez et al. 2025). In contrast, all but one echinoderm species was detected within their known depth range, with the exception of the ophiuroid *Ophiomastix elegans*, which was detected deeper than its known depth range (at 100 and 200 m; Table S3). It should be noted that other species from the genus *Ophiomastix* do inhabit water deeper than 200 m, most notable is *Ophiomastix variabilis* Koehler, 1905, which is known to occur in the Red Sea (WoRMS 2025). These results suggest that contemporary depth ranges are still underestimated for many reef species, particularly the maximal depth range (Menza et al. 2007; Kahng et al. 2014). Our results suggest that this is more apparent in taxa of higher mobility such as fish, and illustrate how eDNA metabarcoding may help bridge this gap, particularly for deep, inaccessible habitats, although improved depth specific sampling methods are needed to alleviate the potential for eDNA carryover across depths. Additionally, our results support the hypothesis that the community structure in the upper mesophotic reef is more similar to the shallow reef than to the lower mesophotic, with significant species overlap between the zones, as was previously shown in the coral reefs of Hawaii (Kahng et al. 2016). Still, the relative proximity of our sampling sites and lack of available regional scale data limit broader interpretation of our results and highlight the need for regional scale collaborations across the GOA and greater Red Sea.

The mesophotic boundaries, on the other hand, appear to be clearer at the deeper depths. The lower mesophotic reef (100 m) revealed a unique ecological community that differed from both the upper mesophotic (40 and 60 m) and the shallow reef (10 m) (Figure 3A, Figure S1). This deeper region of the mesophotic reef is naturally less understood and far less studied than the upper mesophotic (Pinheiro et al. 2016; Bongaerts et al. 2017; Englebert et al. 2017). Interestingly, we identified ASVs of scleractinian corals (families Astrocoeniidae and Pachyseridae) as well as a larger number of Octocorallia

ASVs at this deeper reef region. The presence of both depth generalists and deep species at the 100 m depth mark emphasizes the function of the lower mesophotic as a transition zone between the shallow reef zone and the deep-sea fauna (Stefanoudis et al. 2019). For example, the depth generalist sea urchin *Prionocidaris baculosa* (Lamarck, 1816), which is rare at the shallow reef of the GOA (Eviatar and Bronstein 2025), was detected across all sites at a depth of 100 m, although some overlap in distribution does exist for this genus with upper mesophotic sites (Figure 3B). Furthermore, deep-sea species were often detected at the lower mesophotic (100 m). For example, we detected *Decapterus kurroides* Bleeker, 1855, which is known down to 300 m, at the shallow edge of its known depth distribution (100 m). The rariphotic zone was the least diverse in both unique ASVs and the number of species-assigned taxa, potentially reflecting a more homogeneous environment (e.g., in terms of temperature and light fluctuations; Baldwin et al. 2018). Nevertheless, we detected a few ASVs belonging to octocorals, mainly from the families Xeniidae and Anthogorgiidae. While the presence of azooxanthellate corals like the Anthogorgiidae is expected at the lower mesophotic, the presence of photosynthetic taxa like the Xeniidae is surprising. Extensive ROV studies in the GOA established the Xeniidae depth limit in the region to a depth of 60–70 m (Benayahu et al. 2019). Thus, the presence of colonies at the 100 m and particularly at the 200 m range are unlikely. The detection of xeniid specific ASVs at these greater depths must therefore stem from DNA transport to these depths. As our survey was conducted in July, in the midst of the Xeniidae reproductive season in the GOA (Liberman et al. 2022), the signal we detected may be driven by planulae or gametes transport from adjacent shallow reefs where xeniids are abundant. Nevertheless, these two deeper and distinct zones (i.e., the 100 and 200 m zones) are usually overlooked in marine monitoring, even in comparison to the upper mesophotic (Englebert et al. 2017; Baldwin et al. 2018; Chimienti et al. 2025), and are almost unexplored using traditional censusing methods. Our results show the merit of eDNA metabarcoding in bridging this gap.

Peaks in phylum-specific eDNA concentrations revealed clear and ecologically meaningful structuring of biodiversity across depth (Figure 4). For example, chordate eDNA reached its maximum in the upper mesophotic zone (40 m), whereas Porifera exhibited its highest read abundance in the rariphotic zone (200 m). Although eDNA concentrations do not scale linearly with organismal biomass, a consistent positive relationship between biomass, organismal abundance, and eDNA signals have been demonstrated across marine systems (Minamoto 2022). Accordingly, elevated taxon-specific read counts are commonly interpreted as reflecting higher local abundance or activity of the corresponding taxa (Rees et al. 2014; Fukaya et al. 2021; Liu et al. 2025). Importantly, these depth-associated patterns are not unique to the GOA, but align well with trends reported from other marine regions. For example, eDNA-based monitoring of marine mammals consistently detects stronger cetacean and pinniped signals in depth strata associated with preferred foraging or transit zones, often decoupled from surface visual observations (Foote et al. 2012; Valentini et al. 2016). Similarly, echinoderm and cnidarian eDNA signals have been shown to track benthic habitat zonation and depth-related environmental gradients. eDNA metabarcoding reliably captures depth-structured assemblages of echinoderms, reflecting known bathymetric distributions from shallow reefs to mesophotic and deeper zones, while comparable stratification has been reported for cnidarians, including enhanced detection of mesophotic and deep-reef taxa that are often underrepresented in conventional surveys (Sinniger et al. 2016; Cerrillo-Espinosa et al. 2025).

Several non-mutually exclusive mechanisms may contribute to the observed patterns. First, depth-related gradients in light, temperature, hydrodynamics, and productivity strongly structure benthic and pelagic communities (Lesser et al. 2009), leading to predictable taxonomic turnover from shallow to deep zones. Surface measurements across the three northern GOA sites showed only minor variability in key physicochemical parameters such as temperature, salinity, pH, and nutrient concentrations, indicating relatively homogeneous conditions among sites at shallow depths. In contrast, the depth-resolved profile from the offshore station A, revealed pronounced vertical gradients, including decreasing temperature with depth, alongside marked increases in inorganic nutrients such as nitrate, phosphate, and silicate, and changes in chl-*a* concentrations. These strong depth-dependent shifts in environmental conditions likely structure biological communities along the water column, supporting our eDNA results that biodiversity patterns are driven primarily by depth rather than by spatial differences among sites. The elevated Porifera signal in the rariphotic zone is consistent with the dominance of suspension feeders in low-light, low-energy environments, where sponges often constitute a major fraction of benthic biomass (Raijman-Nagar et al. 2024). Second, eDNA production, transport, and degradation rates are themselves depth-dependent, influenced by reduced UV exposure, lower temperatures, and altered microbial activity at depth, all of which can enhance eDNA persistence in deeper waters (Harrison et al. 2019; Allan et al. 2021). Third, behavioral and life history traits—such as diel vertical migrations, spawning aggregations, or depth-specific feeding strategies—may further amplify eDNA signals at particular depths independent of standing biomass. However, technical limitations and selection of methodologies may also have considerable implications for eDNA monitoring. For example, marker selection (loci and length) is expected to affect the obtained results in both the amount and quality of generated amplicons, and the ability to link ASVs to species (depending on available reference databases) (Ruppert et al. 2019). Water volume filtration is also expected to effect the detection of rare and lowly represented taxa in a given region, thus increasing water volume filtration is a priority in eDNA sampling designs (Juhel et al. 2020), while the number of replicates is essential to eliminate false negative detections and accurately estimate biodiversity (Stauffer et al. 2021).

Together, these findings emphasize that depth is a critical dimension in both eDNA-based and traditional biodiversity surveys. Ignoring depth stratification risks underestimating or entirely missing key components of marine communities, particularly when targeting specific taxonomic groups or functional guilds. Incorporating depth-resolved sampling designs is therefore essential for accurate biodiversity assessments and for maximizing the interpretive power of eDNA metabarcoding across

complex three-dimensional marine environments. The precise boundaries of the mesophotic are still in need of further investigation, and, as our results suggest, are more akin to a gradient shift than a strict boundary. Nevertheless, light attenuation is indeed a strong indicator of community structure, and, as such, needs to be taken into consideration locally, as these factors tend to drastically change between coral reef systems.

4.2 | Taxonomy Independent Approaches: Leveraging Unassigned ASVs for Community Analysis

As community composition is one of the key elements in defining the boundaries of mesophotic reefs, an accurate account of species and their respective depth range is fundamental. Nevertheless, major gaps currently exist in our understanding of bathymetric zonation and species-specific depth ranges. This gap is even more noticeable across mesophotic reefs, where traditional censusing is limited. Broadly, the deeper we look, the broader our knowledge gaps become (Menza et al. 2007). Although most metabarcoding studies use species level as the most commonly reported result, there are challenges for species evaluation in metabarcoding studies, mainly requiring the availability of robust and comprehensive reference databases. The formation of taxonomically validated, species-level genetic reference libraries requires extensive sampling efforts, as well as thorough taxonomic knowledge of local varieties within the studied region (Weigand et al. 2019; Marques et al. 2020), which is often missing for many regions. A practical alternative is the use of one of the publicly available reference databases, most notably those of NCBI or BOLD. However, while these databases offer expansive genetic references, they are known to be prone to taxonomic mistakes or suffer from outdated taxonomic assignments (Machida et al. 2017; Keck et al. 2023). Furthermore, these global databases often lack comprehensive representations of local and often divergent communities. As such, the effectiveness of the aforementioned global databases greatly depends on the target taxa. While some taxa have comprehensive reference databases (mainly vertebrates), other groups often suffer from relatively poor references (Bucklin et al. 2011; Weigand et al. 2019). In the current study, we see that while over 65% of chordate ASVs were successfully assigned to the species level, only 1.75% of the ASVs associated with annelids were successfully assigned to species level. In general, only 14.7% of the metazoan ASVs in the current study could be successfully assigned to their respective species. Although low, this percentage is comparable with other studies that used generalist primers (Nguyen et al. 2020; Shea and Boehm 2024). Nevertheless, this limitation is likely exacerbated in the Red Sea, where a substantial fraction of marine biodiversity remains undescribed and many invertebrate groups suffer from pronounced taxonomic and genetic knowledge gaps. Moreover, the GOA represents a biogeographic edge-of-range for many Red Sea taxa, where local environmental conditions and historical isolation may promote distinct taxonomic and genetic variants that are poorly represented in, or entirely absent from, global reference databases.

This is where the use of clustering units for biodiversity assessment can be useful (Tapolczai et al. 2019; Marques et al. 2020; Mächler et al. 2021). Analysis of the complete ASV dataset provides a more holistic view, not limited by reference databases (Taberlet et al. 2018). While this approach naturally provides coarser taxonomic assignments, it is particularly useful in assigning neglected taxa and understudied regions where genetic references are absent. In the current study, the taxonomically assigned and unassigned (ASV) datasets showed similar trends in describing the community changes between sites and depths. However, the cluster analysis was able to reveal more significant trends than the assigned analysis was not able to detect due to the low assignment rates.

The growing use of eDNA metabarcoding for biodiversity monitoring emphasizes the importance of creating comprehensive and reliable genetic barcode references. Closing the barcode knowledge gap will require extensive efforts, combining taxonomic expertise with large scale genetic screening (Miralles et al. 2020). Thus, even in the midst of the genomic era, taxonomic knowledge and the role of natural collections are more important than ever (Caley et al. 2014). In turn, eDNA metabarcoding can be used to further guide such taxonomic efforts, pointing out understudied groups and habitats.

5 | Conclusions

With an ever-changing climate and growing anthropogenic pressures, the importance of biodiversity monitoring to support informed conservation efforts is greater than ever before. Nevertheless, the depth factor is often overlooked in many conservation initiatives that largely focus on shallow, accessible environments (Bridge et al. 2013). Considering refugia from the effects of climate change, most notably bleaching (Frade et al. 2018; Pérez-Rosales et al. 2021), mesophotic coral reefs are still threatened (Rocha et al. 2018; McWhorter et al. 2024), and, as such, are of the utmost importance for monitoring and protection efforts. As the early days' limitations of metabarcoding, such as absolute quantification (Tsuji et al. 2022) and age structure prediction (Ruiz et al. 2025), are rapidly eroding, and as new fields such as airborne eDNA (Lynggaard et al. 2022; Littlefair et al. 2023) and environmental RNA (Yates et al. 2021) are being established, it appears that eDNA metabarcoding is on the fast track to become a leading method in biodiversity monitoring globally.

When assessing understudied environments, such as mesophotic reefs, eDNA metabarcoding emerges as one of the most robust and effective contemporary survey methods. Our results demonstrate that clear, highly significant depth-dependent community differentiation can be obtained using eDNA metabarcoding even in locations such as the GOA, where, due to its steep bathymetry, distinct depth habitats can be separated by relatively small horizontal distances—indicating exceptional localization of eDNA footprints (e.g., Murakami et al. 2019; West et al. 2020; Shea and Boehm 2024) also along bathymetric gradients.

Author Contributions

A.S. and O.B. conceived and designed the study, acquired, analyzed, and interpreted the data, and wrote the manuscript. O.B. supervised the project and secured funding.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data supporting the findings of this study are provided within the main text and Supporting Information accompanying this publication. Genetic sequence data have been deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1414866. (Reviewer code: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1414866?reviewer=pg7eevnt6m18ovvl3lsa4l5puu).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Heatmap of log-transformed numbers of raw ASV read abundance across the sampled depth gradient and detected phyla. The color gradient represents the read count, with dark blue representing higher read counts and light blue representing lower counts. **Figure S2:** Pair sites Beta diversity scores, the mean Beta diversity dissimilarity within site is marked by a dashed red line. (A) Pair

Beta diversity scores of sites by ASVs. (B) Pair Beta diversity scores of sites by ASVs assigned to genus level. (C) Pair Beta diversity scores of depths by ASVs. (D) Pair Beta diversity scores of depths by ASVs assigned to genus level. **Table S1A:** Environmental measurements by site. All the measurements, excluding Sechi depth, were taken at a depth of 1 m above a bottom depth of 20 m. **Table S1B:** Environmental measurements by depth. Measurements were collected using a CTD at open sea (29°28.023′ N, 34°55.722′ E). **Table S2:** Sampling locations WGS 84 coordinates. **Table S3:** Species known depth recorded. **Table S4:** assignment rate across taxonomic levels by phyla. **Table S5:** Unexpected species records. **Table S6:** species detected outside known depth range, positive values indicate detection in greater depth than known, and negative values indicate detection in shallower depth than known. **Data S1:** Taxa detected in the present study visualized using an interactive sunburst chart, created with the "plotly" package on R.