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Initiating a DNA Barcoding Reference Library of Stony Corals from the Gulf of Eilat (Red Sea)

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Abstract: Accurate identification of scleractinian coral species is fundamental for proper biodiversity estimates, for aiding in efforts of reef monitoring, conservation, restoration, and for the management of coral reefs. Here, we provide the first DNA barcoding reference library for coral species in Eilat, Red Sea, based on the mitochondrial gene cytochrome c oxidase subunit I (*COI*), targeting the identification of stony coral species from shallow (0–12 m) reefs. A total of 191 specimens were collected, depicting 14 families, 39 genera, and 94 species (all are new full species records to the BOLD system). Three species (*Sclerophyllia margariticola*, *Cyphastrea magna*, and *Psammocora profundacella*) are first records for Eilat's coral reef. The results presented here strengthen the claim that *COI* is not universally informative for delimitation of stony coral species, a notion reinforced by the constructed maximum likelihood phylogenetic tree. This library is the first step in a long journey towards elucidating coral biodiversity in the coral reef at Eilat and for improving future management and monitoring efforts.

Keywords: conservation; molecular marker; *COI*; Indian Ocean; reef management; scleractinian corals; biodiversity; taxonomy

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1. Introduction

The need to safeguard the worldwide sustainability of coral reef ecosystems, their biodiversity and reef dwelling organisms, and the management of goods and services, necessitates a well defined biodiversity inventory of the reefs' key species, the hermatypic corals [1–3]. While the species richness of scleractinian corals has not been properly evaluated as of yet, it is roughly estimated at more than 700 valid extant species in contemporary coral reefs [4,5]. Due to key gaps in the current coral taxonomy and biodiversity data [6,7], validated identification of many species is still pending revision [8–11], and a discrepancy comes to light when tallying formally described coral species with the actually existing species [4]. Coral taxonomy is also affected by the lack of experts in taxonomy [12–14], all leading to ambiguous deductions regarding the ill-defined coral traits, lineages, and actual numbers of coral species and their distributions [15–17]. The deficient taxonomy of reef corals also impairs reef monitoring, evaluation of patterns of coral recruitment [3], and reef management towards global climate change impacts [18,19].

The realization that traditional approaches based on morphology alone are not sufficient in scleractinian corals' species delineation [13,16,17] has led to the suggestion for a simultaneous adaptation of molecular-based techniques, collectively termed as DNA barcoding, including the cytochrome b gene (*cytB*), 16S rRNA, and the nuclear ribosomal internal transcribed spacer (ITS) region [12,20,21]. Primarily of all is the 5′ fragment of the mitochondrial cytochrome c oxidase subunit I (*COI*) gene (about 648 bp, the barcoding region), the most commonly used marker [22], that has been extensively utilized in the

past two decades for identifying species including cnidarian species (e.g., [23–25]) and scleractinian corals, in particular [9,26–36].

A species inventory of ca. 100 stony corals in the Gulf of Eilat, Red Sea (reef flat to 30 m depth), has been established in the last decade [37] and is in line with records from the past five decades [38–40]. Yet, this list is most possibly a less representative inventory, taking into consideration additional coral species described in the northern Gulf in general (e.g., [41–48], in Aqaba, Jordan (ca. 80 species: [49–51]) along the Egyptian Red Sea coastline (ca. 110 species; [52]) and from the Saudi Arabian coastline of the Red Sea (ca. 140 species; [53]). In the Gulf of Eilat, Scheer and Pillai [48] described 194 scleractinian coral species, while Devantier et al. [54] reported 260 species from the Saudi Arabian coastline of the Gulf of Eilat. According to Veron et al. [55], ca. 290 species are present in the North and central Red Sea, and an updated checklist [1] reported on 307 species of scleractinian coral species (zooxanthellate and azooxanthellate) in the north and central regions, followd by Berumen et al. [17] that listed 314 scleractinian species.

The Israeli coast of the Gulf of Eilat is known to possess a diverse coral reef [40]. However, it has been continuously degrading in spite of all conservation attempts during the past five decades [3,56–59], primarily due to anthropogenic drivers and occasional natural causes such as southern storms. Monitoring of coral diversity (based on morphological characteristics) is an important component in the management of the coral reefs of Eilat (https://www.gov.il/he/departments/publications/reports/national_monitoring_gulf_of_eilat_reports; accessed on 2 October 2022 in Hebrew), along with approaches that combine molecular methods in the identification of coral species [3], altogether aiding in the creation of a partial list of coral species in the Gulf. For that purpose, this study aims to compile the first inventory of coral species in Eilat, based on their morphological characteristics and *COI* barcoding identities, in order to create a comprehensive and complete future database of the stony corals at the northern Red Sea.

2. Materials and Methods

2.1. Sampling

Four sampling sessions were conducted by SCUBA in three sites along the Israeli Gulf of Eilat in 2013. The first three sampling sessions took place at the Kisoski beach (KIS; 29°32′49.98″ N, 34°57′14.78″ E) and the Dekel beach (DEK; 29°32′24.67″ N, 34°56′51.23″ E) (Figure 1a, Supplementary Table S1) during 18–19 January (down to 6 m depth), 12–15 April (down to 8 m depth), and 13–16 August (down to 7.5 m depth). The fourth session (28 August, down to 6 m depth) was conducted in front of the Inter-University Institute for Marine Sciences in Eilat (IUI; 29°30′07″ N, 34°55′02″ E, Figure 1a, Supplementary Table S1).

Sampled corals were first photographed *in situ* with a plastic ruler (Figure 1b,d), numbered, and a small fragment of about 4 cm was removed from each colony (according to the permit restrictions) and placed in a plastic cup, with its number tag. At the end of each dive, all samples were transported to water tables supplied with continuous flow of natural sea water and under ambient lighting at the national center for mariculture at Eilat, Israel. Specimens were then transported alive to the Israel Oceanographic and Limnological Research (IOLR) laboratory at Haifa, Israel, acclimated for 24–48 h in water tables with continuous flow of natural sea water, and photographed under a binocular (Figure 1c,e). A small live tissue sample was taken for DNA extraction (see below), and the rest of the sample was stored in 70% ethanol as a voucher and then morphologically identified. When needed for detailed morphological characteristics, additional sub-samples were taken from the ethanol-fixed specimens. Their tissues were removed using commercial bleach (1:4 dilution) for 24 h, washed in distilled water, and air dried.

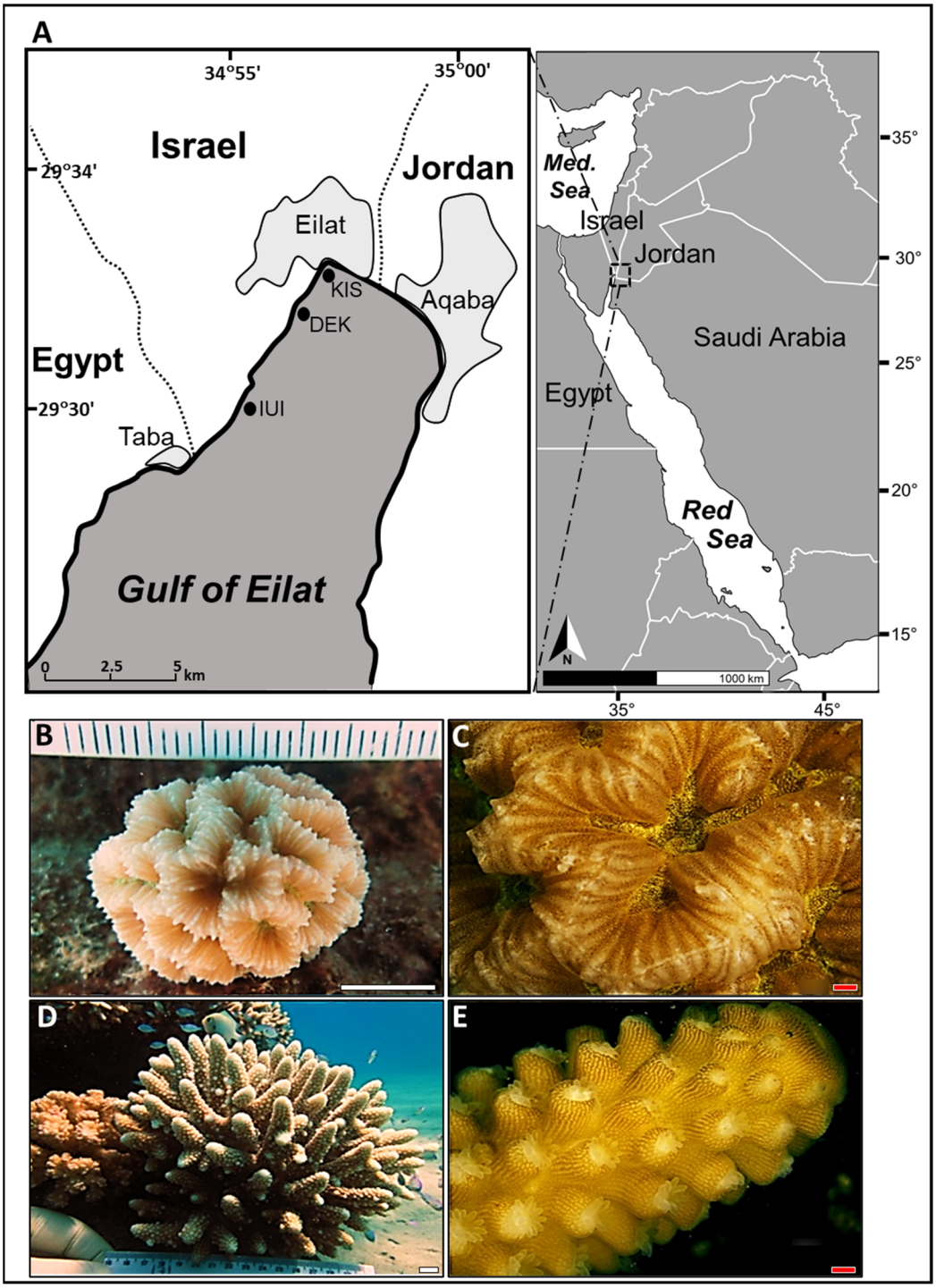

Figure 1. (**A**) Sampling sites at the Gulf of Eilat's reef, Israel: Kisoski beach (KIS), Dekel beach (DEK) and the Interuniversity Institute (IUI). (**B**–**E**) Photographs of coral vouchers. The *in situ* and the close-up photographs of *Platygyra carnosa* Veron, 2000 (**B**,**C**), and *Acropora samoensis* (Brook, 1891) (**D**,**E**). White bars—1.0 cm (**B**,**C**), red bars—1.0 mm (**C**,**E**).

2.2. Traditional Taxonomy

Coral samples were morphologically identified to the lowest taxon level possible, based on Veron [47], and followed the keys to genera and species ([47] volume 3, page 447). Identification was conducted by one team member loudly reading the identification phrases from Veron's 'keys to genera and species' and a second team member simultaneously examining the coral sample. This protocol was repeated until a full identification was

reached and agreed upon. The up-to-date species names were confirmed in the World Register of Marine Species [60] and the Corals of the World website (COTW; [61]). The ethanol-fixed specimens (and accompanying dry skeletal samples, if produced) were registered and archived at the Steinhardt National Natural History Museum and the Research Center at Tel Aviv University.

2.3. DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was isolated from tissue samples according to Graham [62] and Douek et al. [63]. Three sets of primers were used in order to amplify the 650–700 bp *COI* fragments, using Folmer [64] and Fukami et al. [26] and degenerated primers, designed by modifying Folmer [64] primers (Supplementary Table S2). Reactions were carried out in a total volume of 50 µL, which included 1–2 µL of DNA template from each sample, 25 µL of PCR Master Mix 2× (Fermentas) with DreamTaq™, and 2.5 µL of 10 mM of each primer set. The PCR conditions comprised an initial denaturation step of 60 s at 94 °C, followed by 30 cycles (60 s denaturation at 94 °C, 60 s annealing at 48–55 °C, 60 s elongation at 72 °C) and a final 10-min elongation step at 72 °C. The PCR products were screened on 1.3% agarose gel and sent for forward and reverse directions sequencing at Macrogen Inc., Korea, using ABI 3730xl. The DNA samples were kept at 4 °C at IOLR.

2.4. Data Analysis

DNA sequences were assembled using the DNA Baser Sequence Assembler v4 (www.DNABaser.com, accessed on 7 July 2022) and compared to Genbank sequences using BLASTn algorithm in the NCBI website (www.ncbi.nlm.nih.gov; accessed on 15 July 2022). The criteria for a liable sequencing were a mismatch rate of less than 1% between forward and reverse sequences and a high quality of the sequence chromatogram. A match between the taxonomy identification and the NCBI BLASTn algorithm results was made prior to uploading the sample to the Barcode of Life Data (BOLD) site system, under the project name "Scleractinia of the Israeli Red Sea" (SIRS). On the BOLD site, the submitted sequences were translated into amino acids and compared against a Hidden Markov Model of the *COI* protein to verify that they derived from the *COI* gene. Moreover, they were examined for stop codons and compared against possible contaminants to make sure they were all high-quality sequences [22]. All sequences were aligned in ClustalW (EBI) using default settings. According to the model selection test performed, and using MEGA software v11 [65], a maximum likelihood (Hasegawa-Kishino-Yano Gamma distribution with invariant sites) phylogenetic *COI*-based tree was constructed.

3. Results

The four sampling sessions yielded 191 coral samples (17, 80, 54 and 40, respectively for each sampling session) that were assigned (using morphological and *COI* results) to 14 families, 39 genera, and 94 species (Table 1; Supplementary Table S1) [66–68]. Out of these, 177 specimens were fully identified to the species level, of which 14 specimens were identified to a species level but, due to doubts about their identifications, we consider these samples as possible morphological variants of existing species in Veron [47], thus considered as cōnfer (*cf.*; Supplementary Table S1). Ten specimens of the above list were identified to the genus level (5 *Acropora* sp., and single *Dipsastraea* sp., *Echinophyllia* sp., *Echinopora* sp., *Paragoniastrea* sp. and *Pavona* sp.) and four specimens were assigned to the Fungiidae family (specimens were too young and small for an accurate identification) due to incomplete morphological characteristics, as well as *COI* differentiating outcomes. The above reveals the possibility of a higher number of species in our 191 samples.

Table 1. List of coral families, genera, and species from Eilat with counts of vouchers, and of representatives in each taxonomic subgroup. [a]—voucher fully identified, [b]—voucher identified as cf., [c]—voucher not fully identified. *COI* N.I.—*COI* Not Informative (requiring additional molecular markers for complete identification).

Family/Genus	No of Vouchers	Subgroups		Species Details	Remarks
		No.	Types		
Acroporidae	36	3			
Acropora	21	11	5 [a],3 [b],4 [c]	*A. plantaginea* 4 [b], *A. squarrosa* 3 [b], *A. tenuis* 4 [a] + 1 [b], *A. cytherea* 1 [a], *A. humilis* 1 [a], *A. samoensis* 1 [a], *A. valida* 1 [a], *A.* sp. 1–4: 1 [c] 1 [c] 1 [c] 2 [c]	*COI* N.I.
Alveopora	4	3	3 [a]	*A. daedalea* 1 [a], *A. fenestrata* 2 [a], *A. verrilliana* 1 [a]	
Montipora	11	7	3 [a]	*M. cryptus* 1 [a], *M. efflorescens* 1 [a], *M. hemispherica* 1 [a], *M. informis* 2 [a], *M. maeandrina* 3 [a], *M. tuberculosa* 2 [a], *M. verrucosa* 1 [a]	*COI* N.I.
Agariciidae	9	2			
Leptoseris	1	1	1 [a]	*L. yabei* 1 [a]	
Pavona	8	4	3 [a],1 [c]	*P. danai* 1 [a], *P. diffluens* 3 [a], *P. varians* 3 [a], *P.* sp. 1 [c]	*COI* N.I.
Coscinaraeidae	2	1			
Coscinaraea	2	1	1 [a]	*C. monile* 2 [a]	
Dendrophylliidae	4	1			
Turbinaria	4	1	1 [a]	*T. reniformis* 4 [a]	
Euphylliidae	5	1			
Galaxea	5	1	1 [a]	*G. fascicularis* 5 [a]	
Fungiidae	17	3 or 4		Fungiidae #1–4: 1 1 1 1	4 vouchers are too small and young without adequate morphological characteristics and *COI* N.I. to assign a genus or a species
Cycloseris	10	3	3 [a]	*C. cyclolites* 3 [a], *C. fragilis* 1 [a], *C. vaughani* 6 [a]	*COI* N.I.
Danafungia	2	2	2 [a]	*D. horrida* 1 [a], *D. scruposa* 1 [a]	*COI* N.I.
Fungia	1	1	1 [a]	*F. fungites* 1 [a]	*COI* N.I.
Leptastreidae	3	1			
Leptastrea	3	3	3 [a]	*L. inaequalis* 1 [a], *L. purpurea* 1 [a], *L. transversa* 1 [a]	
Lobophylliidae	15	5			
Acanthastrea	2	1	1 [a]	*A. brevis* 2 [a]	
Echinophyllia	3	2	1 [a],1 [c]	*E. aspera* 2 [a], *E.* sp. 1 [c]	
Lobophyllia	5	2	2 [a]	*L. corymbosa* 4 [a], *L. hemprichii* 1 [a]	
Oxypora	3	2	1 [a],2 [b]	*O. crassispinosa* 1 [b], *O. lacera* 1 [a] + 1 [b]	
Sclerophyllia	2	1	1 [a]	*S. margariticola* 2 [a]	
Merulinidae	69	12			*COI* is partly informative in this group
Astraeosmilia	1	1	1 [a]	*A. maxima* 1 [a]	
Coelastrea	1	1	1 [a]	*C. aspera* 1 [a]	
Cyphastrea	5	3	3 [a]	*C. magna* 1 [a], *C. microphthalma* 2 [a], *C. serailia* 2 [a]	

Table 1. *Cont.*

Family/Genus	No of Vouchers	Subgroups		Species Details	Remarks
		No.	Types		
Dipsastraea	27	9	7 [a],2 [b],1 [c]	*D. amicorum* 4 [a], *D. matthaii* 2 [b], *D. speciosa* 2 [a] + 1 [b], *D. danai* 7 [a], *D. faviaformis* 5 [a], *D. lacuna* 1 [a], *D. laxa* 3 [a], *D. veroni* 1 [a], *D.* sp. 1 [c]	
Echinopora	6	3	2 [a],1 [c]	*E. fruticulosa* 2 [a], *E. irregularis* 3 [a], *E.* sp. 1 [c]	*COI* N.I.
Favites	4	2	1 [a],1 [b]	*F. paraflexuosus* 1 [b], *F. pentagona* 3 [a]	*COI* N.I.
Hydnophora	5	1	1 [a]	*H. exesa* 5 [a]	
Merulina	1	1	1 [a]	*M. ampliata* 1 [a]	
Mycedium	4	1	1 [a]	*M. umbra* 4 [a]	
Paragoniastrea	1	1	1 [c]	*P.* sp. 1 [c]	
Paramontastraea	5	1	1 [a]	*P. peresi* 5 [a]	
Platygyra	9	5	5 [a]	*P. acuta* 1 [a], *P. carnosa* 2 [a], *P. crosslandi* 2 [a], *P. daedalea* 1 [a], *P. lamellina* 3 [a]	*COI* N.I.
Plerogyridae	6	2			
Blastomussa	2	2	2 [a]	*B. loyai* 1 [a]. *B. merleti* 1 [a]	
Plerogyra	4	1	1 [a]	*P. sinuosa* 4 [a]	
Plesiastreidae	1	1			
Plesiastrea	1	1	1 [a]	*P. versipora* 1 [a]	
Pocilloporidae	14	3			
Pocillopora	5	1	1 [a]	*P. damicornis* 5 [a]	
Seriatopora	4	1	1 [a]	*S. hystrix* 4 [a]	
Stylophora	5	2	2 [a]	*S. kuehlmanni* 1 [a], *S. pistillata* 4 [a]	
Poritidae	9	2			
Goniopora	3	2	2 [a]	*G. pearsoni* 2 [a], *G. tenuidens* 1 [a]	*COI* N.I.
Porites	6	4	4 [a]	*P. harrisoni* 1 [a], *P. lutea* 2 [a], *P. nodifera* 1 [a], *P. rus* 2 [a]	*COI* N.I.
Psammocoridae	1	1			
Psammocora	1	1	1 [a]	*P. profundacella* 1 [a]	

The most common coral family in our samples was Merulinidae, with 12 genera and 29 species (including two incomplete identifications; a *Dipsastraea* sp. and a *Echinopora* sp.), followed by Lobophylliidae with five genera and eight species (including an unidentified *Echinophyllia* sp.), while the rest of the families included one to three genera each (Table 1). While in most cases Veron's [47] 'keys for genera and species' was satisfactory for full identification, four samples were more challenging. These include *Sclerophyllia margariticola* (Klunzinger, 1879) (samples SIRS-083 and SIRS-129; Figure 2, Supplementary Table S1) that did not match in the first attempt of identification to any known species from the Gulf of Eilat, and partly matched *Cynarina* (pointed septal teeth; yet, *Cynarina* do not occur in the Red Sea) or *Lobophyllia*, with mismatched characteristics to both (our samples were collected as solitary polyps so no colonial characteristics could be evaluated). Yet, BLAST (blast.ncbi.nlm.nih.gov; accessed on 21 August 2022) matching with available *COI* sequences resulted in 99.7% similarity to *Sclerophyllia maxima* (accession number FO904931.1). Then, following Arrigoni et al.'s [69] criteria, the two samples were identified as *Sclerophyllia margariticola*, since they represent a solitary polyp mode of life instead of colonial. Another case was *Cyphastrea magna* (Benzoni and Arrigoni, 2017) (sample SIRS-080; Figure 2, Supplementary Table S1), initially identified as *Cyphastrea chalcidicum* (Forskål, 1775), since it had 12 septa of equal size, and not irregularly exerted septa. Yet, the corallite size and shape did not match Veron's [47] description, and BLAST on the *COI* sequences for *Cyphastrea* sp. revealed discrepancies (see discussion). Referring to the updated literature on *Cyphastrea* from the Red Sea, we followed the identification guide by Arrigoni et al. [70] that labeled this species as *Cyphastrea magna* (likewise confirmed the identifications of all other *Cyphastrea* species in this research). A third case was *Psammocora profundacella* (Gardiner, 1898) (sample SIRS-184; Figure 2, Supplementary Table S1), which was first identified as *P. haimeana*, according to Veron [47]. WoRMS referred to *P. haimeana* as a misspelling of *P. haimiana*, a species that does not occur in the Red Sea and did not resemble our specimen. Following a taxonomic note at the Corals of the World website (www.coralsoftheworld.org/species_factsheets/species_factsheet_summary/psammocora-haimiana/; accessed on 15 September 2022), we referred to Benzoni et al. [71] and identified the specimen as *P. profundacella*. Comparing the *COI* sequences of *P. profundacella* from Benzoni et al. [71] (accession numbers FM865879 and AM494853) to our sequence resulted in 99.34% and 99.56% similarity between the sequences (respectively). Results further revealed the importance of adequate sampling methodology. Samples SIRS-013, 103, 160, 189, 190, and *Acropora* sp. 1–4 (Supplementary Table S1) were too young to exhibit species-specific characteristics. Furthermore, since *COI* is not informative for *Acropora* species, we were not able to identify these species according to DNA sequences.

Polyps in sample SIRS-152 (*Dipsastraea* cf. *matthaii*) were compactly arranged, and the colony was very narrow, hindering us from examining detailed corallite morphological characterizations. *Dipsastraea* cf. *speciosa* (sample SIRS-144) was composed primarily of young polyps in the process of intratentacular budding, or immediately after splitting, hence characteristics of corallites were hard to define. *Dipsastraea* sp. (sample SIRS-178), *Echinopora* sp. (sample SIRS-098), *Paragoniastrea* sp. (sample SIRS-174), and *Pavona* sp. (sample SIRS-047) were all young colonies and, hence, full characteristics of adult colonies were absent.

A *COI*-based phylogenetic tree for the coral families and coral species collected in Eilat (Figure 3, Supplementary Figure S1) revealed two major clades, 'complex' and a 'robust' clades [72]. The complex clade splits into two major monophyletic groups. One holds the families Dendrophylliidae and Poritidae, and the second includes the families Acroporidae, Agariciidae, and Euphylliidae. The robust clade splits into two groups. One includes the monophyletic family Pocilloporidae, and the second that splits holds on one branch the families (Lobophylliidae, Merulinidae, Plerogyridae and Plesiastreidae) and on the second branch the families (Coscinaraeidae, Fungiidae, Leptastreidae and Psammocoridae).

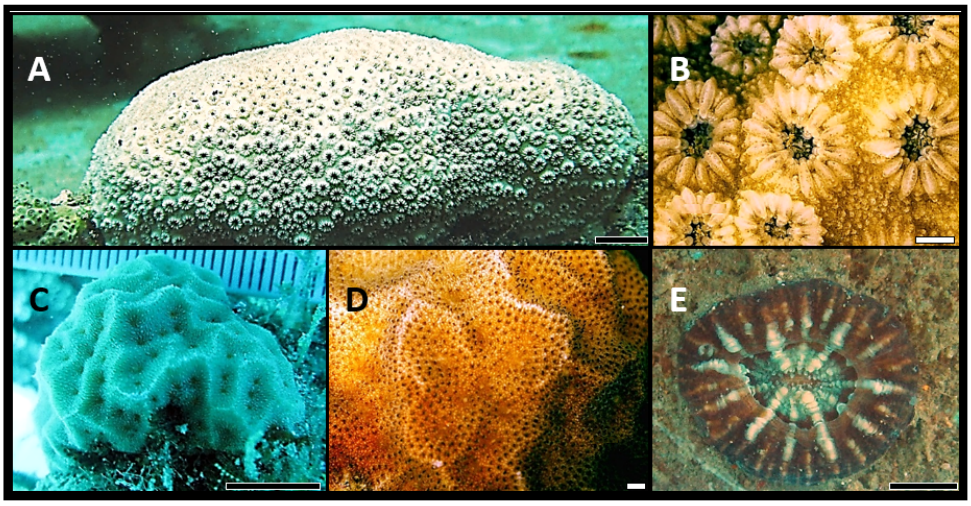

Figure 2. Underwater and close-up documentation of: (**A**,**B**) *Cyphastrea magna* (Benzoni and Arrigoni, 2017) (sample SIRS-080), (**C**,**D**) *Psammocora profundacella* (Gardiner, 1898) (sample SIRS-184) and (**E**) *Sclerophyllia margariticola* (Klunzinger, 1879) (sample SIRS-083). Black bars—1.0 cm (**A**,**C**,**E**), white bars—1.0 mm (**B**,**D**).

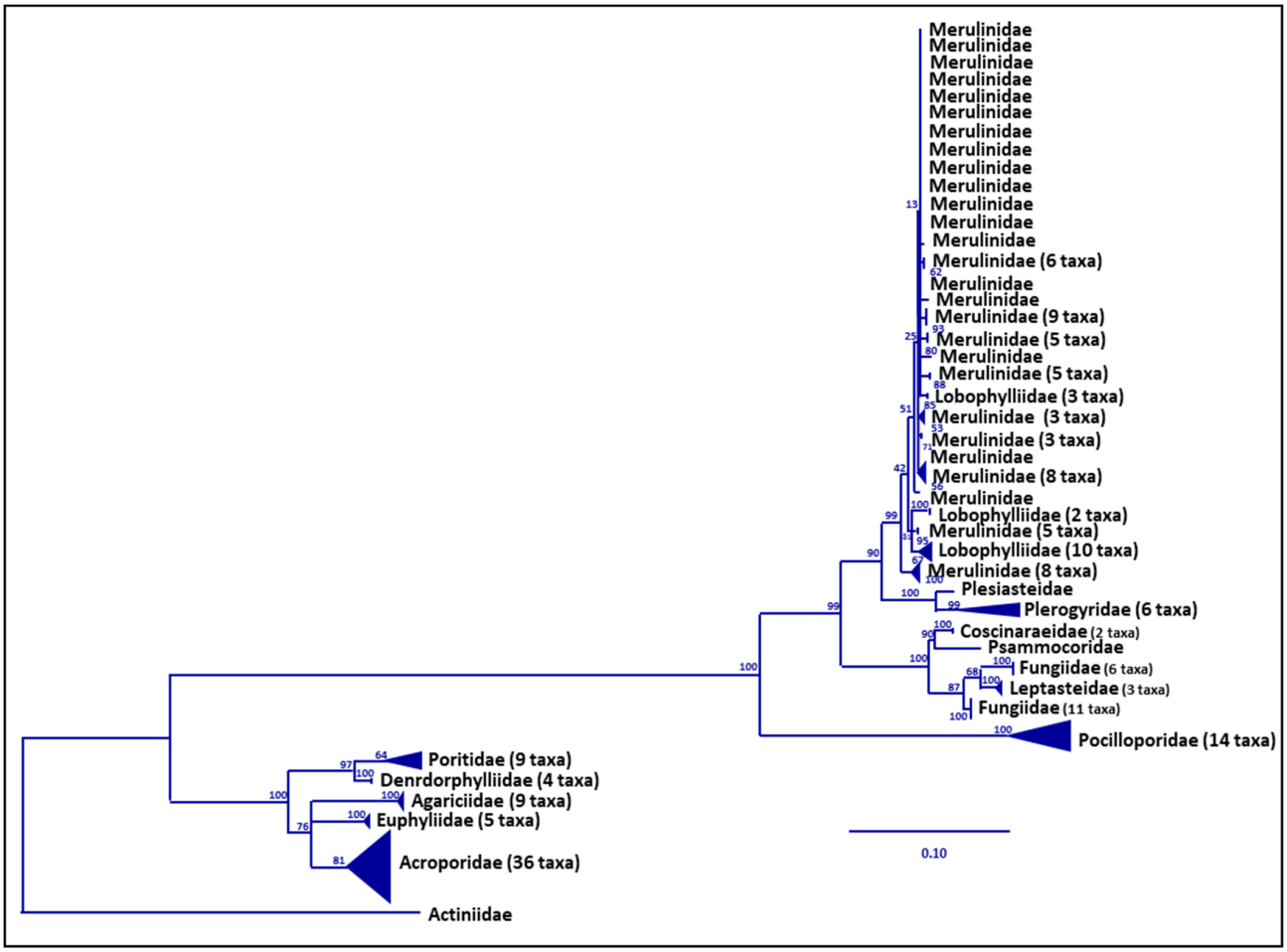

Figure 3. Condensed phylogram of coral samples from Eilat, based on the maximum likelihood analysis of the partial mitochondrial *COI* gene from the 94 species collected in this study. Values at the nodes represent bootstrap values. The number of taxa is given in brackets (a family with no brackets is represented by a single taxon). The tree is rooted by the anemone *Entacmaea quadricolor* (Actiniidae). A detailed phylogenetic tree is provided in Supplementary Figure S1.

4. Discussion

Coral reef conservation, sustainable use of the reefs' goods and services, the rehabilitation of reef resources, its diversity, and the sustainable management of the reefs at local and global scales all necessitate detailed knowledge of reef biodiversity [73–75]. Decisions regarding the objectives and priority areas for actions on coral reef management, including prevention and mitigation of anthropogenic and global climate change impacts, as well as active reef restoration, are based on detailed knowledge on the biodiversity, and on reliable inventory of species for key ecological groups such as corals [75–79]. High diversity among coral species ensures the evolvement and adaptation of coral reefs under the impacts inflicted by anthropogenic activities and environmental changes [80–82], further identifying and treating potentially devastating processes, such as dwindling populations, in particular [3]. Establishing the full species inventory repertoire of scleractinian corals in Eilat is therefore highly important, and the new barcode library initiated here is the first step in a long journey towards the elucidation of coral species biodiversity in the northern Gulf of Eilat. The DNA barcoding library is of utmost importance when deciphering early stage reef recruitments [3,13,83–85], or for foreseen environmental DNA metabarcoding acts [86].

The Israeli coastline of the Red Sea encompasses 12 km of shoreline, out of which ca. 3.5 km are designated as a nature reserve, of which only 1.2 km are fenced and referred to as the Coral Beach Reserve. Previously considered as one of the most diverse reefs in the world [40], the Eilat coral reserve is neighbored by the constantly developing cities of Eilat and Aqaba, perpetrating a wide range of anthropogenic impacts on the coral reef biota. Despite all efforts and management activities engaged, a gradual degradation of the Eilat coral reef has been noted for nearly 40 years [56–59,87,88], some of which is cryptic [3]. Following the development of novel molecular tools for coral identification, it is of prime interest to accurately document the current biodiversity of coral species, as well as to improve monitoring and examination of future trends and anthropogenic/climate change impacts.

This study aims to pioneer the DNA barcoding reference library of shallow water scleractinian corals from the Gulf of Eilat, combining the skills of local taxonomists and the methodology of molecular identification. Four limited collection surveys (permit approval) resulted in a total of 191 vouchered specimens that represent 98 taxa, all of which were successfully barcoded and uploaded onto the BOLD website (www.boldsystems.org; accession numbers SIRS-001-SIRS-191; accessed on 15 October 2022, Supplementary Table S1), creating a new barcode database called "Scleractinia of the Israeli Red Sea" [SIRS]. All vouchered specimens are new full species records in the BOLD system, and three species (*Sclerophyllia margariticola*, *Cyphastrea magna*, and *Psammocora profundacella*) represent first records in Eilat's coral reef, implying that the scientific coverage on the biodiversity and inventory of Eilat's corals is still meagre.

Basal metazoans, such as Cnidarians, have extremely slow mitochondrial evolution rates, around 10–20 times slower than other metazoans [89], but with faster evolving nuclear genes [89–92]. Revealing that the *COI* is unsurprisingly not discriminating between closely related species in all cases is an outcome necessitating reliance on additional molecular markers and/or morphological parameters. This limited ability of *COI* to positively identify coral specimens to the species level is reinforced by the maximum likelihood (HKY + G) phylogenetic tree constructed, clustering almost all *Acropora* spp. on the same branch of the phylogenetic tree and clustering species from the families Merulinidae and Lobophylliidae on the same branch. Furthermore, our phylogenetic tree results (Figure 3; Supplementary Figure S1) revealed some incongruencies when compared with other studies that used several other genetic markers. In the 'robust' clade, our phylogenetic tree (Figure 3) positioned Leptastreidae within the Fungiidae on a branch with the genera *Danafungia* and *Fungia*, separated from the branch of *Cycloseris* (this clustering is only moderately supported by a bootstrap value of 68), while Kitahara et al. [15] placed Leptastreidae as a monophyletic branch, separated from Fungiidae. Kitahara et al. [15] further placed *Danafungia* closer

to *Cycloseris* as a sister group to *Fungia*. The analysis of the monophyletic branch that clusters Merulinidae and Lobophylliidae in our samples (Supplementary Figure S1) reveals more incongruencies, as the genus *Hydnophora* (Merulinidae) was placed within the family Lobophylliidae, while Huang et al. [11] placed it as a sister group to the genus *Favites* based on five nuclear and mitochondrial markers, and Kitahara et al. [15] placed it closer to *Coelastrea* and *Dipsastraea*. Moreover, we found that *Cypastrea* spp. were clustered with *Favites pentagona* on a branch containing Lobophylliidae, rather than within the Merulinidae, while following Huang et al. [11] and Kitahara et al. [15] *Cyphastrea* is nested at the first paraphyletic branch diverging inside the Merulinidae branch, neatly separated from the Lobophylliidae branch. Based on molecular data, *Favites pentagona* was placed on a sister branch to *Dipsastraea* [11], while the morphological data clustered this species with other *Favites* spp. Our molecular data suggest the placement of *F. pentagona* within a different genus, a consideration necessitating support from additional molecular methods.

Since not all scleractinian species are reliably identifiable via *COI*, it is indispensable to use additional markers [13,93]. A combination of barcodes was used in previous studies for separation, including additional mitochondrial and nuclear genes, such as mitochondrial cytochrome B (*cytB*), nuclear β-tubulin, and the rDNA segment containing parts of 18S, internal transcribed spacers (ITS), 5.8S, 28S [26,27,29–31], mitochondrial noncoding intergenic region (*IGR*), and nuclear histone H3 [8,9]. For Acroporidae, nuclear *Pax-C* and the mitochondrial putative control region [94–96] were useful.

Morphological identifications of corals are also associated with difficulties, primarily when samples were taken from colonies too young to present the mature characteristics required for species identification. In our collections, this was mostly noticed in the specimens collected from artificial substrates during the fourth set of sampling (permit constraints). In some genera, the mature form of the colony is critical for the identification process, with *Acropora* being a major genus following this notion ([47] volume 3, page 447). This is also true for Merulinid species, such as *Dipsastraea* and *Coelastrea*, that are identified based on corallite form of mature colonies, while young colonies may present mixed shapes and sizes of corallites, primarily at the colonial peripheries. The preferred sample site when considering reduced damages is inflicted by sampling. A well established and documented DNA barcoding reference library of stony corals may solve the morphological constrictions.

The results of this study thus suggest that sampling of large coral colonies is preferred for morphological identification for the development of corals' DNA barcoding reference library, along with the use of more than a single genetic marker. Since this is a first step in creating a DNA barcoding reference library of stony corals from the Gulf of Eilat, the number of species obtained are clearly lower than the actual number of existing species, including the lists of cryptic species and first records assigned to the area.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse10121917/s1. Table S1. Full list of samples collected in this research. Sample session (1) 18–19 January 2013. (2) 12–15 April 2013. (3) 13–16 August 2013. (4) 28 August 2013. Sample sites include the following. (KIS) Kisoski beach (29°32′49.98″ N, 34°57′14.78″ E). (DEK) Dekel beach (29°32′24.67″ N, 34°56′51.23″ E). (IUI) the Inter-University Institute for Marine Sciences in Eilat (29°30′07″ N, 34°55′02″ E), (Figure 1). N/A—Not Available, Field ID—temporary identification code for sample as given in the field before assigning final voucher number *—in parentheses original scientific name, **—Veron, 2000 volume 3, page 447, bold number—transition to genus. Table S2. Primers used for amplifying the *COI* gene. Figure S1. Phylogenetic tree of coral samples from Eilat, based on the maximum likelihood of the partial mitochondrial *COI* gene from the 191 coral samples collected in this study. Values at the nodes represent bootstrap values. (A) Full tree view. (B) Detailed view of robust clade. (C) Detailed view of complex clade. The number preceding the scientific name is the sample number in the study detailed in Supplementary Table S1. The tree is rooted by the anemone *Entacmaea quadricolor* (Actiniidae).

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O.S.; writing—review and editing, E.N.R., O.S., and B.R.; supervision, B.R.; project administration, B.R.; funding acquisition, B.R. All authors have read and agreed to the published version of the manuscript.

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