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Insights into the occurrence of phylosymbiosis and co-phylogeny in the holobionts of octocorals from the Mediterranean Sea and Red Sea

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Abstract

Background Corals are the foundational species of coral reefs and coralligenous ecosystems. Their success has been linked to symbioses with microorganisms, and a coral host and its symbionts are therefore considered a single entity, called the holobiont. This suggests that there may be evolutionary links between corals and their microbiomes. While there is evidence of phylosymbiosis in scleractinian hexacorals, little is known about the holobionts of Alcyonacean octocorals.

Results *16S rRNA* gene amplicon sequencing revealed differences in the diversity and composition of bacterial communities associated with octocorals collected from the mesophotic zones of the Mediterranean and Red Seas. The low diversity and consistent dominance of *Endozoicomonadaceae* and/or *Spirochaetaceae* in the bacterial communities of Mediterranean octocorals suggest that these corals may have a shared evolutionary history with their microbiota. Phylosymbiotic signals were indeed detected and cophylogeny in associations between several bacterial strains, particularly those belonging to *Endozoicomonadaceae* or *Spirochaetaceae*, and coral species were identified. Conversely, phylosymbiotic patterns were not evident in Red Sea octocorals, likely due to the high bacterial taxonomic diversity in their microbiota, but cophylogeny in associations between certain coral and bacterial species was observed. Noteworthy were the associations with *Endozoicomonadaceae*, suggesting a plausible evolutionary link that warrants further investigations to uncover potential underlying patterns.

Conclusions Overall, our findings emphasize the importance of *Endozoicomonadaceae* and *Spirochaetaceae* in coral symbiosis and the significance of exploring host-microbiome interactions in mesophotic ecosystems for a comprehensive understanding of coral-microbiome evolutionary history.

Keywords Octocorals, Holobiont, Bacteria, Phylosymbiosis, Cophylogeny, Mesophotic zone, *16S rRNA* gene

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Background

Octocorallia is a class of Anthozoans (Phylum Cnidaria) that diverged from the Hexacorallia during the Precambrian and includes over 3500 species that can be found around the world and at all depths, from the littoral zone to the deep sea, and from tropical to polar regions [57]. Corals engage in intricate and complex interactions with a range of microorganisms (e.g., protists, fungi, bacteria, archaea, and viruses). Together, a coral and its microbiota are considered a single entity, which is termed a "holobiont" [35]. Symbionts play important roles in the health of their coral host (e.g., nutrient supply, protection against pathogens) and display an adaptive response to environmental changes (e.g., toxin degradation, thermal tolerance, [73]). The bacterial communities associated with corals, particularly those of the reef-building Scleractinia (Class Hexacorallia), have been extensively studied. These are generally composed of a "core microbiome" of microbial phylotypes that are consistently associated with a host species [1], and various transient microbes whose presence depends on local conditions [30, 100, 103, 104]. These stable associations between a coral species and members of its conserved core microbiome suggest that there may be an evolutionary connection. How coral-microbe symbioses have evolved is, however, still largely unknown.

Phylosymbiosis, defined as 'microbial community relationships that recapitulate the phylogeny of their host' [43], may arise from codiversification, cospeciation, coevolution or even short-term changes in the microbiota of a host, but also from shifts in geographical ranges, or the diet of the host and ecological drift in microbial communities [20, 25, 40, 61, 110]. It has been observed in various systems, such as the roots of plants [99], the gut of mammals [9], insects [41], and sponges [66]. In scleractinian corals, signals of phylosymbiosis have also been observed but at high bacterial taxonomic levels (family and genus [66, 76]), and codiversification has been suggested to have played a role in the development of coral-microbiota interactions in Mediterranean octocorals [100, 104]. While phylosymbiosis considers the whole microbial community associated with a host organism, more detailed analyses assess evolutionary links between host and specific microbial species, such as cophylogeny. Cophylogenetic assessments examine the concordance of phylogenies between two groups of species [4, 68], with congruence suggesting shared evolutionary links [28], although the underlying processes are not fully understood and can be attributed to coevolutionary [23, 89] and biogeographic factors (e.g. co-vicariance [107],). Cophylogenetic patterns have also been observed in scleractinian corals, particularly with bacteria from the genus *Endozoicomonas* [65, 76].

In comparison with Scleractinia, the microbiota of octocorals is relatively understudied, and most studies have been conducted on octocoral populations in shallow waters (reviewed in [100, 104]). Studies on Mediterranean gorgonian corals have shown highly conserved microbiota on both temporal and geographical scales as well as over a wide range of temperatures [97, 98, 100, 102–104]. This suggests that signals of phylosymbiosis and cophylogeny may be present in octocorals as well. Such patterns have indeed been described in octocorals from Australian reefs [65, 66]. It is, however, interesting from a coral holobiont evolutionary perspective to explore this hypothesis further in other regions and climatic zones.

In our study, we analyzed the prokaryotic communities of octocorals from the mesophotic zones (between 60 and 150 m depth) of the temperate Mediterranean Sea, characterized by its hot, dry summers and mild, wet winters [46, 69], and the tropical Red Sea, known for its consistently high temperatures and high salinity [19, 52, 59], emphasizing the different environmental conditions between these two seas. Mesophotic ecosystems are the ideal environment to study potential evolutionary links between corals and their microbiota as there is less disturbance by physical and anthropogenic factors at these depths, but access to these ecosystems is challenging. This dataset, collected during the *Gombessa V* and *VI* (technical diving; 2019, 2021) expeditions and the *ENCOR* campaign (Remotely Operated Vehicle; 2019), encompasses 14 species, belonging to 10 genera, 9 families and both orders of *Octocorallia*. This allowed us to explore whether an evolutionary link between octocorals and their microbiota may exist. The microbiota of several of these octocoral species have, to our knowledge, not been described before.

We show signals of phylosymbiosis in octocoral holobionts from the Mediterranean Sea, but not in those from the Red Sea. All species did, however, appear to have evolved with specific bacterial phylotypes, especially *Endozoicomonas* and *Spirochaetaceae*, as evidenced by cophylogenetic patterns in those coral-microbe associations. Overall, our results provide further insights into the link between the evolution of corals and their associated microbes, and the impact thereon of geographical separation.

Methods

Sample collection

Samples were collected during three scientific expeditions: the *Gombessa V* and *VI* expeditions took place in the temperate Mediterranean Sea, and the *ENCOR* expedition was in the tropical Red Sea (Fig. 1). There is no overlap in coral species between these seas. A

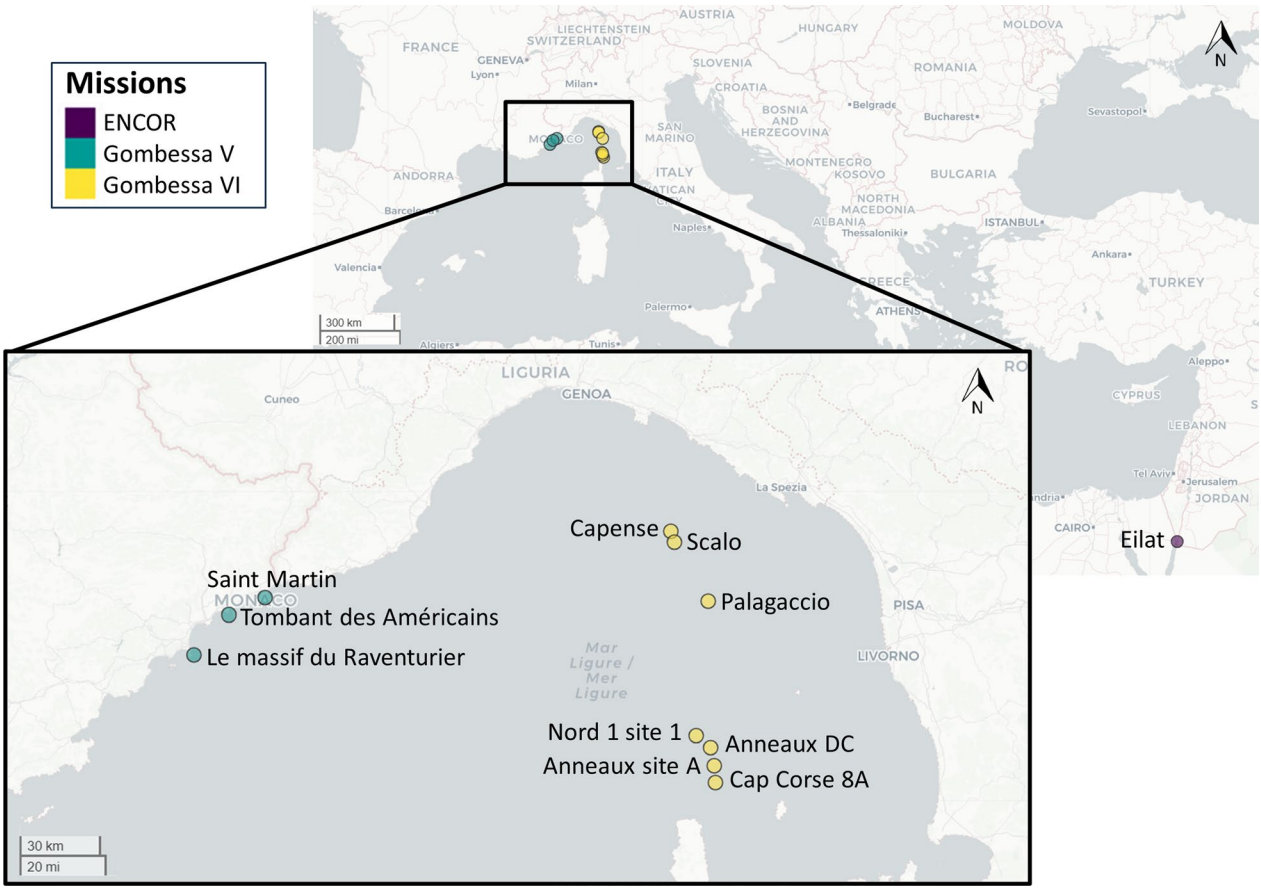

Fig. 1 Locations of the different sampling sites. The corresponding sampling missions are indicated for each site

summary of the samples collected during these expeditions is provided in Suppl. File 1 as Table S1.

Gombessa expeditions V and VI – Corals were sampled along the Côte d'Azur (*Gombessa V*) in August 2019 and near Corsica (*Gombessa VI*) in July 2021 between 70 and 116 m depth. Samples were collected by divers using a combination of saturation diving and electronically controlled rebreather diving with the permission of the Direction Interrégionale de la Méditerranée (DIRM). At each site, fragments of approximately 20 cm were collected from 6 colonies per species. Fragments were placed individually in a plastic bag filled with ambient seawater and brought back to the surface.

ENCOR expedition – Corals were sampled between 65 and 120 m depths near Eilat (Israel) in October 2019, under the permits of the Israel Nature and Parks Authority. For each species, colony fragments of 25–30 cm^2 were collected with a ROV (ECA H800) equipped with an HD video camera (VS300 Eca Robotics) and a manipulative arm for sampling. The ROV was operated from a boat using a fiber-optics umbilical cable. Species were determined with the help of octocoral taxonomist Prof. Yehuda Benayahu (Tel Aviv University).

All samples were rinsed twice with 0.2-µm filtered seawater to remove exogenous, loosely associated microorganisms and stored in RNAlater (ThermoFisher Scientific) at 4 °C. Additionally, we collected multiple replicates of 2 L of seawater at most of the sampling sites, although logistical complications prevented us from collecting seawater at four sites. The water was filtered using 0.2 µm Whatman Nuclepore Track-Etched filters (Sigma-Aldrich), and the retentate was preserved in RNAlater and stored at 4 °C.

DNA extraction

DNA was extracted using the DNeasy PowerBiofilm kit (QIAGEN, Hilden, Germany) with the following modifications: during the cell lysis step, 2 µL of Proteinase K (600 U/ml) was added to the sample and incubated at 60 °C for 2 h, followed by 2 min of bead beating using the CryoMill (Retch, Germany) at a frequency of 30 Hz. Negative extraction control samples (*i.e.,* extraction without

sample material) were processed at the same time as the coral samples to account for contaminants. DNA concentration was measured using an Invitrogen™ Qubit™ 3 Fluorometer and DNA was stored at − 20 °C.

16S rRNA gene amplicon library preparation

DNA was sent to STAB VIDA (Portugal) for amplicon library preparation using Illumina's standard "16S Metagenomic Sequencing Library Preparation" protocol [32] and sequencing. The V3-V4 region (~ 460 base pairs) of the *16S rRNA* gene was amplified using the forward primer 341F 5'-CCTACGGGNGGCWGCAG-3' and the reverse primer 785R 5'-GACTACHVGGGTATCTAATCC-3. PCR-amplified DNA was size-separated via gel electrophoresis and PCR products of the correct amplicon size were extracted using the Qiaquick Gel Extraction Kit (QIAGEN). Libraries were paired-end sequenced on the Illumina MiSeq platform (version 3 chemistry – 2 × 300 bp, 600 cycles). Raw sequence data has been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under accession number PRJNA1045964, PRJNA1047055 and PRJNA1047303.

16S rRNA gene amplicon data analysis

The *16S rRNA* gene amplicon data were processed using the USEARCH v11 software (64-bit version, https://drive5.com/usearch/). In total, 22,911,242 reads were obtained from all 214 samples (coral and seawater), ranging from 158 to 508,818 reads per sample. Negative controls contained 82 to 7,030 reads per sample. Reverse reads were truncated using -fastx_truncate function with a base quality threshold of > Q20 to ensure that high quality reverse read (R2) data was used for merging. Forward (R1) and reverse (R2) reads were merged using -fastq_mergepairs with the following settings: a minimum and maximum length of the merged sequence of 400 bp and 510 bp, and a minimum and maximum base-pair difference of 15 bp. The forward and reverse primer sequences (17 bp and 21 bp) were removed from the merged sequences using -fastq_truncate. The resulting sequences were quality filtered allowing a maximum of one expected error using -fastq_filter, obtaining a total of 15,862,422 sequences with a range of sequences per sample of 3,028 to 379,407 sequences. Unique sequences were identified using the -fastx_uniques command and then denoised using the UNOISE3 algorithm, obtaining 17,474 zero-radius operational taxonomic units' (zOTU, equivalent to an Amplicon Sequence Variant (ASV)). zOTUs were taxonomically annotated using the SINTAX algorithm and the SILVA reference database (version 138) with an assignment confidence cutoff of 0.8. An OTU table was generated by clustering sequences with an identity similarity value of 0.99 using -otu_tab command. Considering the clustering into 99% OTUs, we refer to OTUs from hereon. The OTU table, the metadata as well as the sequences of each zOTU and their taxonomy annotation are available in Suppl. File 1 as Tables S2 and S3 and in Suppl. File 2 as File S1, respectively.

Bacterial community analysis

Analyses were run in the R environment (version 4.1.2, 2021–11-01). The R-package *decontam* [16] was used to identify contaminant OTUs in the samples based on the negative control samples (isContaminant function). A total of 2,386 of the 17,474 OTUs were identified as contaminants and were removed. Another 4,244 OTUs were removed from the dataset as these were annotated as unknown Kingdom, Mitochondria or Chloroplast and thus appeared to be of non-bacterial origin. Measures of the α-diversity, i.e. of the diversity of microbial phylotypes in a sample, were assessed using Chao1 estimations of the richness. This was calculated using the R-package *vegan* ([67], Version 2.6–2, 2022) and analyses of variance (ANOVA) were used to test if the α-diversity of coral-associated bacterial communities was similar among coral species. To fit the assumption of homoscedasticity, a log transformation had been performed on the data prior to ANOVA. Tukey's Honestly Significant Difference (HSD) test was performed to identify potential differences between species. For β-diversity analyses, which compare the microbiota compositions between samples, all samples were rarefied to 9,000 reads using the R function *phyloseq_mult_raref_avg* from the *phyloseq* R-package [58]. Non-metric multidimensional scaling (NMDS) analysis, as well as permutational multivariate analysis of variance (*adonis2*() function from the *vegan R*-package) based on the Bray–Curtis dissimilarity index [10] were used to assess differences in bacterial community β-diversity between the coral species using 9,999 permutations. A pairwise PERMANOVA test was performed to identify species having significantly different bacterial community compositions (*adonis_pairwise*() function from the *metagMisc* R-package; [60]). Hierarchical cluster analysis was used to create a dendrogram (*pvclust* R-package; [94]) using the unweighted pair group method with arithmetic mean (UPGMA) method on the Bray–Curtis distance matrix. *P*-values were computed for each of the clusters via multiscale bootstrap resampling. The core microbiome of each coral species was identified using the *core_members* function (arguments: detection = 0, prevalence = 99/100, include.lowest = FALSE) from the R-package *microbiome* [38].

COI, *mtMutS* and *12S rRNA* mitochondrial genes sequencing

The sequences of three mitochondrial genes (Cytochrome Oxidase Subunit 1 (*COI*), Mutator S (*MutS*), *12S rRNA*) were used to establish a phylogenetic tree of the different coral hosts [21, 49, 56, 88]. PCR amplification of the genes of interest were done on three samples per species. Primers used to specifically target the three genes, and their respective annealing temperatures are given in Table 1.

The Phusion High-Fidelity DNA Polymerase kit (ThermoFisher) was used following the manufacturer's recommendations. The three-step PCR protocol consisted of an initial denaturation step of 98 °C for 3 s followed by 30 amplification cycles (denaturation at 98 °C for 10 s, annealing at 46 °C for COI, 58 °C for MutS and 62 °C for 12S for 30 s and extension at 72 °C for 45 s) then a final extension at 72 °C for 10 min. The PCR products were size separated on an agarose gel and DNA fragments were excised from gel and purified using the GFX™ PCR DNA and Gel Band Purification kit (Illustra™). No PCR amplification was observed in the negative control samples. DNA amplicons were sent to Eurofins Genomics (Germany) for Sanger sequencing. Approximately 25 nucleotides were cut from each sequence on both sides to ensure a good quality and have sequences with identical lengths. Sequences were aligned using the ClustalW Multiple Alignment algorithm in the BioEdit software. For each gene and species, a consensus sequence was created, and the consensus sequences of *COI* (746 bp), *MutS* (779 bp) and *12S rRNA* (583 bp) were concatenated to create a single sequence per coral species. PhyML [27] was used to create the host phylogenetic tree based on the maximum-likelihood principle. The Gamma-distributed General Time-Reversible (GTR) model was selected based on the Akaike's Information Criteria (AIC) using the Smart Model Selection (SMS) software implemented in the PhyML environment [39]. To ensure statistical consistency, 1000 bootstrap replications of the test were performed.

Phylosymbiotic signal detection

Congruence between the topology of the coral host phylogenetic tree and the dendrogram of the bacterial microbiota was quantified using two topological comparison metrics: the Robinson–Foulds metric [84] and the matching cluster metric [7]. The Robinson–Foulds (RF) metric quantifies the structural dissimilarity between trees by counting splits or bipartitions that are unique to each tree. The matching cluster metric evaluates the similarity between two sets of clusters by comparing how well the elements are grouped together or separately, providing a measure of agreement between different clustering outcomes. These two metrics were normalized by dividing the scores by the total possible congruence scores (or maximum congruence) between the two trees to reflect how different the actual trees are relative to that maximum similarity. These metrics were computed using a Python script adapted from Brooks et al. [11] (Suppl. File 2 as File S2) and the TreeCmp program [8], respectively, and then normalized by dividing the resulting scores by the total possible congruency scores for the two trees, giving distance values between 0 (complete congruence) and 1 (complete incongruence). Statistical significance of the phylosymbiotic patterns were assessed by comparing the host phylogenetic tree to 100,000 randomized dendrograms with equivalent topologies, and then estimating the *P*-value as the proportion of random trees giving an equivalent or more congruent result than the bacterial dendrogram. In addition, Mantel tests were performed to assess significance of the correlation between the bacterial composition distance matrix and the cophenetic distance matrix of the host tree using Pearson correlation with 9999 permutations, where a positive r-value indicates potential phylosymbiosis (mantel.rtest function from R-package ade4; [17]).

Cophylogenetic analyses

To identify bacterial phylotypes that may exhibit a concordant evolutionary history with their coral hosts along patterns of cophylogeny, we performed Random Tanglegram Partitions analyses [4, 5] using the R-package RTapas [47]. Given the complexity of the microbiome dataset, we applied a threshold for total relative

Table 1 Characteristics of the primer sets used for Sanger sequencing

Gene	Amplicon length	Primers	References
COI	~1080 bp	COII8068xF 5'-CCATAACAGGRCTWGCAGCATC-3' COIOCTR 5'-ATCATAGCATAGACCATA-3'	[56] [21]
mtMutS	~870 bp	ND42599F 5'-GCCATTATGGTTAACTATTAC-3' MUT3458R 5'-TSGAGCAAAAGCCACTC-3'	[88] [21]
12S rRNA	~600 bp	12SF 5'-GTGCCAGCHNAHGCGGTYA-3' 12SR 5'-RAGDYGACGGGCRRTTTGT-3'	[49]

abundance (> 0.0001), thereby reducing the dataset from 7793 to 244 phylotypes. Next, three input files were generated: (1) the phylogenetic tree of the coral hosts (HH) based on the concatenated consensus sequences of the *COI*, *MutS* and *12S rRNA* genes (see above), (2) a phylogenetic tree of the 244 selected bacterial phylotypes (SS) based on the *16S rRNA* gene amplicon sequences aligned using the ClustalW Multiple Alignment (BioEdit software) and phylogenetic tree construction using PhyML [27] with the Gamma-distributed General Time-Reversible (GTR) model and 100 bootstrap replications (Suppl. File 2 as File S3; and (3 a binary presence-absence matrix (A, containing rows and columns that correspond to the terminals in HH and SS, respectively, and where existing associations between each terminal are encoded as 1, and the absence of associations is represented as 0 (Suppl. File 2 as File S4). The Random Tanglegram Partitions algorithm (function max_incong()) was used on the dataset with N = 10,000 iterations, and randomly selecting n = 7 unique one-to-one associations between hosts (H) and symbionts (S) to maximize incongruence. Subsequently, the Procrustes Approach to Cophylogeny (PACo; [4]) was applied to the selected associations, and the residual frequencies of host-symbionts associations falling within the 1% percentile (res.fq = TRUE) were used to estimate the contribution of each host-symbiont association to the global cophylogenetic signal. Positive values denote host-symbiont associations contributing significantly to cophylogenetic congruence, whereas negative values identify associations leading to phylogenetic incongruence.

Results

Diversity and composition of the octocoral-associated bacterial community

Overall, significant differences in α-diversity (Chao1 index) of the octocoral microbiota were observed between coral species (Fig. S1; Suppl. file 1 Table S4; $P = 6.29.10^{-8}$). Analyses of β-diversity showed that octocoral-associated bacterial communities were significantly different from the seawater bacterial communities ($P = 0.001$), and that the composition differed between coral species ($P = 1.10^{-4}$), particularly between those from the Red Sea and the Mediterranean Sea ($P = 1.10^{-4}$; Fig. 2A, and Suppl. file 1 Table S5). However, the dispersion was also significantly greater among samples of corals from the Red Sea than of corals from the Mediterranean Sea ($P = 0.0003$; Suppl. file 1 Table S6). In addition, pairwise comparisons of the β-diversity of the microbiota showed differences between nearly all species from the Mediterranean Sea ($P < 0.05$; Suppl. file 1 Table S5), apart from the microbiota of *E. verrucosa* which did not differ from that of *E. cavolini* and *P. clavata* ($P = 0.1289$; Suppl. file 1 Table S5). And dispersion was not significantly different between species, except for *E. cavolini*, whose samples were significantly more dispersed than those of *A. coralloides* ($P = 0.012$) and *P. clavata* ($P = 0.0255$; Suppl. file 1 Table S6). Among the corals from the Red Sea, β-diversity of the microbiota was only different between *Ovabunda spp.* and *P. thyrsoides* ($P = 0.03$; Suppl. file 1 Table S5) and dispersion was statistically similar (Suppl. file 1 Table S6).

The hierarchical cluster analysis also showed distinct patterns between the two seas. The octocoral species from the Red Sea, which all belong to the order Malacalcyonaceae, formed a separate cluster from the Mediterranean corals (Fig. S2). Within the two geographic clusters, clustering generally followed the octocoral taxonomy. The Mediterranean cluster was divided into two main clusters corresponding to the orders Malacalcyonaceae (*E. cavolini*, *E. verrucosa*, *P. clavata*) and Scleralcyonaceae (*C. verticillata*, *C. rubrum;* Fig. S2). Samples were mainly grouped along genus and species lineages, although a few exceptions were observed. For example, the Mediterranean *A. coralloides* clustered with the Scleralcyonaceae although it belonged to the Malacalcyonaceae order and among the Red Sea corals, *K. utinomi* was grouped with various *Sclerophytum* species (Fig. S2).

The bacterial community compositions (Fig. 2B) indeed showed differences between the species. Mediterranean gorgonians of the genera *Paramuricea* and *Eunicella* harbored a bacterial community dominated (52% to 98%) by *Endozoicomonas* (OTU1, OTU2, OTU3 and OTU5; Class *Gammaproteobacteria*; Order *Oceanospirillales*). The microbiota of *Ovabunda* spp. (65%), *P. thyrsoides* (83%) and *S. glaucum* (48%) also largely consisted of *Endozoicomonas* but harbored different OTUs (e.g., OTU11, OTU6 and OTU7 respectively) than the Mediterranean gorgonians. The microbiota of all other octocoral species also contained *Endozoicomonas* but generally in very low (< 1%) relative abundance. In contrast, the microbiota of both Scleralcyonaceae *C. rubrum* (48% to 67%, mainly OTU4 and OTU15) and *C. verticillata* (21%, mainly OTU24) consisted primarily of *Spirochaetota*, especially from the *Spirochaetaceae* family. *Spirochaetota* were also observed in some Malacalcyonaceae from the Mediterranean Sea (*A. coralloides* (15%, mainly OTU4)) and the Red Sea (S. *glaucum* (35%, mainly OTU29), *S. eilatense* (22%, mainly OTU147 and OTU223) and *S. vrijmoethi* (4%, mainly OTU140)). Other bacterial taxa were also found to be relatively abundant in certain coral species. For instance, *Bacillota* were frequently found in association with *C. rubrum* (11%), *C. verticillata* (4%) and the Red Sea species *Ovabunda* spp. (7%) and *K. utinomii* (25%; Fig. 2B). *C. rubrum* had a high percentage of *Gammaproteobacteria* of the order BD72BR169 (between 2

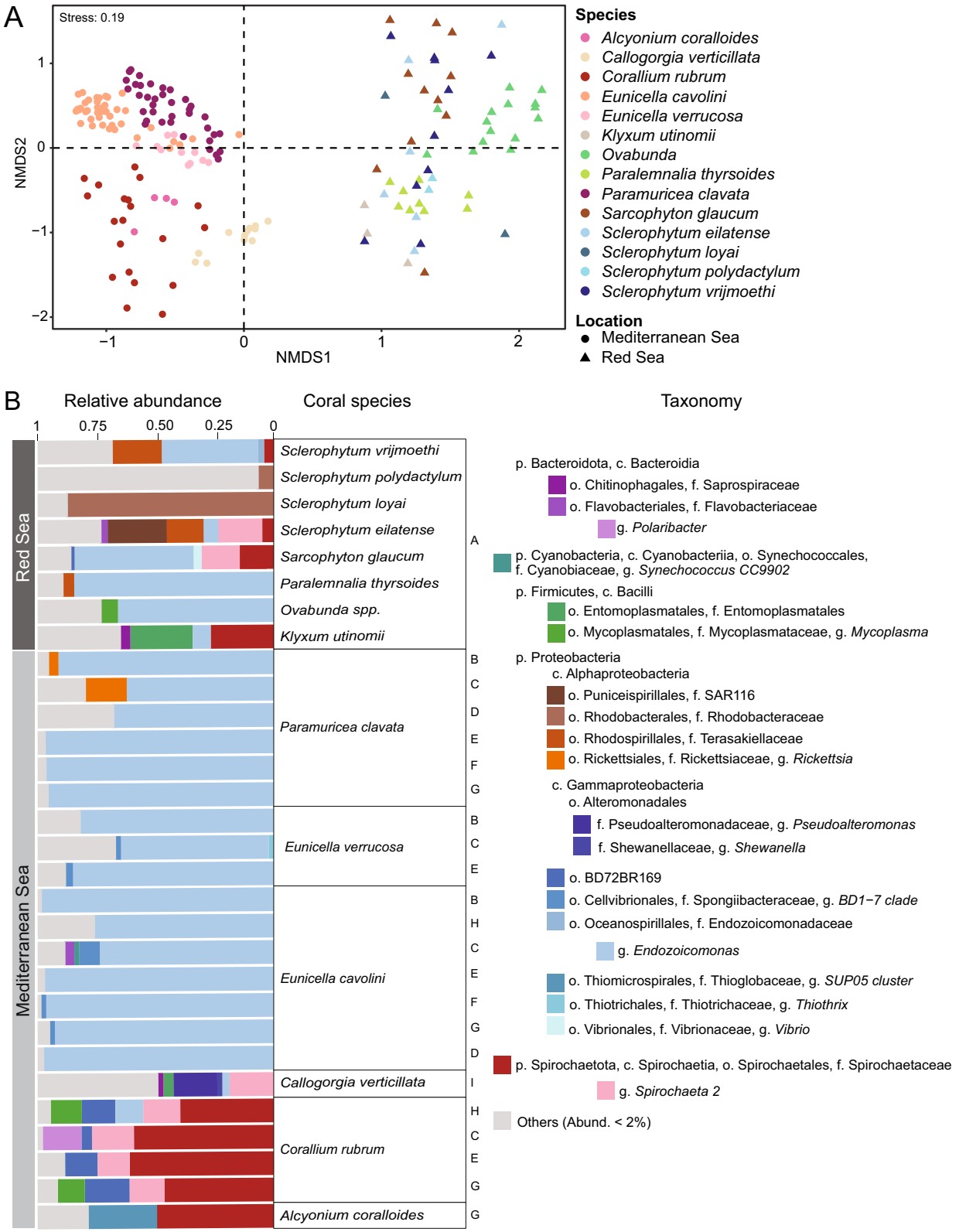

Fig. 2 **A** NMDS on Bray–Curtis distance matrix showing ordination of coral samples; **B** Bacterial community composition for each species per location (taxa less abundant than 2% are grouped together under Others; Lowercase letters correspond to taxonomic levels, p: Phylum, c: Class, f: Family, g: Genus; Uppercase letters correspond to sampling locations, A: Eilat, B: Tombants des Américains, C: Saint Martin, D: Nord 1 Site 1, E: Massif du Raventurier, F: Capense, G: Cap Corse 8A, H: Scalo, I: Anneaux Site A)

and 20% in average) as well, and the SUP05 cluster from the *Thioglobaceae* family accounted for about 30% of the total bacterial abundance of *A. coralloides.* Furthermore, *Pseudoalteromonas* represented about 20% of the bacterial abundance in *C. verticillata*, and similar proportions of *Terasakiellaceae* were found in the microbiota of the Red Sea species *S. eilatense* and *S. vrijmoethi* (Fig. 2B).

The analysis of the core microbiome highlighted the importance of certain symbionts, as only few OTUs composed the core microbiome of most coral species (between 0 and 33 OTUs; for *S. polydactylum* a core microbiome could not be accurately determined as there were only 2 samples present in the dataset; Suppl. file 1 Table S7). Notably, abundant OTUs annotated as *Endozoicomonas* (Suppl. file 1 Table S7), were part of the core microbiome of the Mediterranean gorgonians *E. cavolini* (OTU1, OTU3 and OTU5), *E. verrucosa* (OTU1, OTU3, OTU5 and OTU8) and *P. clavata* (OTU1, OTU2, OTU3 and OTU8). OTU1, OTU2 and OTU5 were also found in the core microbiome of *A. coralloides*. Contrastingly, *Spirochaetaceae* (OTU4 and OTU15) were the main members of the core microbiome of *C. rubrum*. *Spirochaetaceae* OTUs were also present in the core microbiome of *A. coralloides* (OTU4, OTU53, OTU135 and OTU1476) and *C. verticillata* (OTU24). Overall, the core microbiome of all species harboured OTUs belonging to the *Endozoicomonas* genus and/or the *Spirochaetaceae* family. However, OTUs belonging to other bacterial taxa were also part of the core microbiome of various coral species. For example, *Pseudoalteromonas* OTU397 and OTU458 are abundant in the microbiota of *C. verticillata* and are part of its core microbiome, while *Thioglobaceae* SUP05 cluster OTU73 was found in the core microbiome of *A. coralloides*. No core microbiome could be identified for *S. eilatense* (Suppl. file 1 Table S7).

Signals of phylosymbiosis

Since the coral-associated bacterial communities were relatively well clustered according to host taxonomy, we assessed whether patterns of phylosymbiosis could be observed. When comparing the host phylogenetic tree to a simplified dendrogram representing bacterial community composition (Fig. 3AB), the extent of congruence between the two structures is indicated by distance values close to 0 (complete congruence) and 1 (complete incongruence). Here, no signal of phylosymbiosis was observed when all coral species were considered given the high positive correlation values (Fig. 3A and Suppl. file 1 Table S8; Robinson-Foulds distance of 0.68 ($P=0.00033$); matching cluster distance of 0.85 ($P=0.045$); Mantel test r-value = 0.1 ($P=0.1$)). However, patterns of phylosymbiosis were observed in the Mediterranean species (Fig. 3B and Suppl. file 1 Table S8; Robinson-Foulds distance of 0.28 ($P=0.01$); matching cluster distance of 0.32 ($P=0.001$); Mantel test r-value of 0.6 ($P=0.003$)). It should be noted though that the position of *A. coralloides* and *C. verticillata* in the microbiome dendrogram was "reversed" in comparison with host phylogeny; and that the bacterial community compositions of *C. rubrum* and *A. coralloides* clustered despite their distant phylogenetic relationship (Fig. 2B). In the Red Sea, phylosymbiosis patterns could not be discerned between the corals and their bacterial communities (Fig. S3 and Suppl. file 1 Table S8; Robinson-Foulds distance of 1.08 ($P=0.99$); matching cluster distance of 0.85 ($P=0.11$); Mantel test r-value = 0.52 ($P=0.1$)).

Cophylogeny between coral hosts and bacterial phylotypes

The Random TaPas algorithm was used to assess whether the evolutionary histories of both the octocoral species and certain bacterial phylotypes were correlated. A total of 120 host-symbiont associations, comprising 101 OTUs out of the 244 OTUs analyzed, contributed significantly to cophylogenetic congruence with the coral holobionts (Fig. 4 and Suppl. file 1 Table S9). This was particularly true for associations with phylotypes belonging to the classes *Gammaproteobacteria*, *Alphaproteobacteria* and *Spirochaetia*. No signals of cophylogeny with bacterial phylotypes were observed in *K. utinomii*.

Associations with *Gammaproteobacteria* were the main contributors to cophylogenetic congruence (66% of all associations). This was particularly the case for *Endozoicomonas* phylotypes, which showed cophylogenetic links with 9 of the 13 octocoral species investigated. *Ovabunda* had the most associations with *Endozoicomonas* OTUs ($n=23$) contributing to cophylogeny, whereas most other Red Sea octocoral species (except *S. loyai* and *S. polydactylum*) had cophylogenetic associations with one to seven *Endozoicomonas* OTUs. Mediterranean octocorals had cophylogenetic associations with other *Endozoicomonas* OTUs (e.g. OTU1 and OTU3 in *E. cavolini*, OTU1 and OTU5 in *E. verrucosa* and OTU3 in *P. clavata*), which were also part of their respective core microbiomes (Fig. 4 and Suppl. file 1 Table S9). Other Gammaproteobacterial taxa also presented cophylogenetic associations with several octocorals. Notably, the gorgonian *C. verticillata* had 8 cophylogenetic links with phylotypes from the order *Coxiellales,* the family *Shewanellaceae,* the *Spongiibacteraceae* clade *BD1-7* and the genus *Pseudoalteromonas.* Furthermore, 4 of these phylotypes were also found to be part of *C. verticillata* core microbiome (2 *Pseudoalteromonas* OTU397 and OTU458, OTU24 annotated as *Spirochaeta 2* and the *Spongiibacteraceae* clade *BD1-7* OTU103). Other cophylogenetic associations were observed between (1) *Vibrio* OTUs and Red

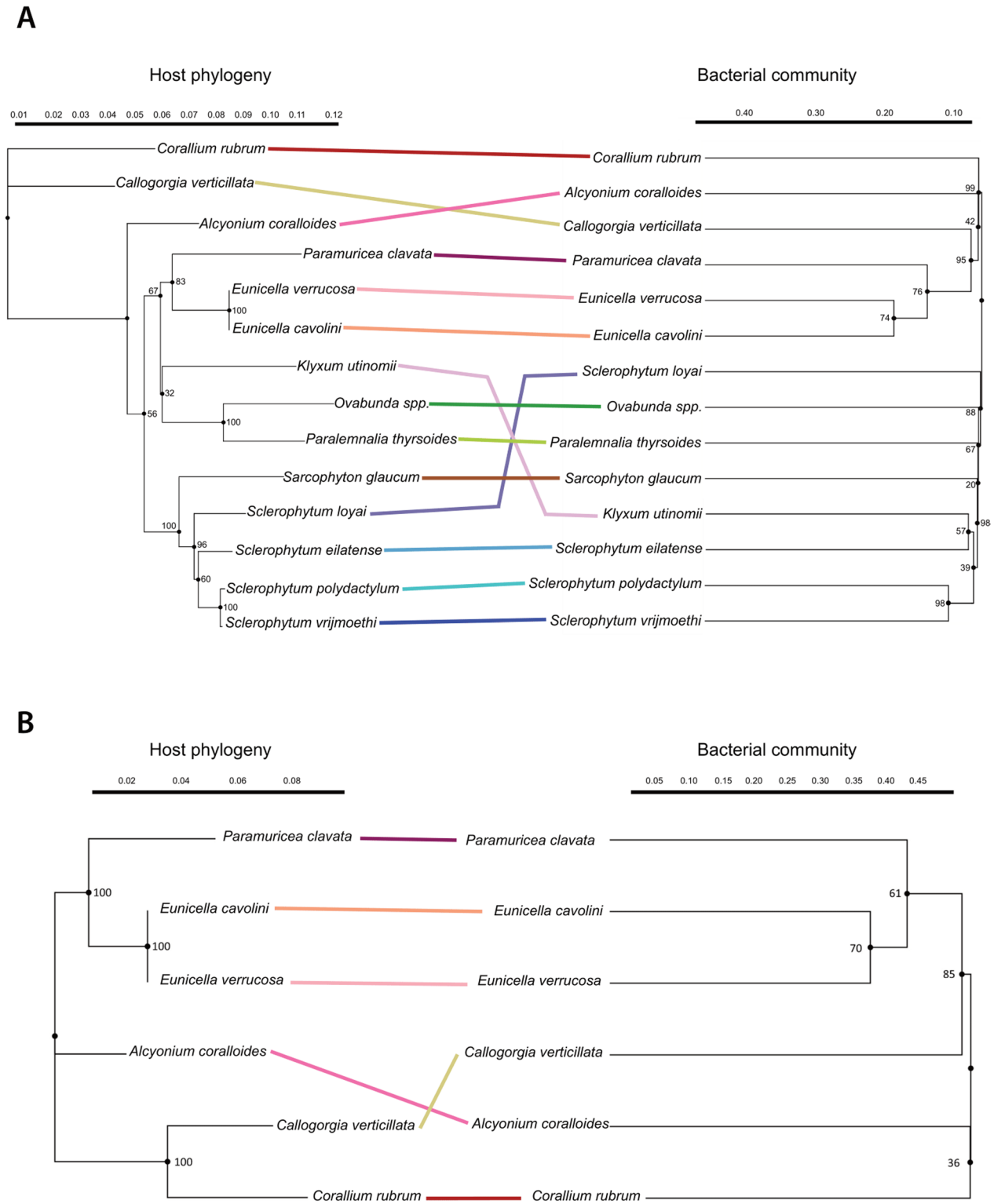

Fig. 3 Phylosymbiosis in octocorals. Juxtaposition of the coral host phylogenetic tree and the microbiota dendrogram for **A** all the coral samples and **B** a subset of the Mediterranean coral species only

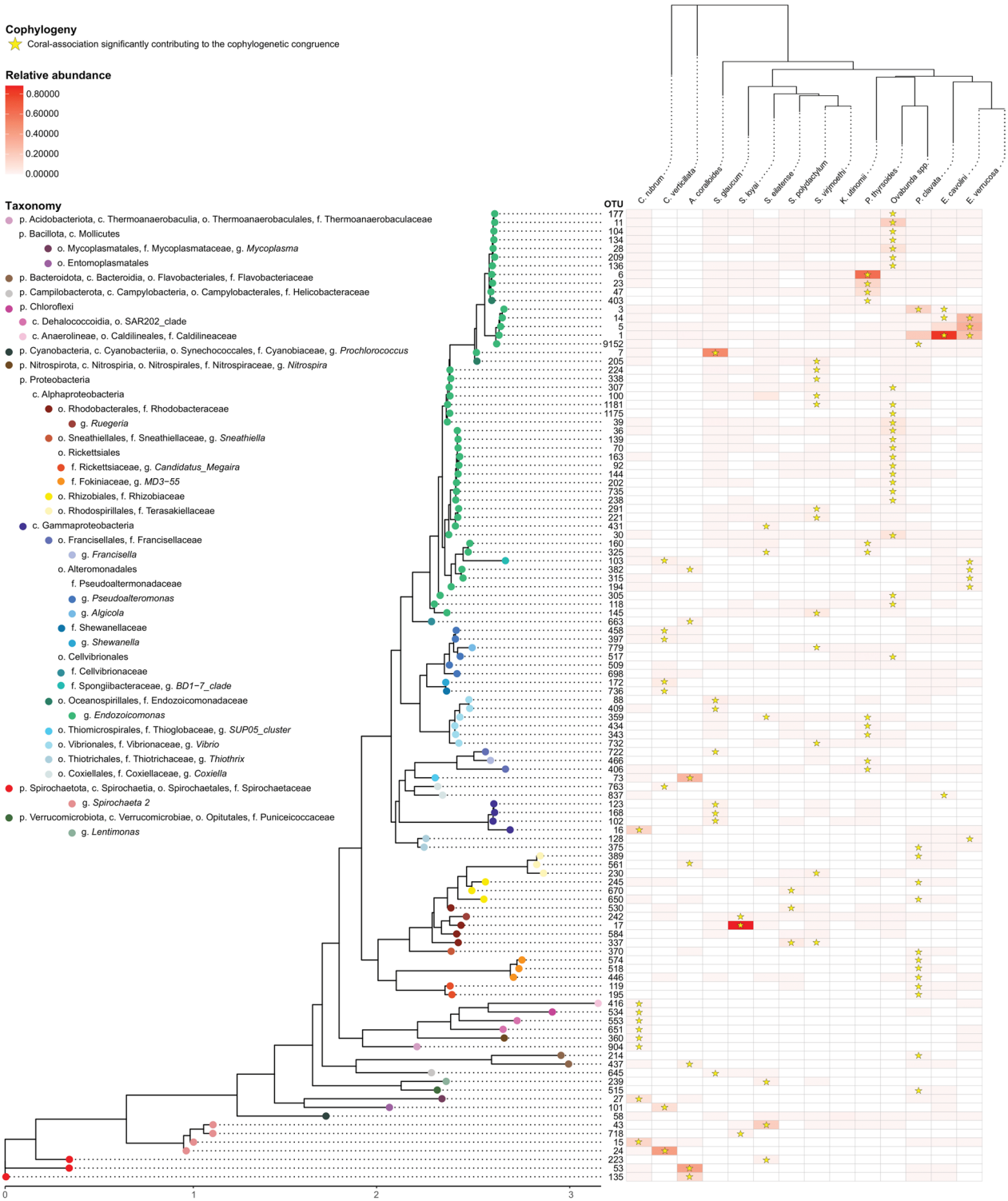

Fig. 4 Relative abundance of the OTUs involved in host-symbiont associations contributing significantly to cophylogenetic congruence (positive residual frequencies values) for all Mediterranean and Red Sea coral species (the stars correspond to the contributing associations between a host and an OTU) along with their respective phylogeny. Taxonomic annotation: p – phylum; o – order; f – family; g – genus

Sea coral species (*S. vrijmoethi, S. eilatense, S. glaucum* and *P. thyrsoides*), (2) the *Spongiibacteraceae* clade *BD1-7* (OTU103) and *E. verrucosa*, and (3) the order *BD72BR169* (OTU16) and *C. rubrum* (Fig. 4 and Suppl. file 1 Table S9).

Associations with *Alphaproteobacteria* also contributed significantly to cophylogenetic congruence (15 OTUs, 13% of all associations) with octocorals. Cophylogenetic signals were found between *P. clavata* and 8 *Alphaproteobacteria* OTUs belonging to the families *Rhizobiaceae*, and *Terasakiellaceae*, the *Fokiniaceae* MD3-55, and the genera *Sneathiella* and *Candidatus Megaira*. Other octocoral species had only 1–3 cophylogenetic associations with *Alphaproteobacteria*, mainly between *Rhodobacteraceae* and *Sclerophytum* spp.

The class *Spirochaetia* accounted for 6% of the associations contributing to cophylogenetic congruence. *C. rubrum* showed cophylogeny with a *Spirochaetaceae* (OTU 15, Fig. 4 and Suppl. file 1 Table S9), which was an abundant member of its core microbiome. Other *Spirochaetaceae* phylotypes also showed signals of cophylogeny in the Mediterranean coral species *A. coralloides* (OTU 135 and OTU 53) and *C. verticillata* (OTU 24), and these OTUs were also found in the core microbiomes of these corals. In octocorals from the Red Sea, two associations with *Spirochaetaceae* were found to contribute to cophylogenetic congruence, one in *S. eilatense* (OTU 43) and one in *S. loyai* (OTU 718; Suppl. file 1 Table S9).

Octocorals also showed cophylogenetic signals with phylotypes belonging to various other bacterial classes (14 out of the 120 contributing associations (11,6%); Suppl. file 1 Table S9). Notably, *C. rubrum* had associations with phylotypes belonging to different other classes, such as *Mollicutes* (OTU27 from the genus *Mycoplasma*), *Nitrospiria* (OTU360) and *Thermoanaaerobaculia* (OTU904) and two OTU from the phylum *Chloroflexi* (OTU416 and OTU534). The other *Scleralcyonaceae*, *C. verticillata*, showed cophylogeny with a *Mollicutes* phylotype (OTU101; order *Entomoplasmatales*) as well. *Verrucomicrobiae* phylotypes contributed to cophylogenetic congruence in *P. clavata* (OTU515; family *Puniceicoccaceae*) and *S. eilatense* (OTU239; genus *Lentimonas*). Cophylogeny between a phylotype from the *Helicobacteraceae* family (OTU645) and *S. glaucum* as well as between *Cyanobacteria* from the *Prochlorococcus* genus (OTU58) and *S. eilatense* were observed.

Discussion

We investigated the diversity and composition of bacterial communities associated with octocorals from the mesophotic zone of the Mediterranean Sea and Red Sea and assessed whether we can detect evolutionary patterns in these host-microbe associations. Our study revealed that Mediterranean and Red Sea octocoral holobionts harbor specific bacterial communities, but signals of phylosymbiosis were found only in octocorals from the Mediterranean Sea. However, indications of cophylogeny were detected in 13 of the 14 octocoral species investigated, suggesting that octocorals share a common evolutionary history with a few specific bacterial symbionts, primarily belonging to the *Endozoicomonadaceae* and *Spirochaetaceae*.

Octocorals are associated with specific bacterial communities

The composition of the coral-associated bacterial communities differed significantly between corals from the Mediterranean Sea and those from the Red Sea. This difference may be related to the about 17 million-years separation of the Mediterranean Sea and the Red Sea before the opening of the Suez Canal in 1869 [91]. The different light, temperature, and salinity conditions in these two seas [51, 96] may have contributed to the divergence observed in the microbiota of Mediterranean and Red Sea octocorals. Similarly, differences have been observed in the microbiota of coral species from different reefs or oceans [45, 70, 85, 103, 105].

Mediterranean octocorals from the mesophotic zone had species-specific microbiota, but two distinct 'clusters' could be observed – corals with a microbiota dominated by *Endozoicomonas* (*P. clavata* and *Eunicella* spp.) and corals with a *Spirochaetaceae*-dominated microbiota (*A. coralloides*, *C. rubrum*, *C. verticillata*). These patterns are consistent with reports on shallow populations [6, 37, 81, 100, 102–104], suggesting that depth has little influence on the microbiota of these octocoral species. The latter 'cluster' was rather surprising as it was comprised of corals that belong to two different orders—the *Malacalcyonaceae* and *Scleralcyonaceae*, respectively. Each coral species did, however, harbor its own microbial phylotypes and taxa, explaining the overall species-specific hierarchical clustering observed here, but some species also shared phylotypes. For example, the microbiota of *Eunicella* spp. and *P. clavata* had high abundances of *Endozoicomonas* OTU1 and OTU3, and the core microbiome of *A. coralloides* contained both phylotypes of *C. rubrum* (e.g., *Spirochaetaceae* OTU4) as well as gorgonians (e.g., *Endozoicomonas* OTU1, OTU2, OTU5). This overlap in the core microbiota is rather striking as *A. coralloides* is called the 'false red coral' in common language because of the similarities in appearance with *C. rubrum*, while it is in fact a 'parasite' or 'epibiont' that overgrows gorgonians, like *P. clavata* and *Eunicella* spp. [26, 55. 79]. This suggests that evolutionary patterns in host-microbiota associations may be masked by differences in species-specific life strategies and/or that associations with

specific symbionts may have arisen multiple times during host evolution.

Other taxa found at relatively high abundances in the microbiota of Mediterranean octocorals here and in previous studies belonged to the genus *Rickettsia* in *P. clavata*, the *Spongiibacteraceae* BD1-7 clade in *C. rubrum* [78, 98], and the *Gammaproteobacteria* BD72BR169 in *E. cavolini* and *E. verrucosa* 100, 104]. However, bacteria from these taxa are also present in the microbiota of various other Mediterranean gorgonians [6, 97, 100, 104].

The microbiota of octocorals from the Red Sea were distinct from those from the Mediterranean, but *Endozoicomonas* and *Spirochaetaceae* were also frequent and abundant symbionts of several of these tropical soft coral species. Overall, they could be divided into three main groups based on their microbiota composition: (1) *Endozoicomonas-Spirochaetaceae*-dominated (*K. utinomi*, *S. glaucum*, *S. eilatensis*, *S. vrijmoethi*); (2) *Endozoicomonas*-dominated (*Ovabunda* spp., *P. thyrsoides*); and (3) others with relatively high abundances of *Rhodobacteraceae* (*S. polydactylum*, *S. loyai*). While matching relatively well with the hierarchical clustering analysis, these groups were not as clear-cut, as *Mollicutes* were abundant in *K. utinomi* (*Entoplasmatales*) and *Ovabunda* spp. (*Mycoplasmatales*), whereas *P. thyrsoides*, *S. eilatensis* and *S. vrijmoethi* contained a relatively high abundance of *Rhodospirillales* (family *Terasakiellaceae*) in their microbiota. The microbial taxa identified here as the main symbionts of octocorals from the Red Sea are consistent with reports on octocorals from other tropical locations. For example, *Endozoicomonas* is a highly abundant symbiont of *Sclerophytum* and *Sarcophyton* species from the Indo-Pacific [18, 29, 66, 71], but also, in many other octocorals from the Indo-Pacific [109] and Caribbean [54, 62, 74, 83, 93]. Spirochaetes have also been found relatively abundant in the microbiota of Indo-Pacific *Sarcophyton* spp. [29, 66], *Lobophytum* [109] and in some *Sclerophytum* species [66, 71]. But the holobionts of *Sclerophytum* species do not always harbor Spirochaetes in this region [29]. However, *Sclerophytum* spp. from both the Red Sea as the Indo-Pacific do associate with *Rhodospirillales* [29] and *Rhodobacteraceae* [3, 14, 48].

Mollicutes, such as *Entoplasmatales* and *Mycoplasmatales*, were also highly abundant in the microbiota of *C. verticillata* and *K. utinomii*, and of *Ovabunda* spp. and *C. rubrum*, respectively. Members of these orders have previously been observed in association with deep-sea corals and gorgonians (*Entoplasmatales*: [13, 24, 108, 100, 104]), and with other octocorals (*Mycoplasmatales*: [24, 31, 77, 100, 103, 104]).

Overall, as for the hexacorals, octocorals are consistently found to have dominant associations with bacteria belonging to a few main taxa, particularly *Endozoicomonas* and *Spirochaetaceae*, but also harbor numerous other rare taxa. Of particular interest, are those phylotypes that belong to a coral species' core microbiome and those that are unique to a coral species. Such consistent associations of various microbial taxa with octocorals underscore their importance as symbionts and suggest that there may be an evolutionary link between these holobiont partners.

Unclear signals of phylosymbiosis in octocoral holobionts

Phylosymbiosis refers to a pattern in which the evolutionary relationships between hosts are reflected in the composition of their microbiomes, with closely related hosts harboring more similar microbial communities [43]. This pattern suggests that host evolution has played a role in shaping the structure of their microbiomes, possibly through processes like co-diversification. However, phylosymbiosis does not necessarily imply coevolution between hosts and microbes, as various ecological and environmental factors can also influence microbial community composition. Understanding phylosymbiosis provides important context for interpreting host-microbiome associations in an evolutionary framework.

A phylosymbiotic signal has been observed in scleractinian corals [76] and octocorals from Australia [66]. However, we did not observe phylosymbiotic signals between octocorals and their microbiota, when we considered all octocoral species studied here. This may be explained by a clear separation in bacterial community composition according to the two geographic locations, the Mediterranean Sea and the Red Sea. These seas have been isolated for at least 17 million years [91], suggesting that the geographical separation of these coral populations (i.e., vicariance) influenced patterns in the structure of the microbiota more than host phylogeny.

However, when the coral populations from the two seas were assessed separately, signals of phylosymbiosis were observed in the temperate Mediterranean octocorals but not in corals from the tropical Red Sea. The Mediterranean octocorals *C. rubrum*, *P. clavata* and several *Eunicella* spp., have been extensively studied and possess a microbiota that is specific as well as temporally and spatially stable [100, 102–104]. It was therefore previously hypothesized that the microbiota may have evolved closely with the host via co-diversification and/or via vertical transmission (maternal inheritance) of bacterial communities between coral generations in these larval-brooding species [100, 104]. Here, we confirm that phylosymbiosis exists in Mediterranean coral holobionts, but the mechanisms explaining this pattern remain to be investigated.

On the contrary, the tropical octocorals from the Red Sea and their microbiota did not demonstrate phylosymbiotic relationships. This contrasts with the findings in octocorals from the Great Barrier Reef [66] and to the patterns observed in the temperate Mediterranean in this study. Particularly the positioning of *K. utinomi* and *S. loyai* in the bacterial community dendrogram did not match with the host phylogeny. O'Brien et al. [66] also observed three such 'mismatches' but phylosymbiosis signals were still detected. However, their dataset contained a greater diversity of octocoral species representing a wider range of octocoral taxonomy, which may have contributed to better detection of phylosymbiotic patterns [43]. The relatively thick layer of mucus, which contains numerous environmental microbes, surrounding the tissues of these tropical octocorals is another factor that might have masked the phylosymbiosis signal in the Red Sea octocorals. In scleractinian corals, phylosymbiosis was in fact only found in bacterial communities associated with host animal tissues and skeleton, but not in the mucus [76]. To obtain a better understanding of the relationships between octocorals and their microbiome, it may be important to target different anatomical regions of the coral hosts to determine if this would result in different phylosymbiosis patterns. Besides, future studies into evolutionary links between tropical octocorals and their microbiota would likely benefit from expanding the dataset to include additional octocoral species representing higher diversity in octocoral taxonomy.

The observation that phylosymbiosis was only detected in octocoral holobionts from the Mediterranean Sea but not the Red Sea, and that these seas are geographically separated, suggest that vicariance may be a driver of the divergent evolutionary patterns between host phylogeny and microbiota composition. It further underscores the role of environmental conditions and evolutionary histories in shaping host-microbe interactions. In particular, the more extreme conditions in the Red Sea, such as higher temperatures and higher salinity could have significantly influenced how symbiosis between corals and their microbial associates established and evolved, as well as the nature of these interactions. As such, it may have affected the evolution of microbial communities in a way that differs from those in the Mediterranean. Comprehensive meta-analyses using standardized methods are necessary to further clarify these dynamics.

Cophylogenetic interactions are restricted to only a few host-symbiont associations.

While phylosymbiosis sheds light on how species that are closely related tend to have more similar microbiota, fine-scale tests of host-microbe cophylogeny help to identify specific microbial lineages, that may have a matching evolutionary history with their hosts. This could indicate shared events, such as cospeciation and codiversification, or other ecological or evolutionary relationships. Although studies of cophylogeny do not identify the processes by which host-symbiont associations arose, studying these symbionts is particularly valuable as they could play key roles in shaping microbiota dynamics.

Of the 244 most abundant OTUs (with a relative abundance of > 0.1%) that were examined, 101 OTUs were found to have significant associations contributing to the cophylogenetic signal with one or more of the 14 octocoral species investigated (0–24 phylotypes per coral species). This finding supports the idea that a limited group of bacterial phylotypes might play a significant role in host fitness. Similar findings were observed in two previous studies on octocorals, scleractinian corals and marine sponges, where only a small number of bacterial genera, such as *Endozoicomonas*, displayed cophylogenetic patterns and contributed to a phylosymbiotic signal [65, 76]. The cophylogenetic signals observed for the host-bacteria associations listed below suggest that codiversification (i.e., parallel evolutionary changes and speciation events in two or more host and symbiont lineages, leading to a shared evolutionary history) is likely significant between corals and certain bacterial lineages. It can result from different mechanisms such as coevolution but also vicariance (specificity of certain bacterial strains according to geographical and ecological factors) and vertical transmission.

In this study, nearly half of the 101 OTUs that showed a cophylogenetic signal with octocorals belonged to the genus *Endozoicomonas*, and such associations were present in 9 of the 14 octocoral species studied here. Interestingly, a cophylogenetic signal was observed between the abundant *Endozoicomonas* OTU3 and both *P. clavata* and *E. cavolini*, and between OTU1 and both *E. cavolini* and *E. verrucosa*. And in octocorals from the Red Sea, cophylogenetic associations with *Endozoicomonas* were particularly found in *Ovabunda* spp. (24), *S. vrijmoethi* (6) and *P. thyrsoides* (7). This shows the importance of the *Endozoicomonas*-coral associations in the evolutionary history of octocorals from both seas and highlights the possibly important contributions of these symbionts to their host's fitness, such as the digestion of complex molecules [2, 90], the provision of amino acids [63], vitamins [50], and involvement in sulfur cycling [95]. However, others question the nature of the symbiosis between corals and *Endozoicomonas* [75].

Spirochaetaceae were another main microbial taxon identified as contributing to the cophylogenetic congruence in our study. This was the case in three temperate octocorals (*C. rubrum*, *C. verticillata* and *A. coralloides*) and in two tropical octocorals (*S. loyai* and *S. eilatense*).

The microbial associations showing a fit with cophylogeny were different for each coral species, and primarily involved the genus *Spirochaeta* as well as unclassified *Spirochaetaceae*. While some coral species may share a concordant evolutionary history with their most abundant *Spirochaetaceae* symbionts, others may have an evolutionary relationship with lower abundant or rare phylotypes, as previously observed in other coral species and sponges from the Great Barrier Reef [65]. For example, the most abundant *Spirochaetaceae* in *C. rubrum* did not contribute to cophylogenetic congruence, whereas a member of the genus *Spirochaeta* did. This cophylogenetic link is interesting as the *Spirochaeta* of *C. rubrum* has been hypothesized to be involved in the characteristic color of this red coral [101]. Surprisingly, however, most of the tropical octocorals assigned to the '*Endozoicomonas-Spirochaetaceae*'-dominated microbiota group did not show cophylogenetic relationships with their *Spirochaetes* symbionts. The role of *Spirochaetaceae* within the coral holobiont remains to be investigated, but other symbiotic Spirochaetes are known to fix nitrogen [42], metabolize carbon sources [44], and produce vitamin B6 and the antimicrobial compound pyrroloiminoquinone [106].

Rhodobacteraceae also showed cophylogeny with octocorals in which they were a main symbionts (*S. loyai*, *S. polydactylum*) as well as with *S. vrijmoethi*. O'Brien et al. [65] also found cophylogenetic relationships between this taxon and corals from the Indo-Pacific. The function of these symbionts is yet unknown, but members of this taxon have been suspected to be opportunistic pathogens as they are often found in stress-impacted corals [3, 15, 78, 86, 87]. This also shows that it is difficult to interpret the results on evolutionary links between corals and microbes, as these analyses cannot distinguish between the nature of these host-microbe relationships, and thus the importance of these microbes in host health.

Besides members of the three taxa that dominated the bacterial communities of the octocorals studied here, phylotypes belonging to other taxa were also found to contribute to cophylogenetic congruence. For example, cophylogeny was observed between *A. coralloides* and *Thioglobaceae* SUP05 cluster OTU73. This microbe is part of *A. coralloides*' core microbiome, and may be of importance for the health of this coral because of its amino acid and B vitamin biosynthesis capacities, as well as for its antiviral defense system and chemoautotrophic metabolism has been demonstrated [33]. Cophylogenetic signals were also detected between *Mollicutes* and two corals from the Mediterranean Sea, particularly OTU101(*Entomoplasmatales*) and *C. verticillata*, and OTU27 (*Mycoplasma*) and *C. rubrum*. *Mycoplasma* has been proposed to feed commensally on remnants of prey captured by scleractinian cold-water corals [34, 64], and may explain how this type of association evolved. It has also been shown that *Mycoplasma* have co-diversified with salmonids [82]. Moreover, associations between *Spongiibacteraceae* OTUs from clade BD 1–7 and the corals *C. verticillata* and *E. verrucosa* were detected to contribute to cophylogeny, which may not be surprising as these bacteria are commonly found at relatively high abundances in various gorgonian coral species [80, 100, 103, 104]. Characteristic for *P. clavata* were its cophylogenetic associations with various *Alphaproteobacteria* (*Rhizobiaceae*, *Terasakiellaceae*, *Fokiniaceae*, *Sneathiella* and *Candidatus Megaira*), which are not very abundant in its microbiome. These taxa have been observed, although in low abundance, within the microbiome of Mediterranean gorgonians [78, 97, 98]. *Terasakiellaceae* were also detected across multiple coral species [72, 80, 108]. *Terasakiellaceae* as well as *Sneathiellaceae* may play a role in the cycling of nitrogenous compounds [12, 36] within the coral holobiont.

Interestingly, we also found cophylogenetic associations between the octocorals studied here from the Mediterranean Sea and Red Sea and several bacterial taxa that had previously been shown to have cophylogeny with octocorals from the tropical Indo-Pacific. Notably, O'Brien et al. [65] found evolutionary links between *Sclerophytum* and *Sarcophyton* coral species and *Cyanobacteria*, *Chloroflexi*, *Verrucomicrobiae* and *Thermoanaerobaculaceae*. Compared with scleractinian corals, few similarities have been observed. While Pollock et al. [76] found strong cophylogenetic signals between scleractinians and *Endozoicomonas*, they also revealed cophylogeny with *Clostridiaceae*, *Kiloniellales* and *Myxococcales*. Such relationships have, however, not been identified in octocorals so far.

The absence of cophylogenetic signals between certain high abundant phylotypes and their coral hosts raises intriguing questions about the dynamics of host-bacteria interactions. For example, neither OTU1 in *P. clavata* nor OTU4 in *C. rubrum* exhibited significant cophylogenetic associations with their respective hosts, despite their high abundance within the holobionts. This suggests a lack of specific evolutionary relationships between them. This finding challenges the conventional assumption that dominance within the microbiome implies a strong and persistent relationship with the host organism. Other factors could thus play important roles in shaping the nature and efficacy of these associations.

The observations by us and others highlight the potential importance of relatively few but taxonomically diverse microbial taxa in the evolutionary history of coral-bacteria associations. Corals throughout the world appear to engage in symbioses with several specific

microbial taxa, particularly *Endozoicomonas*. Altogether, these observations also suggest that it is not necessarily the most abundant symbiont or all members of the consistently associated core microbiome that share similar evolution patterns with their host.

Limitations in cophylogeny studies into microbe-coral associations

Our investigation of cophylogenetic patterns in host-bacteria relationships may present some limitations. First, as with most cophylogenetic studies, our assessment of bacterial phylogeny is based on variations within the variable regions of the *16S rRNA* gene [65, 76, 111]. Although this approach is currently the most practical, it involves the use of amplicons, which are relatively short marker sequences with limited phylogenetic information and the results may have to be interpreted with some caution. For example, no overlap in *Endozoicomonas* symbionts was found between *P. clavata* and *Eunicella* spp. previously [103], whereas these corals share a dominant *Endozoicomonas* phylotype (OTU1) in this study. The main difference between these studies is the use of a different primer set (targeting V5-V6 and V3-V4, respectively) to generate the *16S rRNA* gene amplicons. Using full-length *16S rRNA* gene sequencing approaches may provide better resolution and insights into evolutionary aspects in coral-microbe symbiosis.

Second, as emphasized by O'Brien et al. [65], cophylogenetic models do not provide insights into the nature of the symbiotic interaction between host and microbe (i.e., whether it is mutualistic, parasitic, or commensal), nor into the underlying mechanisms responsible for these observed patterns. It is thus essential to recognize that associations showing cophylogenetic congruence may not always be beneficial. For instance, some *Vibrionaceae* and *Rhodobacteraceae* significantly contributed to the cophylogenetic signal in various coral species from both seas. Although their role in these coral holobionts is unclear, members of these families have been implicated in coral disease and mortality [15, 22, 53, 87, 92], and their evolutionary relationship may thus also indicate a potentially harmful relationship. To understand the effect of symbiosis on host fitness, it is crucial to consider both parasitic and mutualistic symbiotic relationships as equally important factors.

Conclusion

While many bacteria associated with corals are host-specific, only a minority of bacterial phylotypes associated with corals display cophylogenetic patterns indicative of long-term host-microbe relationship, especially with *Endozoicomonas*. This result emphasizes the idea that, although host-microbe cophylogeny likely plays a role in phylosymbiosis, other factors, such as biogeographic influences, also play an important role in shaping this pattern. Furthermore, the different degrees of cophylogeny between coral microbes and their hosts underscore the fact that the microbiome is not a singular entity subject to uniform selection and that the abundance of the symbionts within the holobiont does not correlate with their common evolutionary history. Instead, it comprises a multitude of distinct participants with varying degrees of historical association with both the host and each other.

This study reveals important patterns between coral hosts and their microbiota, offering valuable insight into their potential evolutionary history. However, to better understand whether these patterns indicate coevolution or are driven by other factors, such as vicariance, further research is needed. Investigating whether these patterns remain consistent throughout the coral's life cycle and how environmental factors influence host-microbe interactions would add more clarity.

Abbreviations

A. coralloides	Alcyonium coralloides
C. verticillata	Callogorgia verticillata
C. rubrum	Corallium rubrum
E. cavolini	Eunicella cavolini
E. verrucosa	Eunicella verrucosa
K. utinomii	Klyxum utinomii
OTU	Operational Taxonomic Unit
P. thyrsoides	Paralemnalia thyrsoides
P. clavata	Paramuricea clavata
S. glaucum	Sarcophyton glaucum
S. eilatense	Sclerophytum eilatense
S. loyai	Sclerophytum loyai
S. polydactylum	Sclerophytum polydactylum
S. vrijmoethi	Sclerophytum vrijmoethi

Supplementary Information

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Additional file 1.

Additional file 2.

Additional file 3.

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Author contributions
JvdW, CFP and JD designed the study; AG, LB, JD, CR, JvdW, CFP collected and processed samples during the expeditions; RTP performed the DNA extractions and preliminary data analyses; CP and JvdW analyzed the data; CP and JvdW wrote the manuscript. All authors edited and approved the manuscript.

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Availability of data and materials
The raw sequencing data is available via the NCBI Sequence Read Archive under accession numbers PRJNA1045964, PRJNA1047055 and PRJNA1047303. Processed data (OTU table, metadata and OTU sequences) are in the Supplementary Information.

Declarations

Competing interests
The authors declare no competing interests.

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