RESEARCH ARTICLE

High diversity of microplankton surrounds deep-water coral reef in the Norwegian Sea

Sigmund Jensen[1], David G. Bourne[2], Martin Hovland[3,4] & J. Colin Murrell[5]

[1]Department of Biology, University of Bergen, Bergen, Norway; [2]Australian Institute of Marine Science, Townsville, Qld, Australia; [3]Centre for Geobiology, University of Bergen, Bergen, Norway; [4]Ambio ASA, Stavanger, Norway; and [5]School of Environmental Sciences, University of East Anglia, Norwich, UK

Correspondence: Sigmund Jensen, Department of Biology, University of Bergen, Norway. Tel.: +4755584400; fax: +4755584450; e-mail: sigmund.jensen@bio.uib.no

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Abstract

Coral reefs that exist in the depths of the oceans are surrounded by *Eukarya*, *Archaea* and bacterial communities that may play an important role in the nutrition and health of the reef. The first interdomain community structure of planktonic organisms in seawater from a deep-water coral reef is described. Community profiling and analysis of ribosomal RNA gene sequences from a coral reef system at 350 m depth in the Norwegian Sea revealed a rich diversity of *Eukarya* and *Bacteria* and a moderate diversity of *Archaea*. Most sequences affiliated with marine microplankton from deep-sea to cold-surface regions, with many sequences being similar to those described in studies of mesopelagic and oxygen minimum zones. Dominant phylotypes belonged to the *Alveolata* (group I, II, dinoflagellates), *Stramenopiles* (silicoflagellates), *Alphaproteobacteria* (*Pelagibacter ubique*), *Gammaproteobacteria* (ARCTIC96BD-19), *Bacteroidetes* (*Flavobacteria*) and mesophilic *Crenarchaeota* (*Nitrosopumilus maritimus*). Several rare and novel members of the community fell into distinct phylogenetic groups. The inferred function of dominant community members suggested autotrophs that utilise light, ammonium or sulphide, and lifestyles based on host associations. The high diversity reflected a microplankton community structure, which is significantly different from that of microplankton collected at the same depth at a pelagic station away from reefs.

Introduction

Deep-water coral reefs are unique environments with rich biological communities surrounded by cold permanent darkness (Hovland, 2008; Roberts *et al.*, 2009). These reefs have been found down to 3000 m depth but typically occur at continental margins and at depths that corresponds to the ocean mesopelagic zone (200–1000 m). At continental margins, biological production is generated to support over 90% of global fish catches (Pauly *et al.*, 2002). The continental margin of the Norwegian Sea harbours the largest deep-water coral reef known (the Røst reef) along with thousands of smaller reefs (Buhl-Mortensen *et al.*, 2010). Unlike tropical shallow-water reefs, reefs in the depths of the oceans have no direct benefit of sunlight. Deep-living corals host no phototrophic dinoflagellates (*Symbiodinium*), but instead host different cold- and dark-adapted microorganisms, which like the surrounding microplankton are energized by organic (heterotrophy) and inorganic (autotrophy) substances. Studies of deep-water coral–associated microorganisms have analysed 16S rRNA genes in total nucleic extracts from the major reef builder *Lophelia pertusa* (Yakimov *et al.*, 2006; Neulinger *et al.*, 2008; Kellogg *et al.*, 2009) and from octocorals (Penn *et al.*, 2006), *Madrepora oculata* (Hansson *et al.*, 2009) and gorgonians including *Paragorgia arborea* (Gray *et al.*, 2011). Corals have a diverse diet and consume organic matter as well as micro- and macroplankton (Carlier *et al.*, 2009). Corals can filter-feed and ingest planktonic bacteria and dissolved organic matter (Sorokin, 1973). Planktonic bacteria may also be trapped in coral mucus surfaces and ingested during absorption of the mucus (Neulinger *et al.*, 2008). Isotope signatures point to a nutritional link with phytodetritus and zooplankton, which reflect signatures of coral tissues (Griffin & Druffel, 1989; Duineveld *et al.*, 2004).

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Bacterioplankton in the seawater that surrounded deep-water coral reefs in the Gulf of Alaska (Penn *et al.*, 2006), in the Mediterranean (Yakimov *et al.*, 2006), in the Trondheimsfjord (Neulinger *et al.*, 2008), in the Norwegian Sea (Jensen *et al.*, 2008) and off Ireland (Hansson *et al.*, 2009) were dominated by lineages of *Alphaproteobacteria* and *Gammaproteobacteria*, coexisting with lineages such as the *Bacteroidetes*. It is unclear whether this bacterioplankton is different from bacterioplankton collected away from reefs, but clearly it differs from coral-associated bacteria. This is supported by comparisons of ribosomal RNA intergenic regions (Schöttner *et al.*, 2009) and is consistent with observations from similar studies of shallow-water coral reefs (Rohwer *et al.*, 2001; Bourne & Munn, 2005).

Environmental surveys have rarely analysed prokaryotes and microeukaryotes together (Brown *et al.*, 2009), and there are few reports on the identity of non-bacterial microorganisms from deep-water coral reefs. For example, microeukaryotes (protists) have yet to be studied and the diversity of *Archaea* in these environments is poorly understood (Yakimov *et al.*, 2006). *Archaea* can constitute 2% of coastal seawater plankton, as demonstrated for mesophilic members of the lineages *Crenarchaeota* (*Thaumarchaeota*) and *Euryarchaeota* (DeLong, 1992). *Thaumarchaeota* are widespread planktonic *Archaea* that have been analysed in seawater from the deep North Atlantic (Agogue *et al.*, 2008), from the Namibian upwelling (Woebken *et al.*, 2007) and from sediment below *L. pertusa* corals (Yakimov *et al.*, 2006). *Eukarya* can constitute 50% of coastal seawater plankton (DeLong, 1992) and resemble prokaryotes like the tiny green alga *Ostreococcus tauri* (Vaulot *et al.*, 2008). Microeukaryotes have received much attention since observations of their high diversity in seawater from the equatorial Pacific Ocean (Moon-van der Staay *et al.*, 2001) and the deep Antarctic Ocean (López-García *et al.*, 2001). Given that seawater contains myriads of microorganisms that exert vital control on marine life, we performed the first simultaneous description of the structure and composition of these seawater *Eukarya*, *Archaea* and *Bacteria* from a deep-water coral reef.

Materials and methods

Seawater sampling and storage

Samples of seawater were collected from depths of 300–370 m on the continental margin of the fishing ground Haltenbanken in the Norwegian Sea. The coral reef is located partly inside one of the many pockmarks of the hydrocarbon field Morvin (Hovland *et al.*, 2010) and is referred to as MRR08 (65°08′10″N, 06°28′02″E; 6°C; Fig. 1). Pockmarks are seafloor depressions suspected to have been formed as a consequence of gas seepage (Hovland, 2008). Dives to MRR08 were performed by the remotely operated vehicle 'ACV03'. Three seawater samples W1, W2 and W3 were collected in Niskin bottles *c.* 1 m above the seafloor and adjacent to live coral colonies in June 2008 (Fig. 1). Onboard, 0.25 L from each sample was immediately filtered through 0.2-μm Sterivex cartridges and frozen at −20 °C. Water samples were also frozen for subsequent nutrient analysis (in 50-mL sterile Nunc tubes). In April 2009, MRR08 was revisited for visual observations of the corals and for seawater methane and sulphide sampling. Onboard, 120-mL serum flasks were immediately filled to overflowing from the Niskin sampler and closed with red butyl rubber stoppers perforated with a needle to purge trapped air bubbles. Triplicate flasks were crimp-capped and the needles removed. For preservation, $ZnCl_2$ was added to 5 mM to precipitate sulphide and $HgCl_2$ was added to 20 μM to poison organisms. Flasks were stored in the dark at 8–10 °C before H_2S and CH_4 analyses. To look at plankton diversity away from coral reefs, seawater 80 km SSW of MRR08 and upstream the prevailing current (Gulf Stream) was collected from 300 to 350 m depth at a pelagic station (64°41′04″N, 05°15′51″E; 711 m bottom depth; 6 °C; Fig. 1) above the methane seep Nyegga, in August 2008 using a CTD 0356 equipped with Niskin flasks. Onboard, samples were pooled and 1 L immediately filtered through 0.2-μm Sterivex cartridges and then frozen at −20 °C.

Measurements of methane, sulphide and nutrients

Methane was analysed by flame-ionization gas chromatography using a MS 13× molecular sieve column, an oven temperature of 150 °C and a detector temperature of 80 °C with N_2 as the carrier gas (SRI 8610C; SRI Instruments). The method of Tavormina *et al.* (2008) was modified to fit the 120-mL flask volume by injecting 5 mL N_2 (99.999%) and shaking to equilibrium (for 15 min). Headspace samples (1 mL) were withdrawn from triplicate flasks of $HgCl_2$ preserved seawater and analysed in the gas chromatograph. Flasks prepared with accurate concentrations of methane served to convert readings into molar concentrations, taking into account the headspace volume, the seawater volume and the solubility of methane in seawater (Yamamoto *et al.*, 1976). Sulphide was analysed spectrophotometrically by the method of Fonselius *et al.* (1999). To triplicate flasks of $ZnCl_2$ preserved seawater, 0.5 mL of *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride (95.6 mM in 6 M HCl) and 0.5 mL Fe^{3+} (185 mM in 6 M HCl) was added

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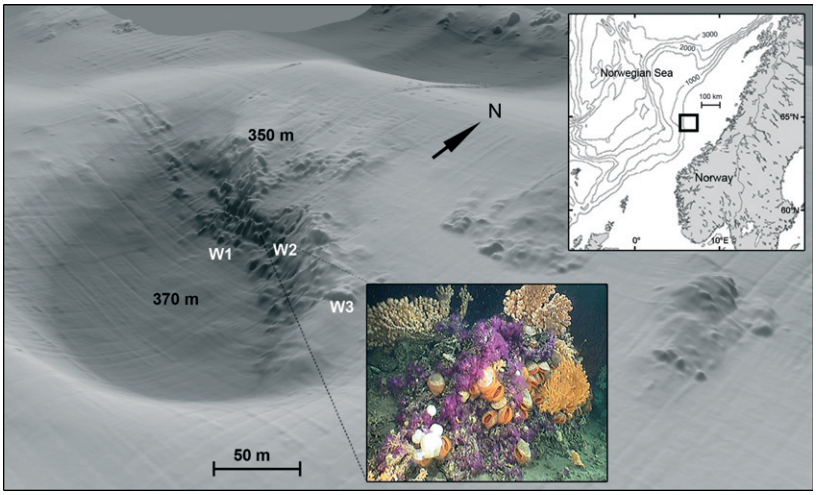

Fig. 1. Map of region sampled in the Norwegian Sea, including a photograph of the deep-water coral reef MRR08 and a 1.5× enhanced bathymetry of the seafloor indicating positions W1, W2 and W3 collected for seawater (65°08′10″N, 06°28′02″E). The photograph shows corals of the *Paragorgia arborea* (cream white), *Primnoa resedaeformis* (orange), *Anthelia borealis* (purple) the bivalve *Acesta excavata* and sponges. Seawater 80 km SSW of the coral reef was collected from the water column at 300–350 m depth (64°41′04″N, 05°15′51″E).

and following 2 h of colour development, absorbance measured at 670 nm (Varian Cary 100). Standards obtained from accurate concentrations of sulphide (titrated with thiosulphate) served to convert readings into molar concentrations. Additional nutrients were measured using a colorimetric autoanalyzer system (Skalar) at the Institute of Marine Research in Bergen.

DNA extraction and PCR amplification

Nucleic acids were extracted from the plankton by the method of Somerville *et al.* (1989) modified for a 1.5-mL Eppendorf tube. Briefly, 400 μL of SET lysis buffer (20% w/v sucrose, 40 mM EDTA pH 8.0, 50 mM Tris–HCl pH 9.0) and 45-μL fresh lysozyme (9 mg mL^{-1} in 10 mM Tris–HCl pH 8.0) was added to the thawed Sterivex cartridges, and the inlet and outlet were capped. Contents were mixed by inversion and cartridges incubated at 37 °C for 30 min with rotation in a Hybaid oven. Fifty microlitres of 10% SDS and 14 μL of fresh proteinase K (20 mg mL^{-1} in 50 mM Tris–HCl pH 8.0) were added. Incubation continued with rotation at 55 °C for 2 h. Crude lysate was collected, and 400 μL of phenol/chloroform/isoamyl alcohol (25 : 24 : 1; pH 8.0) was added and the tube gently shaken and then centrifuged at 6500 ***g*** for 10 min. Another 400 μL of phenol/chloroform/isoamyl alcohol was added and the shaking and centrifugation repeated. To the aqueous phase (*c.* 400 μL), 3 μL glycogen (Roche) and 800 μL PEG (30% w/v PEG 6000 in 1.6 M NaCl) was added. The tube contents were mixed gently and nucleic acids precipitated at room temperature overnight. Nucleic acids were pelleted by centrifugation at 14 000 ***g*** for 30 min, the pellet was washed in ice cold 70% (v/v) ethanol, resuspended in sterile water and stored at −20 °C.

Reaction mixtures (20–50 μL) contained 10–100 ng DNA, 0.5 μM of each primer, 250 μM of each dNTP, 20 μg mL^{-1} bovine serum albumin and 0.4 U Phusion DNA polymerase in 1× HF buffer (Finnzymes). PCR was performed using a PTC-200 thermal cycler (MJ Research) with denaturation at 95 °C for 5 min then 30 cycles of 94 °C for 1 min, primer-dependent annealing, primer-dependent extension at 72 °C and a final extension at 72 °C for 7 min. The following annealing and extension conditions were applied for primers targeting ribosomal RNA gene sequences to be cloned from *Bacteria*, *Archaea* and *Eukarya*, respectively: 27f/1492r (Lane, 1991) 55 °C for 1, 1 min; A751f/UA1406r (Baker *et al.*, 2003) 60 °C for 0.5, 0.5 min; EukF/EukR (DeLong, 1992) 60 °C for 0.5, 1 min. Shorter rRNA gene fragments were PCR-amplified for profiling in denaturing gradient gels (DGGE) using primers 338fGC/518r (Muyzer *et al.*, 1993), Parch519F/Arch915rGC (Vissers *et al.*, 2009) and euk1209fGC/uni1392r (Diez *et al.*, 2001). The following annealing and extension conditions were 55 °C for 1 and 1 min, using the Dynazyme II DNA polymerase in 1× of the supplied buffer (Finnzymes). All amplicons were checked for size and purity by agarose gel electrophoresis, followed by staining with ethidum bromide (Sambrook & Russell, 2001).

DGGE, cloning and sequencing

DGGE was performed as before (Jensen *et al.*, 2010) using the V20-HCDC system of Scie-Plas Limited

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(Southam) and photographed using a ChemiDoc XRS+ gel documentation system (Bio-Rad). Dominant DGGE bands were excised from the gel and eluted in 20 μL sterile water overnight. Eluates were PCR-amplified as described for DGGE above, and an aliquot of the amplicons was re-run on the DGGE.

PCR products for cloning were purified using a GenElute PCR cleanup kit (Sigma), and if accompanied with unspecific bands (EukF/EukR yielded a shorter band), bands of the expected sizes were cut from the agarose gel and purified using a Gen Elute Gel Extraction kit (Sigma). A-overhangs were added according to Finnzymes in 60-μL reaction mixtures of 1 U Dynazyme II DNA polymerase in the supplied buffer (Finnzymes) and 0.2 mM dATP, at 72 °C for 20 min. Following the protocol of Invitrogen, amplicons from the W1, W2 and W3 DNA (1 : 1 : 1 v/v) pooled from 3 to 4 independent reactions, were ligated into the pCR4 TOPO TA vector, transformed into competent *Escherichia coli* hosts and spread on Luria Bertani plates containing ampicillin (100 μg mg^{-1}) and a cover of X-gal (0.8 mg $plate^{-1}$).

White colonies were randomly picked as template for M13 amplification using the Dynzyme II DNA polymerase in 1x buffer (Finnzymes) and an annealing temperature of 60 °C for 1 min and extension at 72 °C for 1 min, at PCR conditions as described for cloning above. Amplicons from inserts of the expected size and from DGGE bands of the expected migration property were cleaned (USB Amersham) and sequenced using the BigDye 3.1 chemistry (Perkin Elmer) and an ABI 3700 PE sequencer (Applied Biosystems). All eukaryal and bacterial amplicons were read from the forward PCR primer and also from the reverse primer for the most repeatedly sampled sequences and dominant DGGE bands. Archaeal amplicons were read in full from the forward M13 primer.

Phylogenetic analyses

The rRNA gene sequences were analysed in ARB (Ludwig *et al.*, 2004) and in MrBayes (Ronquist & Huelsenbeck, 2003) with bootstrapping performed in Phylip (Felsenstein, 2004). Alignments were performed in SILVA (Pruesse *et al.*, 2007) and imported into ARB for manual refinements. Related sequences were found by searches in GenBank using BLASTN (Altschul *et al.*, 1990) and by adding the aligned sequences to a SILVA Guide Tree using the quick add maximum-parsimony tool in ARB. Topologies were evaluated by reconstructing phylogenies using evolutionary distance, maximum-parsimony, maximum-likelihood and Bayesan models. For Bayesan trees, a general time reversible evolutionary model (GTR) of four chains with gamma-distributed rate variation across sites and a proportion of invariable sites was used. The model was stopped as standard deviation of split frequencies approached 0.01. Burnin was set to discard samples from generations obtained before convergence was reached. The sequences were inspected for potential chimeras using Pintail (Schloss *et al.*, 2009). Sequences flagged suspicious were further inspected for bias in the alignment and for position in the phylogenetic tree. Sequences that passed quality checks were used to construct the phylogenetic trees presented, and in mothur, used to calculate the distances underlying the operational taxonomic units (OTUs), the diversity indexes and the community similarities (Schloss *et al.*, 2009). Additional sequences of acceptable quality from mismatching EukF/EukR (11 archaeal sequences) and A751f/UA1406r (80 bacterial sequences) amplifications were categorised into existing and new OTUs by using the quick add tool in ARB and by inspections of the subsequent alignments. DGGE band sequences were added to the trees using the quick add tool.

Nucleotide sequence accession numbers

Sequences analysed in this study are available in GenBank under the accession numbers JN832705–JN832922 (*Eukarya*), JN832923–JN833213 (*Bacteria*) and JN833214–JN833463 (*Archaea*). Short sequences are available from the authors (< 200 nt; 13 DGGE bands).

Results

Physicochemical and biological characteristics of the sampling sites

The living structure of the MRR08 reef extends *c.* 10 m high, 70 m long and 20 m wide. The reef fauna is dominated by filter-feeding invertebrates. Most of these are smaller than the megafauna that dominate photographic images of the reef (Fig. 1). *Sebastes mentella* (redfish) were observed swimming in-between the corals and Euphasid shrimps (krill) and myriads of copepods (crustaceans) down to the deepest location (W1). Several other coral reefs occur in this area and appear as rugged steep-sided textures on bathymetry images (Fig. 1; Hovland *et al.*, 2010). Marine snow drifted in the water column but no gas bubbles or other direct evidence of seepage was observed during the sampling. The methane concentration in top sediment near the centre of the pockmark was 200 nM (Hovland *et al.*, 2010). The MRR08 seawater yielded 8.2 nM CH_4, 75 nM NO_2^-, 300 nM H_2S, 725 nM PO_4^{3-}, 8500 nM SiO_4^- and 12 350 nM NO_3^-. At the pelagic station 80 km SSW of the reef, sampling depth and seawater temperature was similar (*c.* 350 m, 6 °C). The methane concentration was elevated two to threefold

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above the background (pers com Frida Lise Daae). The Nyegga area is located on the shelf break at *c.* 700 m depth and harbour complex pockmarks with documented methane seepage (Hovland, 2008).

Microplankton community profiles

The reef and mid-water microplankton community structure was profiled using DGGE analysis of rRNA genes, which demonstrated more bands indicative of higher diversity for profiles of *Eukarya* and *Bacteria* compared to profiles of the *Archaea* (Fig. 2). Identified eukaryal band sequences (Fig. 4a) belonged to *Alveolata* (Guillou *et al.*, 2008) from cold marine environments and most sequences (bands 3, 5, 6, 7) affiliated with the poorly understood but widespread marine group I *Alveolata* (López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001). One sequence affiliated with the characterised dinoflagellate *Gyrodinium* (band 4). Sequences near the top of the profiles affiliated with the parasitic *Alveolata* group II (band 1) and *Apicomplexa* (band 2). Identified archaeal band sequences (Fig. 4c) belonged to marine *Thaumarchaeota* and *Euryarchaeota* (DeLong, 1992) with relatives occuring in polar oceans (six bands) and in the deep sea (band 14), respectively. Bacterial sequences retrieved (11 bands) belonged to *Proteobacteria* (Fig. 4d) and the cyanobacterium *Synechococcus* (Fig. 4e) from cold marine waters and coral reefs. From DGGE analysis, the most prominent differences between the sites were distinct bands in the bacterial W1 profile (bands 19, 20, 24, 25; *Oceanospirillales*) and in the eukaryal W3 profile (2–3 bands; unidentifed) (Fig. 2).

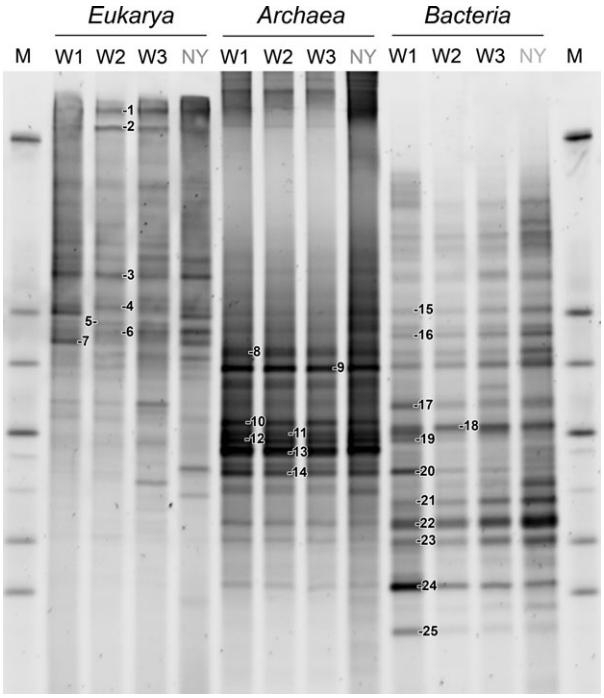

Fig. 2. Community profiles of planktonic *Eukarya*, *Archaea* and *Bacteria* from deep-water coral reef MRR08 (W1, W2, W3) and from a mid-water station at same depth away from reefs (NY). The organisms are represented by 18S rRNA and 16S rRNA gene sequences separated into bands by electrophoresis in a denaturing gradient gel (DGGE) ranging 30–65% denaturant. Numbers indicate bands identified by sequencing. M is a marker made from cloned rRNA gene sequences.

Community structure and composition

Analysis of a total of 747 rRNA gene sequences from free living and aggregate associated seawater microorganisms from the coral reef and the mid-water samples indicated a reef seawater diversity ranking *Archaea* < *Bacteria* < *Eukarya* (clone libraries). Sequences from the reef yielded higher diversity indices (Table 1), steeper rarefaction curves (Fig. 3) and more representative OTUs (37 of 52 boxed in Fig. 4) than recovered from a mid-water sample. The reef sequences dominated the lineages of *Alveolata* group I, *Dinophycea*, *Ellobiopsidae*, *Ciliophora*, *Cryptophyta*, *Stramenopiles*, *Euryarchaeota*, *Cyanobacteria* and *Actinobacteria* (Fig. 3). Libshuff analysis of community similarities using homologous and heterologous coverage curves (Schloss *et al.*, 2009) resulted in a low *P*-value

Table 1. Diversity indices and community similarities of plankton from deep-water coral reef MRR08 (MO) and from a mid-water station at the same depth away from the reef (NY)

Library	Sample	Sequences	OTUs	Shannon	Chao 1	Singletons	Coverage (%)
Eukarya	MO	115	57	3.8	101	33	71
	NY	98	40	2.8	82	29	70
Archaea	MO	121	20	1.9	40	9	93
	NY	122	15	1.5	14	7	94
Bacteria	MO	155	67	3.4	100	34	78
	NY	136	51	3.3	76	29	79

The data were calculated from rRNA gene alignments using a 3% distance cut-off.
Coverage is calculated as (1 − (singletons/clones)) × 100%.

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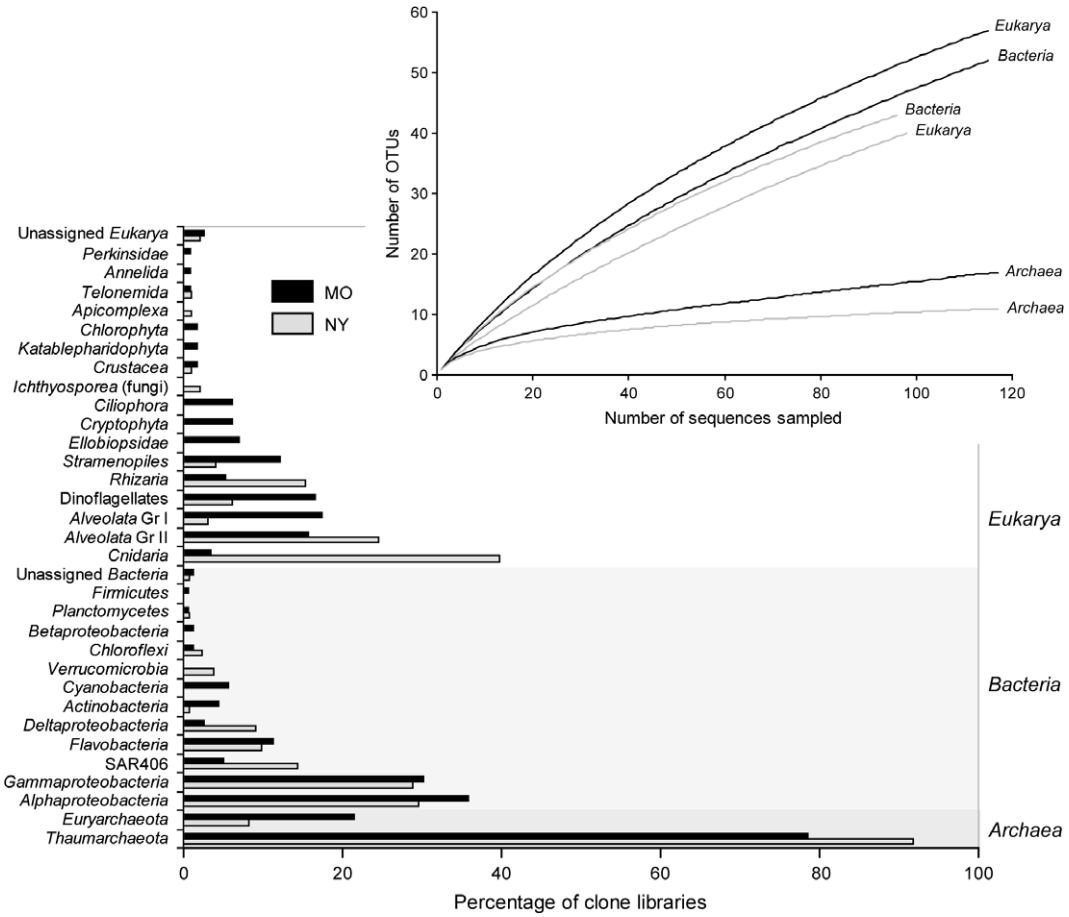

Fig. 3. Rarefaction curves (inset) and lineage identities of plankton from deep-water coral reef MRR08 (MO) and from a mid-water station at same depth away from reefs (NY), as represented by rRNA gene clones from the different libraries.

($P = 0.021$, $P < 0.025$ indicates a significant difference) for the archaeal community within the reef (MO) and mid-water samples (NY), suggesting that the reef library has many archaeal phylotypes not present in the mid-water library (e.g. OTUs 11, 14, 19). The reverse heterologous comparisons produced a high *P*-value ($P = 0.614$), suggesting that the mid-water library has many archaeal phylotypes in common with the reef library (e.g. the major OTU3). For the *Eukarya*, the corresponding *P*-values were 0.009 and < 0.001, suggesting that the reef library has phylotypes (e.g. OTUs 6, 15, 37) not present in the mid-water library. Coverage curves indicated a distinct reef seawater microeukaryote diversity, yielding a $\Delta C_{MO/NY} = 4.2$, which is nearly ten times the reciprocal $\Delta C_{NY/MO}$ comparison and ΔC comparisons from the *Bacteria* and *Archaea*. Libshuff *P*-values for the *Bacteria* were 0.001 for both, suggesting little

Fig. 4. Bayesan phylogenetic trees of plankton surrounding deep-water coral reef MRR08 (MO), plankton present at a mid-water station at the same depth away from reefs (NY) and organisms from the GenBank. The trees show *Alveolata* (a) and other *Eukarya* (b), *Archaea* (c), *Proteobacteria* (d) and other *Bacteria* (e). The organisms are represented by rRNA gene sequences. Sequences from this study are shown in bold and are from dominant DGGE bands from the reef and from the repeatedly sampled OTUs, as identified by site (MO, NY), a prefix (clone number), the number of sequences and the OTU. DGGE bands distinct for the reef and OTUs exclusively represented by sequences from the reef are boxed. Grey boxes indicate OTUs exclusively represented by sequences from the station away from reefs. Sequences of unboxed OTUs were represented by sequences from both sites. The trees were constructed from alignments filtered to > 1200 nucleotide positions (*Archaea* > 600 nt), excluding ambiguities, missing data and positions, where the frequency of a nucleotide occurring was < 50%. Posterior probabilities (numbers) and maximum-likelihood bootstraps (open circles 50–74%, filled circles 75–100%) are indicated at the branch points. Shorter sequences subsequently added into the trees are indicated by asterisks. Outgroups were selected from *Nitrosopumilus maritimus*, *Pelagibacter ubique* and *Gyrodinium rubrum*. Scale bars indicate 0.10 changes per nucleotide.

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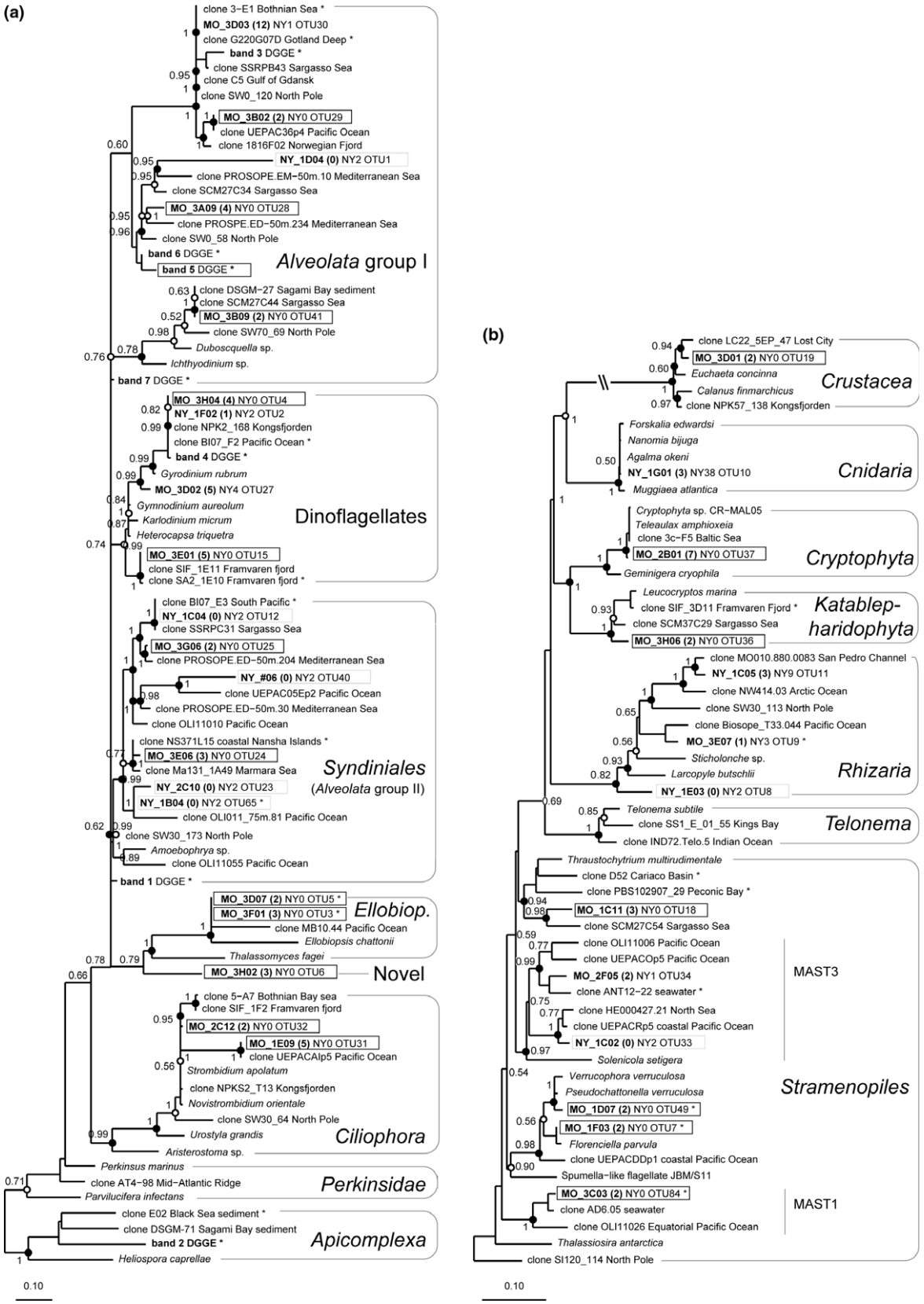

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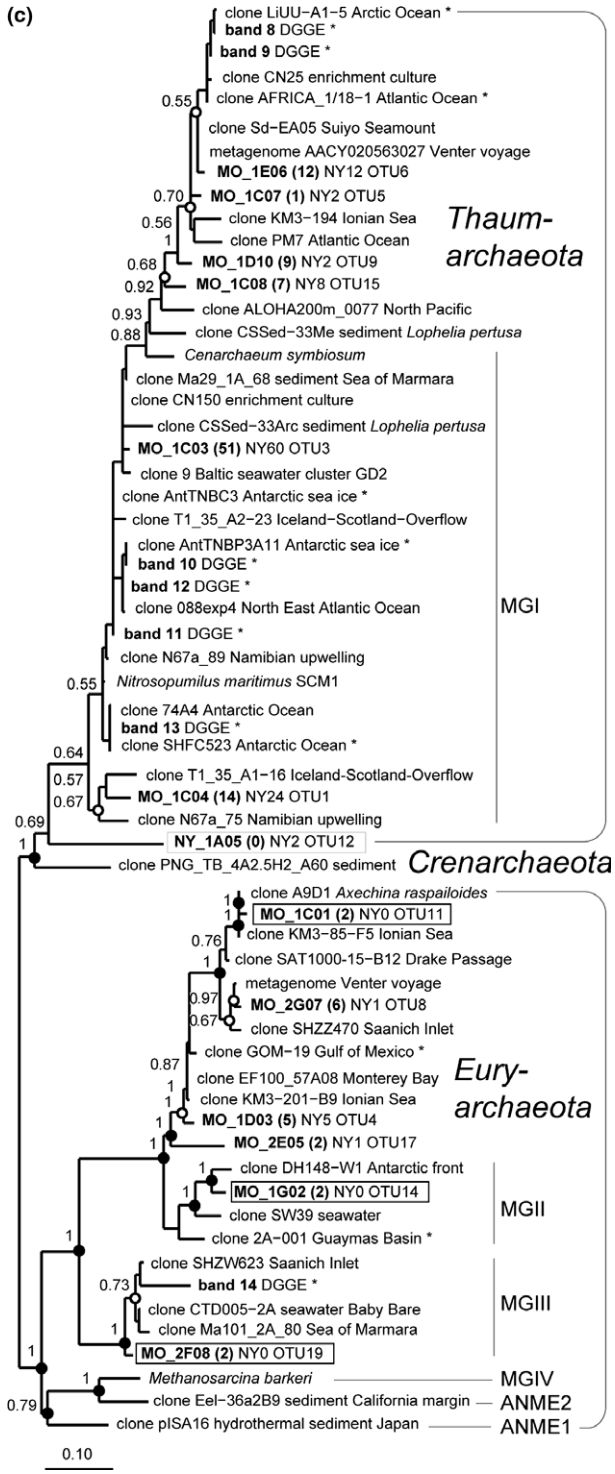

Fig. 4. (Continued)

overlap and that both bacterial libraries contained unique phylotypes (e.g. OTUs 1, 7, 17). The 3% sequence cut-off used to approximate species revealed a total of 207 OTUs (Table 1). Excluding singletons, 68 reef and 42 mid-water OTUs were repeatedly sampled.

Eukarya

The 213 18S rRNA gene sequences analysed fell into 89 OTUs (Table. 1). *Alveolata* was the most abundant lineage in the reef library (64%) and the second most abundant lineage in the mid-water library (35%) (Fig. 3). *Cnidaria* was the most abundant lineage in the mid-water library (40%) but was a minor lineage in the reef library (< 4%). These 43 sequences (including OTU10) are closely related to the small siphonophoran jellyfish *Nanomia bijuga* (Fig. 4b). Three additional metazoan sequences were detected and these affiliated with the copepod *Calanus finmarchicus* (including OTU19). Seven distinct clades of *Alveolata* were recognised (Fig. 3). The largest (Fig. 4a) contained 18 reef and 24 mid-water sequences in a total of 34 OTUs, which fell into the parasitic *Alveolata* group II (Guillou *et al.*, 2008). The second largest clade contained 20 reef and three mid-water sequences in a total of five OTUs of the group I *Alveolata* (López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001). The major OTU30 had 13 sequences closely related to sequences from surface waters of the Southern Baltic Sea (Piwosz & Pernthaler, 2010), the anoxic Gotland Deep (A. Stock, K. Juergens, J. A. Bunge, T. Stoeck, unpublised data) and from below the North Pole sea ice (Bachy *et al.*, 2011). The third largest clade contained 19 reef and six reference sequences in a total of eight OTUs of dinoflagellates such as the *Gyrodinium*, *Gymnodinium* and *Karlodinium* (Vaulot *et al.*, 2008). Three exclusively reef clones represented a novel alveolate with 82% sequence identity to its tentatively closest relative the phototrophic *Gymnodinium* (OTU6). Eight reef sequences affiliated with parasites of *Ellobiopsidae* (including OTUs 3 and 5) and seven reef sequences affiliated with ciliates resembling *Strombidium* (OTUs 31, 32). Non-alveolata sequences fell into ten lineages (Fig. 3) dominated by *Stramenopiles* including the recovery of 14 reef and four mid-water sequences (Fig. 4b). Four reef *Stramenopiles* affiliated with the silicoflagellates *Florenciella parvula* (OTU7) and *Pseudochattonella* (OTU49) and distant to three divergent reef sequences (OTU18) (Vaulot *et al.*, 2008). Coastal and open-ocean flagellates of the MAST-1 (OTU84) and MAST-3 (OTUs 33, 34) clades included uncultured *Stramenopiles* (Massana *et al.*, 2004). Seven exclusively reef sequences were related to sea-ice plankton and the alga *Cryptophyta* (OTU37). The heterotrophic flagellates *Katablepharidophyta* included two reef sequences (Fig. 4b). The *Rhizaria* included in total six reef and 15 mid-water sequences (seven OTUs) related to the *Sticholonchidae* (Fig. 4b).

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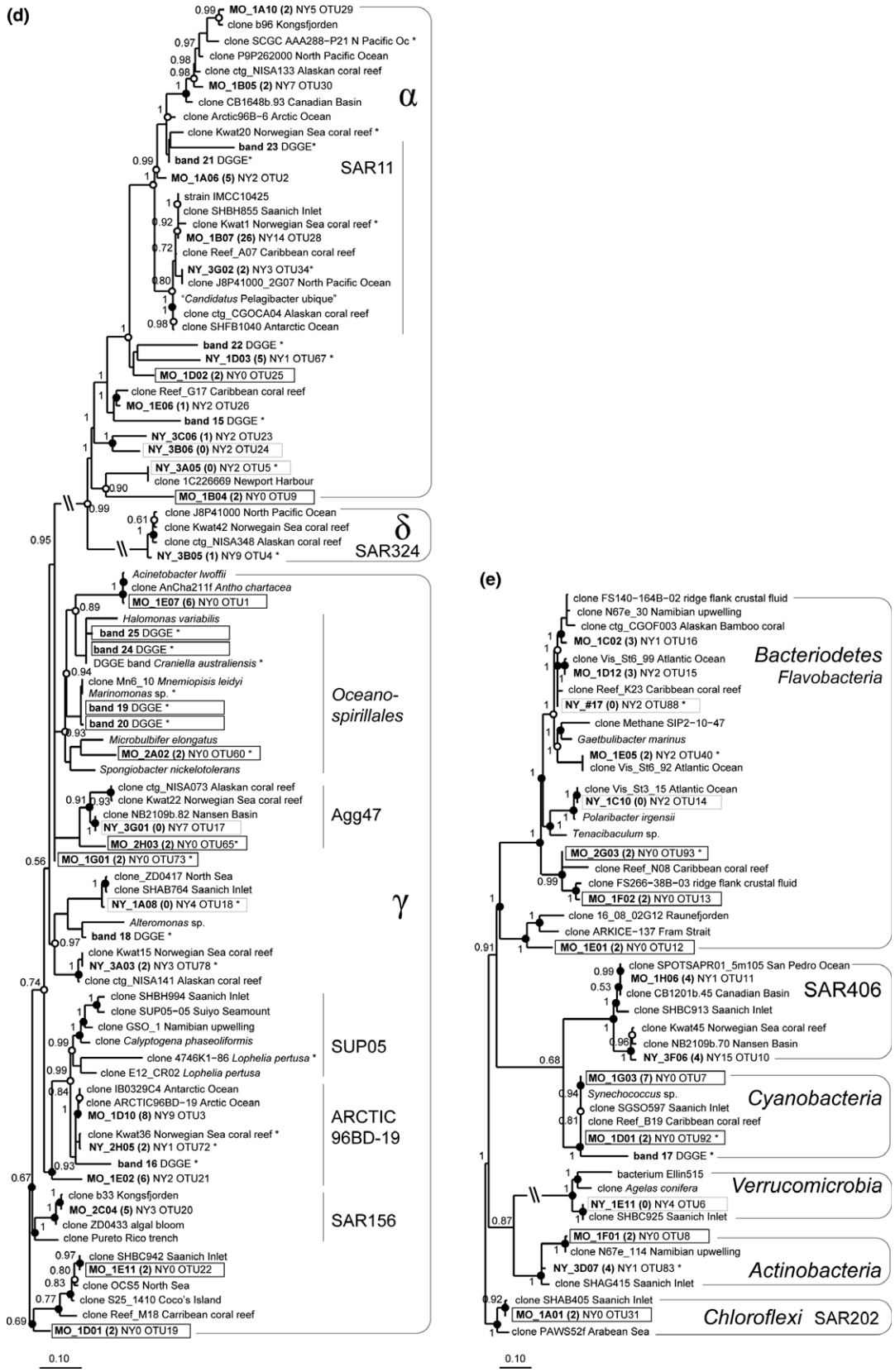

Fig. 4. (Continued)

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Archaea

A large portion of the 16S rRNA gene sequences from both clone libraries (207 of the 243 analysed) fell into the marine group I *Thaumarchaeota* (MGI; Fig. 4c). Sequences from the two largest OTUs 3 and 1 affiliate with the ammonium oxidiser *Nitrosopumilus maritimus* (Könneke *et al.*, 2005). The MGI includes sequences from the deep Iceland-Scotland-Overflow (Agogue *et al.*, 2008), the Namibian upwelling (Woebken *et al.*, 2007), the Arctic (Galand *et al.*, 2009) and Antarctic Oceans (Béjà *et al.*, 2002) and sediments from below *L. pertusa* corals (Yakimov *et al.*, 2006). A different group of *Thaumarchaeota* were sequences found in the japanese Suiyo Seamount hydrothermal vent (OTUs 5, 6). Two mid-water sequences grouped away from MGI (OTU12). Remaining archaeal sequences were more diverse and fell into the *Euryarchaeota* that included deep-sea MGII and MGIII (Galand *et al.*, 2009), harboured a higher proportion of sequences from the reef and affiliated with deep seawater plankton from the Saanich Inlet, the Ionian Sea and other less cold habitats (Fig. 4c). No thermophilic marine *Crenarchaeota*, anaerobic methane oxidisers (ANME) or methanogens were detected.

Bacteria

The 291 bacterial 16S rRNA gene sequences analysed fell into 93 OTUs (Table 1). *Proteobacteria* represented 67–70% of the sequences in the libraries (Fig. 3). The *Alphaproteobacteria* dominated with a total of 57 reef and 39 mid-water sequences (Fig. 4d). The major OTU28 grouped with several smaller OTUs into the SAR11 clade that includes *Pelagibacter ubique* (Rappé *et al.*, 2002). Some novel alphaproteobacterial sequences occurred. These OTUs 25, 67 and relatives were different to bacterioplankton sequences previously obtained from deep-water coral reefs (Penn *et al.*, 2006; Jensen *et al.*, 2008; Neulinger *et al.*, 2008). Nearly as many sequences from the libraries were *Gammaproteobacteria*, with a total of 48 reef and 38 mid-water sequences, including OTUs 3, 21 and 72 which affiliated with uncultivated ARCTIC96BD-19 from the Polar Ocean (Bano & Hollibaugh, 2002). Six exclusively reef sequences were > 99% identical to the *Acinetobacter lwoffii* (OTU1). Non-proteobacterial sequences fell into eight lineages (Fig. 3). Dominant of these was the *Bacteriodetes* (Fig. 4e) which were, as in the North Atlantic seawater study of Gómez-Pereira *et al.* (2010), all *Flavobacteriaceae*, including a total of 18 reef and 13 mid-water sequences (15 OTUs). Their closest cultivated relative was the gas-vacuolated sea-ice bacterium *Polaribacter irgensii*. The cyanobacterium *Synechococcus* was exclusively represented by nine sequences from the reef (OTUs 7, 92). Seven reef sequences and one mid-water sequence affiliated with the *Actinobacteria* (including OTUs 8, 83). Two reef sequences (OTU 31) and three mid-water singletons affiliated with the *Chloroflexi* SAR202 and 8 reef and 19 mid-water sequences affiliated with SAR406 (including OTUs 10, 11) (Gordon & Giovannoni, 1996). Four reef and 12 mid-water sequences affiliated with the *Deltaproteobacteria* including OTU4 from the SAR324 (Wright *et al.*, 1997). The *Verrucomicrobia* was only represented by sequences from the mid-water library (including OTU6). *Epsilonproteobacteria* and species of *Vibrio* were undetected.

Rarer microplankton

Sequences of OTUs sampled once accounted for 56 *Eukarya*, 50 *Bacteria* and 11 *Archaea* (Table 1). Most singletons fell into lineages represented by OTUs of more than one sequence. Lineages exclusively represented by singletons were from the reef libraries: the *Chlorophyta* (2), *Telonemida* (1), *Perkinsidae* (1), *Annelida* (1), *Betaproteobacteria* (2), *Planctomycetes* (1) and *Firmicutes* (1), and from the mid-water libraries: the *Ichthyosporea* (2), *Telonemida* (1), *Apicomplexa* (1) and *Planctomycetes* (1).

Discussion

Patterns of microplankton diversity

This study performed the first interdomain analysis of planktonic organisms from a deep-water coral reef by documenting the diversity of *Eukarya* and *Archaea* along with the diversity of *Bacteria*. Ribosomal RNA gene sequences from community profiles (DGGE) and clone libraries (OTUs) correlated and reflected a diversity spanning jellyfish and important fish prey copepods to widespread and novel marine microorganisms. Many of the bacterial sequences had previously been retrieved from deep-water coral reef seawater (Penn *et al.*, 2006; Jensen *et al.*, 2008; Neulinger *et al.*, 2008) and included members from the lower ocean surface layer SAR202, SAR324 and SAR406 enriched below the deep chloropyll maximum (Gordon & Giovannoni, 1996; Wright *et al.*, 1997). Other bacterial and also eukaryal and archaeal sequences were similar to those from studies of oxygen minimum zones (Behnke *et al.*, 2006; Woebken *et al.*, 2007; Agogue *et al.*, 2008; Brown *et al.*, 2009; Lavik *et al.*, 2009; Walsh *et al.*, 2009). Seafloor oxygen minimum zones typically occur at depths between 200 and 1000 m where mid-water oxygen minimum zones ($O_2 < 0.5$ mL L^{-1}) intersect the continental margin (Reeburgh, 2007). The oxygen concentration of 6.2 mL L^{-1} near deep-water corals in the Gulf of Mexico (Kellogg *et al.*, 2009) indicates

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bulk aerobic conditions. Microplankton may, however, form abundant compacted marine snow aggregates at the bottom of the euphotic zone. *Flavobacteria* have, for example, been associated with phytoplankton (Gómez-Pereira *et al.*, 2010), Agg47 bacteria with aggregates (DeLong *et al.*, 1993) and *Thaumarchaeota* with the mesopelagic oxygen minimum (Agogue *et al.*, 2008), while *Alveolata* group I dominated samples from anoxic and suboxic ecosystems (Guillou *et al.*, 2008). There were many similarites in the community structure of the sampled seawater organisms, yet diversity at the bottom of a deep-water column near a coral reef was significantly different from that at a similar mid-water depth away from reef complex.

Physicochemical drivers of the microplankton diversity

Samples analysed in this study were from the same water mass, depth, temperature and darkness, though distinct and important differences exist. Whereas reef seawater was collected 1 m above the seafloor, the mid-water seawater was collected *c.* 400 m above the seafloor. NO_2^-, PO_4^{3-}, SiO_4^- and NO_3^- of the nearest 200–400 m station investigated was from off the Nyegga shelf break (64°8′ 42″N, 1°16′19″E; 2465 m bottom depth) and yielded 25, 905, 6.420 and 12.945 nM, respectively, concetrations which are comparable to nutrients of the MRR08 seawater. Complex environmental factors, however, characterise the benthic environment, especially in the presence of coral reefs and pockmarks. Plankton experience more turbulence near the seafloor, resulting in higher exposure to inorganic and organic constituents. Such factors drive structure–function relationships and likely contribute to niche partitioning, leading to high diversity.

Sediment samples and other indicators suggest cyclic methane, ethane and propane seeps in sediments at MRR08, most probably as dissolved gases in porewater (Hovland *et al.*, 2010). The *A. lwoffii* sequence recovered in this study has been implicated with the degradation of crude oil (Amund & Higgins, 1985) and enriched near the bottom of the pockmark hydrocarbon utilising *Oceanospirillales* from the related genera *Marinomonas* and *Halomonas* were detected. Few sequences from the mid-water station affiliated with hydrocarbon utilisers. Above Nyegga methane peaked at 250 m depth to about fourfold the background concentration (pers com Frida Lise Daae, Reeburgh, 2007), indicating seepage persisted up into the mesopelagic zone. This would suggest a methane concentration in the same order of magnitude in seawater of reef and mid-water sites. Ten nM is in the lower range of that found associated with pelagic methanotrophs following specific amplification of the methane monooxygenase encoding gene *pmoA*, whereas in this study methanotrophs were not detected by 16S rRNA gene amplification (Tavormina *et al.*, 2008). The detection of pelagic methanotrophs appear to require a sensitive assay even from seawater with methane in the μM range (Tavormina *et al.*, 2008). Few sequences affiliated with extant one-carbon compound (C1) cycling organisms, except for a mid-water singleton, *Methylophaga*.

Sulphide is chemically oxidised to sulphate in aerobic seawater (Fonselius *et al.*, 1999) and low sulphide was expected in the mid-water sample and measured in the reef sample, comparable to the < 1000 nM seawater sulphide of *Bathymodiolus* beds on the Gabon margin (Duperron *et al.*, 2005). Distinct members of the *Gammaproteobacteria* from reef and mid-water samples resembled sulphur-oxidising chemoautotrophs in bivalve symbionts (Dubilier *et al.*, 2008) and in *L. pertusa* from the Trondheimsfjord (Neulinger *et al.*, 2008) and the Gulf of Mexico (Kellogg *et al.*, 2009). Sequences from three OTUs and a DGGE band affiliated with 16S rRNA gene sequences of these thiotrophs and most fell into the ARCTIC96BD-19 from the central Arctic Ocean (Bano & Hollibaugh, 2002). This clade is closely related to the SUP05 clade from the Suiyo Seamount hydrothermal plume (Sunamura *et al.*, 2004). ARCTIC96BD-19 and SUP05 sequences occur in oxygen minimum zones in the Saanich Inlet (Walsh *et al.*, 2009), in the Namibian upwelling (Lavik *et al.*, 2009) and in another Norwegian Sea coral reef (Jensen *et al.*, 2008). Such sequences may represent thiotrophs important for fuelling of carbon into mesopelagic plankton networks. Interestingly, some *Oceanospirillales* and members from the ARCTIC96BD-19 and Agg47 have recently been suggested to be involved with autotrophy in the dark, along with the SAR324 which contain genes potentially supporting the oxidation of methane and one-carbon cycling (Swan *et al.*, 2011).

No bacterial sequences were affiliated with ammonia oxidisers except for a reef singleton *Nitrosospira,* suggesting that *Archaea* may be the important reef seawater and mid-water ammonia oxidisers, despite only relatively low diversity of archaeal sequences being retrieved in this study. *Thaumarchaeota* are abundant and ubiquitous in the pelagic deep sea, including in the deep North Atlantic and Arctic (Agogue *et al.*, 2008; Galand *et al.*, 2009), and have been found in sediment beneath *Lophelia* corals from the Ionian Sea (Yakimov *et al.*, 2006). Ammonia from fermentation in aggregates, excreted by zooplankton and benthic animals, may support ammonia oxidisers such as *N. maritimus* and *Nitrosospira*, which in turn produces nitrite. The reef seawater nitrite concentration was 75 nM when measured in this study and twice the mid-water nitrite concentration measured. Nitrite is oxidised to nitrate by bacteria such as the *Nitrospina*, although

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only four reef singleton sequences related to these organisms were recovered despite nitrate levels of 12–13 μM.

The occurrence of phototrophic organisms in deep waters can be explained by heterotrophic capabilities of especially *Stramenopiles* (Behnke *et al.*, 2006), by the sinking of phototrophs from surface waters (López-García *et al.*, 2001) and possibly vertical mixing. Present in the reef seawater, but less frequently detected mid-water, were phototrophs like relatives of silicoflagellate algae from European coastal waters and relatives of *Cryptophyta* from the Baltic Sea (Piwosz & Pernthaler, 2010). The cryptomonads contain phycobiliproteins in addition to chlorophyll and carotenoids (Vaulot *et al.*, 2008) and may as the *Synechococcus* be efficient in harvesting dim light. Additional reef seawater photoautotrophs were represented by the green algae *Clorophyta* (two singletons). Phototrophy also occur among many characterised dinoflagellates (Vaulot *et al.*, 2008).

Specialised host associates

Interestingly, there were no 16S rRNA gene sequences retrieved from host associates comparable to the '*Candidatus* Mycoplasma corallicola' (Neulinger *et al.*, 2009), '*Ca.* Acestibacter aggregatus' (Jensen *et al.*, 2010), *Bdellovibrio*, *Rickettsia* or species of the genus *Vibrio*. Instead sequences from the reef affiliated with *A. lwoffii* from *Pocillopora damicornis* corals from the Great Barrier Reef (Bourne & Munn, 2005) and *Antho chartacea* sponges from the Sydney's Bare Island (Taylor *et al.*, 2007). The reef sequences also affiliated with uncultured *Euryarchaeota* from the sponge *Axechina raspailoides* (Holmes & Blanch, 2007). Most specialised host associates were detected from sequences in the 18S rRNA gene libraries, as organisms of the *Alveolata* group I and group II. There was a rich diversity of *Alveolata* in both reef and mid-water samples, which is consistent with pyrosequencing and clone library studies from the North Atlantic Ocean (Lovejoy *et al.*, 2006), from the Equatorial Pacific Ocean (López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001; Brown *et al.*, 2009), from a Norwegian sulphidic Framvaren fjord (Behnke *et al.*, 2006) and from deep-sea hydrothermal vents (López-García *et al.*, 2007). Relatively abundant in oxygen minimum zones and in the reef library was the *Alveolata* group I. These organisms have been described from environmental sequences only (López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001), and there is little information about their morphology and lifestyle (López-García *et al.*, 2007). Guillou *et al.* (2008) refer to two species as parasites of fish eggs (*Ichthyodinium*) and ciliates (*Duboscquella*) and proposed the *Alveolata* group I to be included into the order *Syndiniales* (*Alveolata* group II). The novel reef seawater OTU6 fell in an unstable phylogenetic position in between crustacean-infecting *Ellobiopsidae* (Gómez *et al.*, 2009) and dinoflagellate-infecting *Amoebophrya* (Guillou *et al.*, 2008).

Comparison with other deep-water coral reefs

Few DNA sequences have been obtained from deep-water coral reefs. The 16S rRNA gene sequences from microplankton fell into eleven phyla (Penn *et al.*, 2006; Jensen *et al.*, 2008; Neulinger *et al.*, 2008). All phyla were represented by sequences from the MRR08 seawater except for the *Acidobacteria*, *Deferribacteres*, *Spirochaetes* and *Verrucomicrobia*. Additionally present in the MRR08 seawater was the clade SAR406. Many more sequences have been obtained from comparable depths of open-ocean seawater and especially from oxygen minimum zones (e.g. Behnke *et al.*, 2006; Woebken *et al.*, 2007; Agogue *et al.*, 2008; Brown *et al.*, 2009; Lavik *et al.*, 2009; Walsh *et al.*, 2009). The reason for the affiliation with such sequences may be that some organisms identified in this study were embedded in oxygen-deficient aggregates and that there were no planktonic *Eukarya* and *Archaea* rRNA gene sequences from other deep-water coral reefs to compare with. Given that the MRR08 is a representive deep-water coral reef, comparison with planktonic organisms away from reefs suggests that seawater from deep-water coral reefs harbour more distinct phylotypes, more phylotypes from mesopelagic and oxygen minimum zones and are enriched in algae and cyanobacteria.

Conclusions

Alveolata, *Proteobacteria* and *Thaumarchaeota* represent major lineages in driving the high planktonic diversity observed in seawater from a deep-water coral reef off mid-Norway. This diversity relates to mesopelagic zones, and insight into the organisms' phylogeny was revealed. The functional diversity remains largely unknown as interpreting functions become increasingly uncertain as the distance from characterised phylotypes increases. Versatile parasites could be an important component of the microplankton. Others are potential utilisers of ammonium, sulphur and hydrocarbons. Detection of phototrophs suggests import from surface waters. Taken together, microplankton may exert complex control on the nutrition and health of deep-water coral reefs. Further studies are required to determine seasonal variation and activity of these microbes and identify reef seawater-specific ecotypes.

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