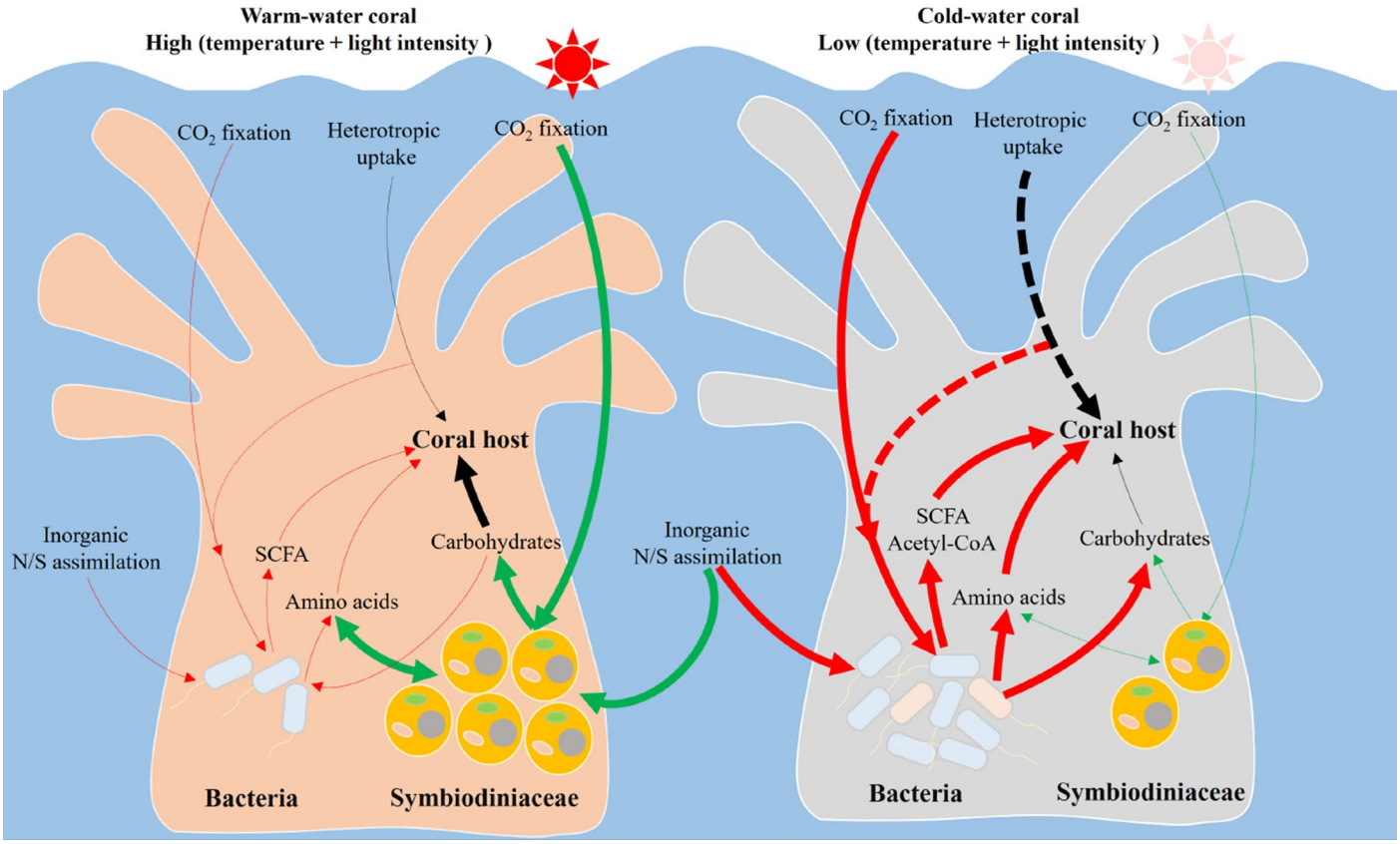


Rapid Review: Recent Evidence for Zooxanthellae in Deep-Dwelling Cold Water Corals (CWCs)

Chris Aslett

A swift objective summary of the findings of the seminal study of Gong and allies who investigated the bacterial and "algal" microbiomes integral to the holobionts of deep-ocean cold water Scleractinia (Gong et al. 2023).

Intuition spawned the longstanding deepwater corals in frigid aphotic zones are azooxanthellate paradigm, and thus refuting empirical evidence is somewhat revelatory. Advancements in deep underwater exploration have uncovered numerous and diverse ecosystems whose sessile Cnidaria include hexacorallian Scleractinia, octocorallian "soft" corals, antipatharian black corals, and hydroid corals of the order Hydrocorallina and family Stylasteridae. Although less prone to erecting the elaborate edifices common to warm water corals (WWCs), cold water corals (CWCs) fashion three-dimensional deepwater niches that are ripe for macro- and micro-scopic inhabitation. Found from 50 to 4000 m, they favour temperatures ranging from 4 to 12°C (Gong et al. 2023). Several are zooxanthellate where those of some like *Leptoseris hawaiiensis* can photosynthesise at 172 m (Rouzé et al. 2021, cited in Gong et al. 2023) when exposed to profoundly narrow-wavelength light with an intensity of near to 1 percent of surface downwelling (Gong et al. 2023).

The study analysed the CWCs *Narella versluysi*, *Heterogorgia uatumani*, and species of *Muriceides* sampled from 260 to 370 m and compared the findings with those of the shallow WWC model control group: *Acropora pruinosa*, *Galaxea fascicularis*, and *Pocillopora damicornis* (Fig 1.). Ascertaining the microbial commensals and mutualists of each coral was facilitated by metabarcoding the internally transcribed spacer 2 (ITS2) regions of Symbiodiniacea's ribosomal RNA (rRNA) and the 16 svedberg (16S) subunits of prokaryotic rRNA, while metatranscriptome analyses ascribed and contextualised each taxon's putative role. Moreover, microscopic examinations of coral tissue assisted the classification of microbiome communities and their anatomical compartmentalisations (Gong et al. 2023).

The prokaryotic populations of some CWCs such as *Leptoseris* and *Madrepora* may be host-specific and unlike those found environmentally. CWCs nurture diverse endodermal communities of bacteria and Symbiodiniaceae many of which are novel taxa like the phyla Coralsanbacteria, Coralqiangbacteria, Coralgsqaceae, Coralgongineae, and the Symbiodiniaceae strain, C-new of the genus *Cladcopium*. W- and C-WCs are home to Symbiodiniaceae of the genera *Cladocopium* and *Durusdinium*. *Cladocopium* strains C1, C42u, and C-new were the most common amongst CWCs, whilst *Cladcopium* strain C50c and *Durusdinium* strains D1 and D4 were prevalent in WWCs. Excluding *N. versluysi*, the number of zooxanthellae with detectable ITS2 regions were scarce among CWCs. Microscopy revealed both coccoid and actively dividing endodermal Symbiodiniaceae in corals from either kind of environment (Fig 2.).

The bacterial microbiomes of both types of coral comprised 29 to 51 percent of the phylum Proteobacteria, 9 to 45 percent Firmicutes, 15 to 18 percent Acidobacteria, and 2 to 10 percent archaean Euryarchaeota, whereas the phylum Tenericutes was comparatively enriched in CWCs at merely 5 percent. The amplicon sequence variants (ASVs; ≒phylotypes; ≒taxa) *Acetilactobacillus jinshanensis*, *Fusobacterium canifelinu*, and *Veillonella dispar*, together with species of *Actinomyces*, *Campylobacter*, *Leptotrichia*, *Prevotella*, and *Streptococcus* were abundant in CWCs. Furthermore, novel taxa were identified in deep- and shallow-water corals such as members of the phylum Candidatus Coralqiangbacteria, the nascent Candidatus genus *Coralgsqia*, and the new Candidatus

Keywords: bacterial communities; microbial profiles; cold water corals; metabolome; prokaryotes; warm water corals; zooxanthellae; zooxanthellar profiles.

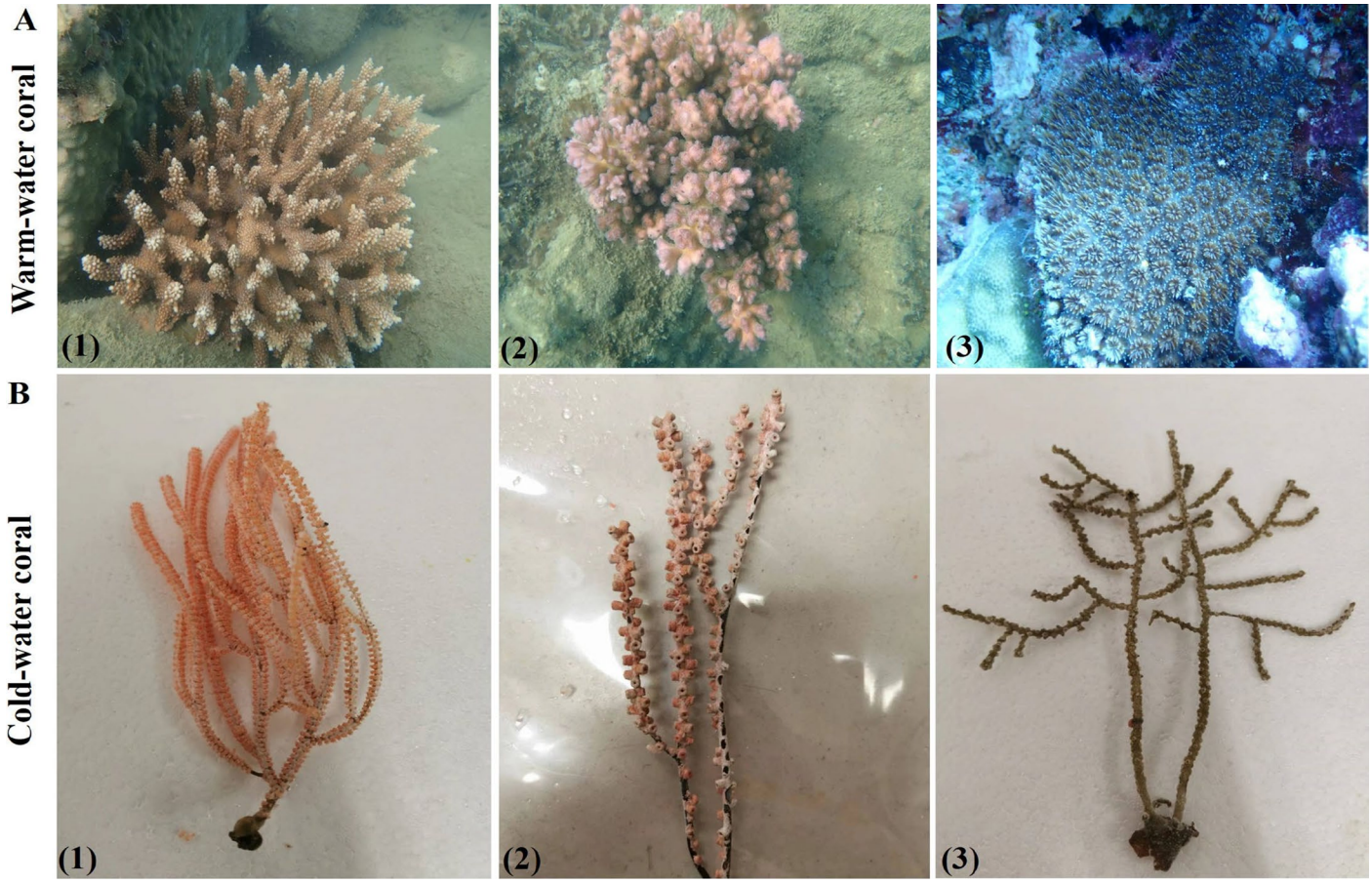


Fig 1. Photographs of WWCs [A], and CWCs [B]. [A1] *Acropora pruinosa*; [A2] *Pocillopora damicornis*, and [A3] *Galaxea fascicularis*. [B1] *Narella versluysi*; [B2] *Heterogorgia uatumani*, and [B3] a colony of *Muriceides*. Courtesy of Gong et al. 2023 and The Creative Commons Attribution Licence. https://creativecommons.org/licenses/by/4.0/

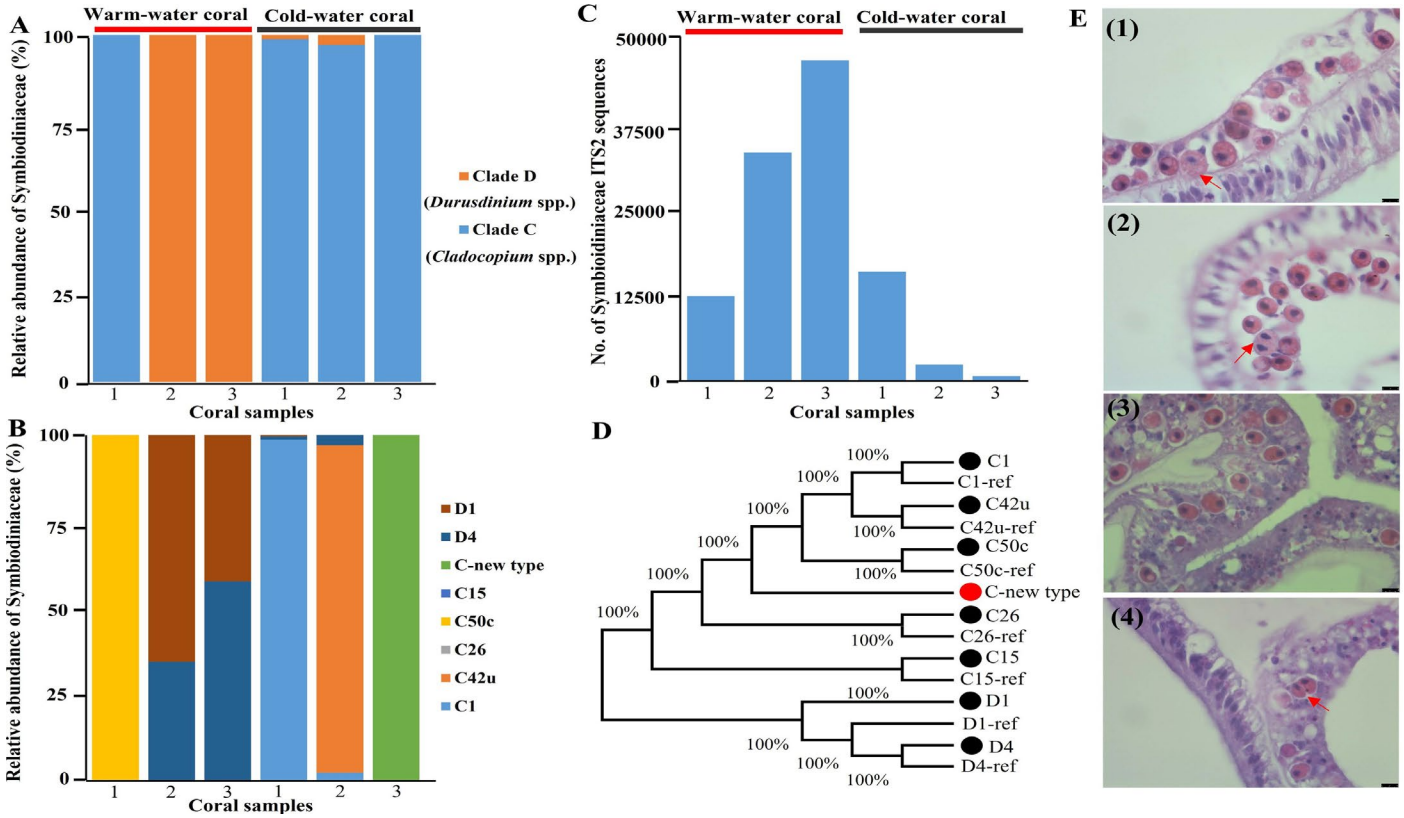


Fig 2. The profiles of Symbiodiniaceae in the analysed W- and C-WCs. Bar plots of the zooxanthellar genera [A] and types [B] in the studied W- and C-WCs. [C] bar plots of the total abundances of Symbiodiniaceae's amplicon sequence variants (ASVs) in the examined W- and C-WCs according to the sequence analyses of their secondary internally transcribed spacers (ITS2s). [D] an ITS2 sequence analysis-discerned tree, contextualising the phylogeny of zooxanthellar types in the studied W- and C-WCs with node-associated percent Bayesian posterior probabilities and maximum likelihood (ML) bootstraps. [●] new taxon. [1 & 2] brightfield micrographs of tissue cross sections of WWCs, and [3 & 4] those of CWCs where red arrows indicate actively mitotic Symbiodiniaceae. Courtesy of Gong et al. 2023 and The Creative Commons Attribution Licence. https://creativecommons.org/licenses/by/4.0/

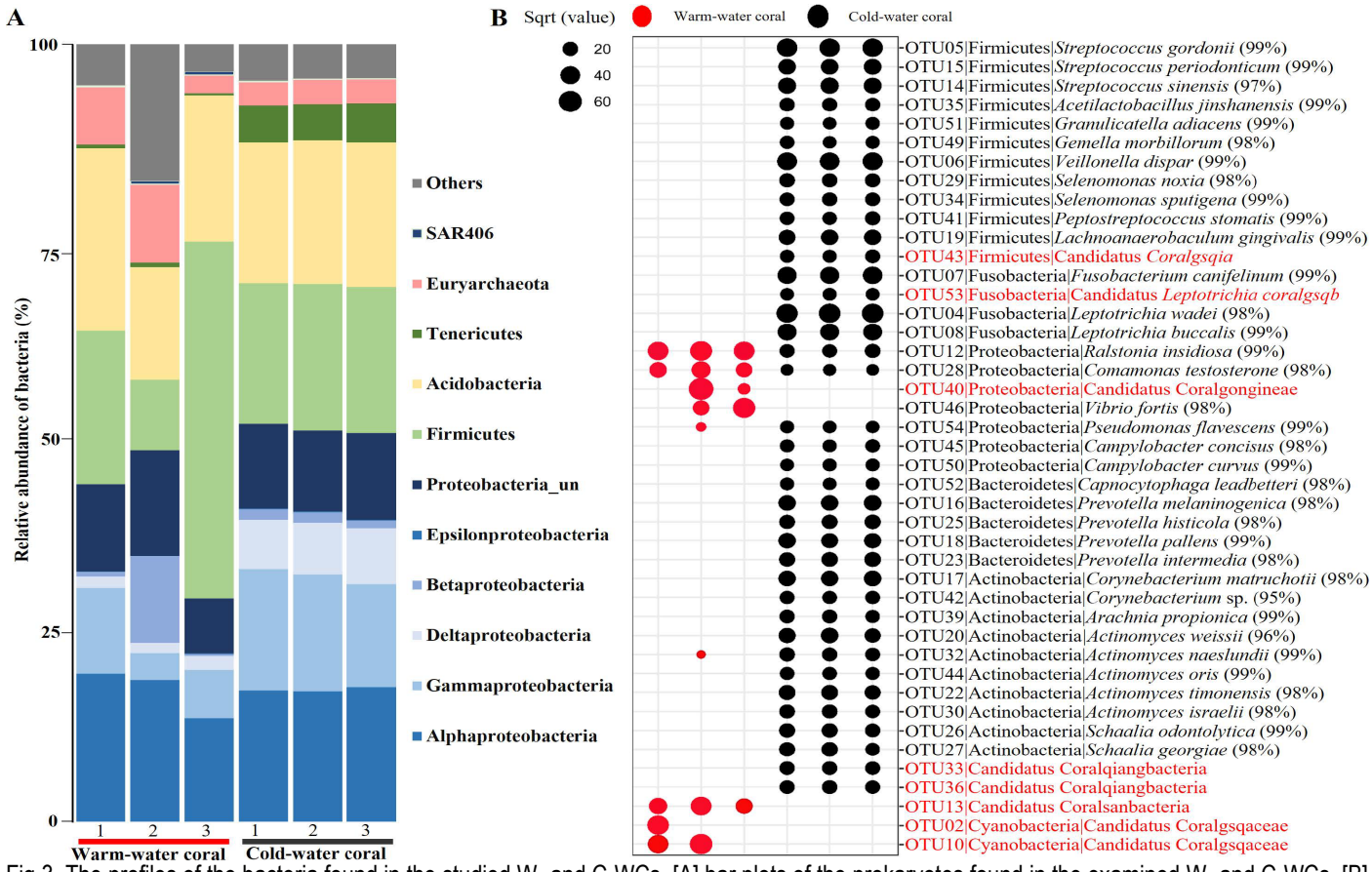


Fig 3. The profiles of the bacteria found in the studied W- and C-WCs. [A] bar plots of the prokaryotes found in the examined W- and C-WCs. [B] sqrt transformation pheatmaps illustrating the enumerations of the most prevalent operational taxonomic units (OTUs; ≅ASVs; ≅phylotypes) observed in W- or C-WCs with novel OTUs highlighted in red. Courtesy of Gong et al. 2023 and The Creative Commons Attribution Licence. https://creativecommons.org/licenses/by/4.0/

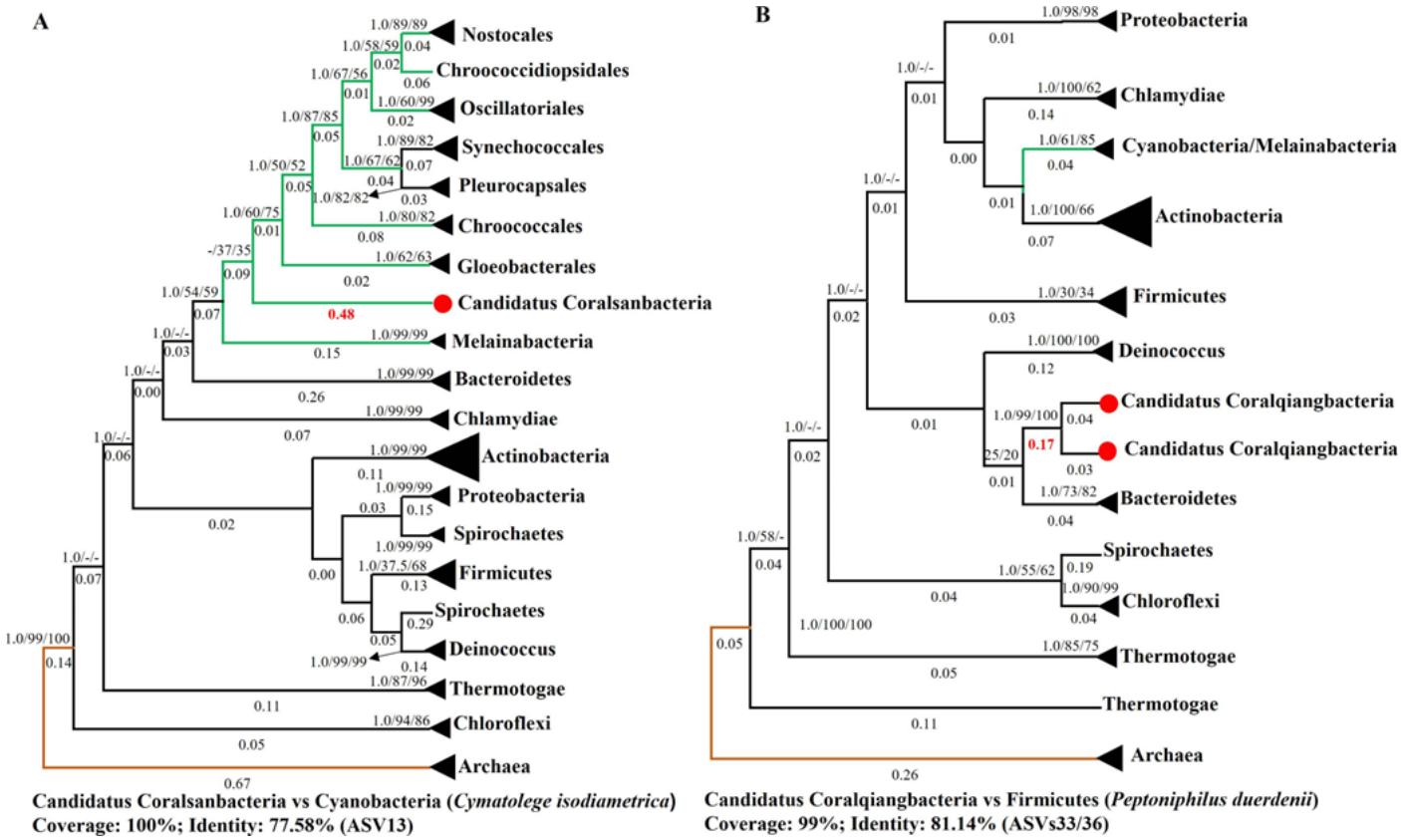


Fig 4. The ancestral lineages of the two new candidate bacterial phyla [●] delimited using 16 svedberg (S) ribosomal ribonucleic acid (rRNA) comparative sequence analyses contextualised with the data of Konstantinou and allies' from 2021, where Bayesian posterior probability/maximum likelihood (ML) bootstrap/neighbour-joining (NJ) support are associated with each node. [A] illustrates the fairly deep divergence of the phylum Candidatus Coralsanbacteria from Cyanobacteria clustered with seven orders of cyanobacteria and melainabacteria. [B] features the recent sequential divergence of the phylum Candidatus Coralqiangbacteria from Deinococcus and Bacteroidetes. Courtesy of Gong et al. 2023 and The Creative Commons Attribution Licence. https://creativecommons.org/licenses/by/4.0/

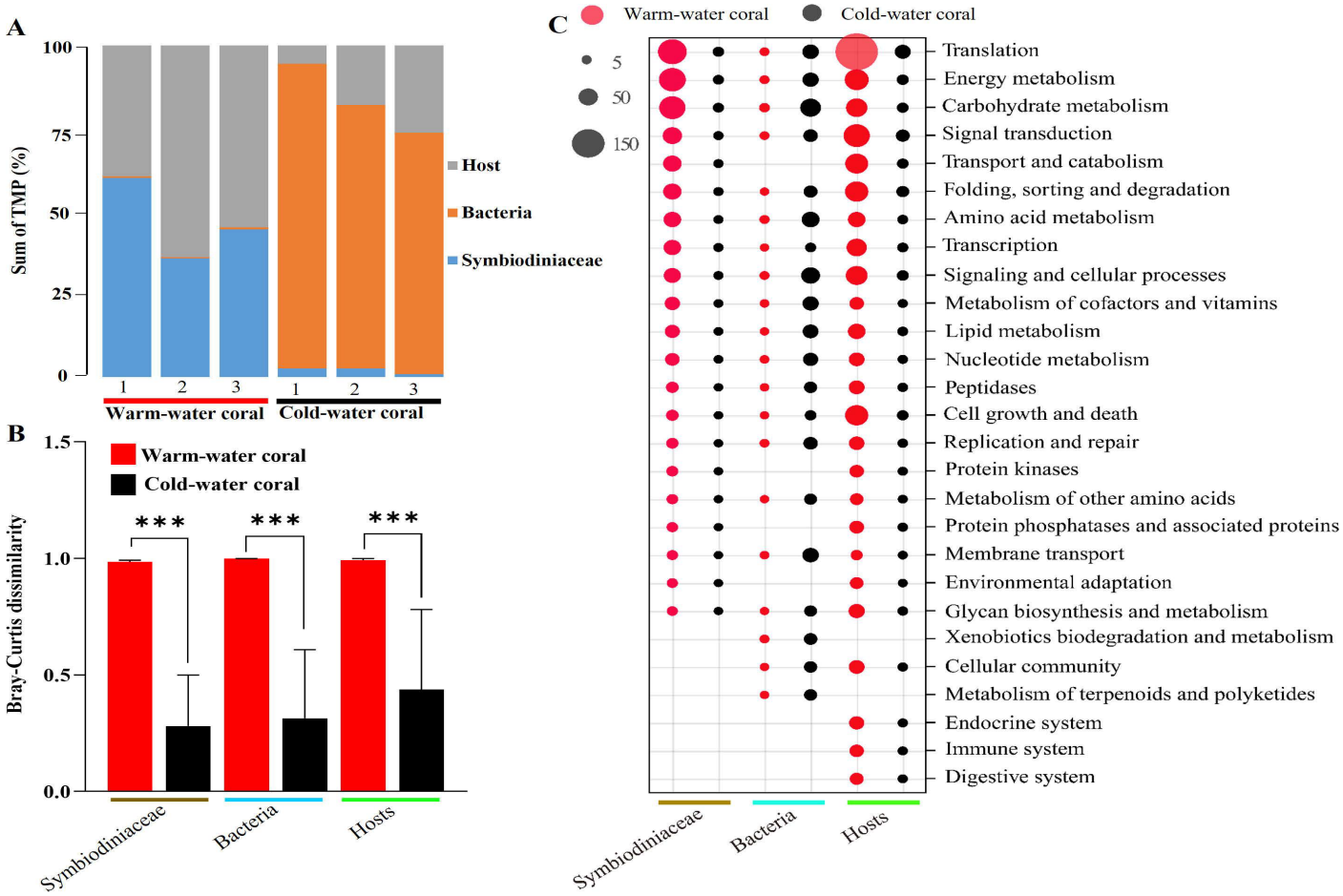
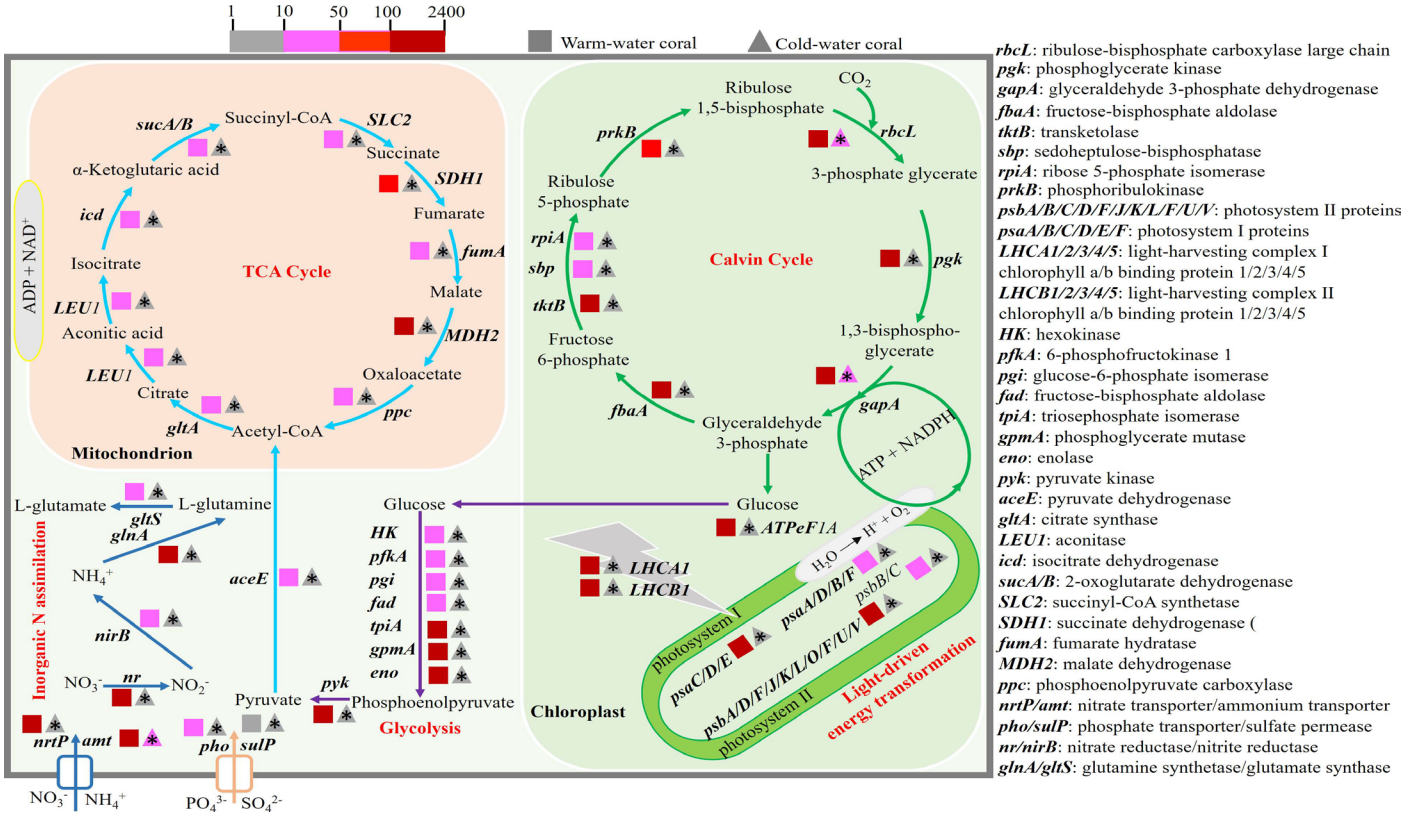
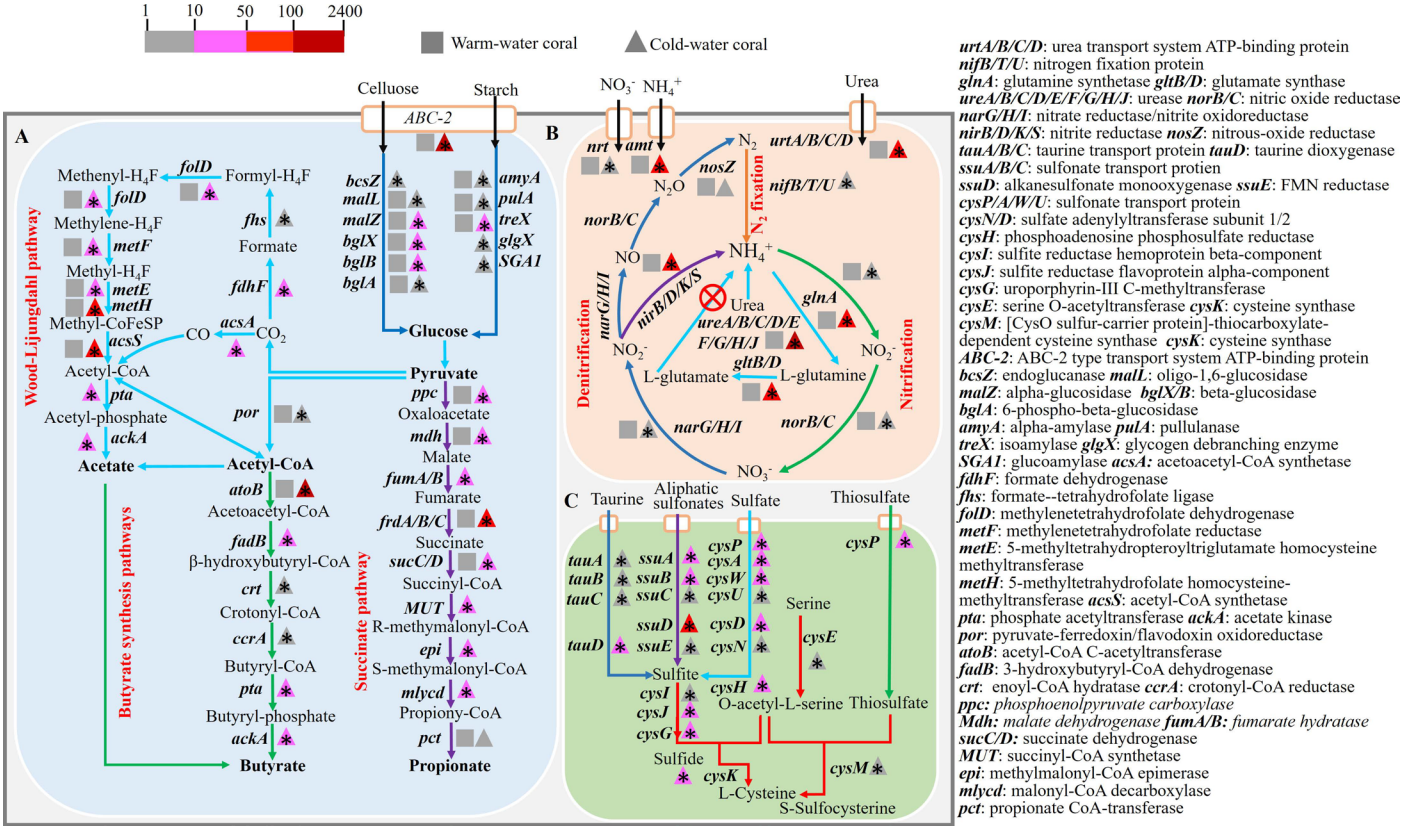


Fig 5. The transcriptomic profiles of the holobionts of W- and C-WCs. [A] bar plots of the relative abundances of zooxanthellar-, prokaryotic-, and host-expressed genes. [B] bar plots of the Bray Curtis distance-discerned dissimilarities of the gene transcriptional profiles of Symbiodiniaceae, bacteria, and hosts. [C] pheatmaps exemplifying the relative abundances of transcripts associated with the metabolic pathways that monopolised the resources of Symbiodiniaceae, bacteria, and hosts. Courtesy of Gong et al. 2023 and The Creative Commons Attribution Licence. https://creativecommons.org/licenses/by/4.0/



rbcL: ribulose-bisphosphate carboxylase large chain
pgk: phosphoglycerate kinase
gapA: glyceraldehyde 3-phosphate dehydrogenase
fbaA: fructose-bisphosphate aldolase
tktB: transketolase
sbp: sedoheptulose-bisphosphatase
rpiA: ribose 5-phosphate isomerase
prkB: phosphoribulokinase
psbA/B/C/D/F/J/K/L/F/U/V: photosystem II proteins
psaA/B/C/D/E/F: photosystem I proteins
LHCA1/2/3/4/5: light-harvesting complex I chlorophyll a/b binding protein 1/2/3/4/5
LHCB1/2/3/4/5: light-harvesting complex II chlorophyll a/b binding protein 1/2/3/4/5
HK: hexokinase
pfkA: 6-phosphofructokinase 1
pgi: glucose-6-phosphate isomerase
fad: fructose-bisphosphate aldolase
tpiA: triosephosphate isomerase
gpmA: phosphoglycerate mutase
eno: enolase
pyk: pyruvate kinase
aceE: pyruvate dehydrogenase
gltA: citrate synthase
LEU1: aconitase
icd: isocitrate dehydrogenase
sucA/B: 2-oxoglutarate dehydrogenase
SLC2: succinyl-CoA synthetase
SDH1: succinate dehydrogenase (
fumA: fumarate hydratase
MDH2: malate dehydrogenase
ppc: phosphoenolpyruvate carboxylase
nrtP/amt: nitrate transporter/ammonium transporter
pho/sulP: phosphate transporter/sulfate permease
nr/nirB: nitrate reductase/nitrite reductase
glnA/gltS: glutamine synthetase/glutamate synthase

Fig 6. The light-driven energy transformation, CO_2 fixation, glycolysis, citric acid cycle, and nitrogen (N) and phosphorus (P) metabolisms of Symbiodiniaceae in W- and C-WCs. The lines of each pathway have distinctive colours while squares and triangles indicate the respective zooxanthellae of W- and C-WCs, whose colours represent the comparative scale-commensurate predominance of each gene's transcript. [*] ANOVA probabilities. Courtesy of Gong et al. 2023 and The Creative Commons Attribution Licence. https://creativecommons.org/licenses/by/4.0/



urtA/B/C/D: urea transport system ATP-binding protein
nifB/T/U: nitrogen fixation protein
glnA: glutamine synthetase ***gltB/D***: glutamate synthase
ureA/B/C/D/E/F/G/H/J: urease ***norB/C***: nitric oxide reductase
narG/H/I: nitrate reductase/nitrite oxidoreductase
nirB/D/K/S: nitrite reductase ***nosZ***: nitrous-oxide reductase
tauA/B/C: taurine transport protein ***tauD***: taurine dioxygenase
ssuA/B/C: sulfonate transport protien
ssuD: alkanesulfonate monooxygenase ***ssuE***: FMN reductase
cysP/A/W/U: sulfonate transport protein
cysN/D: sulfate adenylyltransferase subunit 1/2
cysH: phosphoadenosine phosphosulfate reductase
cysI: sulfite reductase hemoprotein beta-component
cysJ: sulfite reductase flavoprotein alpha-component
cysG: uroporphyrin-III C-methyltransferase
cysE: serine O-acetyltransferase ***cysK***: cysteine synthase
cysM: [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase ***cysK***: cysteine synthase
ABC-2: ABC-2 type transport system ATP-binding protein
bcsZ: endoglucanase ***malL***: oligo-1,6-glucosidase
malZ: alpha-glucosidase ***bglX/B***: beta-glucosidase
bglA: 6-phospho-beta-glucosidase
amyA: alpha-amylase ***pulA***: pullulanase
treX: isoamylase ***glgX***: glycogen debranching enzyme
SGA1: glucoamylase ***acsA***: acetoacetyl-CoA synthetase
fdhF: formate dehydrogenase
fhs: formate--tetrahydrofolate ligase
folD: methylenetetrahydrofolate dehydrogenase
metF: methylenetetrahydrofolate reductase
metE: 5-methyltetrahydropteroyltriglutamate homocysteine methyltransferase
metH: 5-methyltetrahydrofolate homocysteine-methyltransferase ***acsS***: acetyl-CoA synthetase
pta: phosphate acetyltransferase ***ackA***: acetate kinase
por: pyruvate-ferredoxin/flavodoxin oxidoreductase
atoB: acetyl-CoA C-acetyltransferase
fadB: 3-hydroxybutyryl-CoA dehydrogenase
crt: enoyl-CoA hydratase ***ccrA***: crotonyl-CoA reductase
ppc: *phosphoenolpyruvate carboxylase*
Mdh: *malate dehydrogenase* ***fumA/B:*** *fumarate hydratase*
sucC/D: succinate dehydrogenase
MUT: succinyl-CoA synthetase
epi: methylmalonyl-CoA epimerase
mlycd: malonyl-CoA decarboxylase
pct: propionate CoA-transferase

Fig 7. The carbon (C)-, nitrogen (N)-, and sulphur (S)-associated metabolic pathways monopolised by the prokaryotic communities of W- and C-WCs. The lines of each pathway have distinctive colours while squares and triangles indicate the respective bacteria of W- and C-WCs, whose colours represent the comparative scale-commensurate predominance of each gene's transcript. [A] genes implicated in the transportation and degradation of exogenous carbon sources, CO_2 fixation, and the production of short chain fatty acids (SCFAs). [B] genes linked to the degradation and utilisation of inorganic and organic N. [C] genes associated with the assimilation of inorganic- and the disincorporation of organic-sulphur. [*] ANOVA probabilities. Courtesy of Gong et al. 2023 and The Creative Commons Attribution Licence. https://creativecommons.org/licenses/by/4.0/

species, *Leptotrichia coralgsqb* in CWCs, while WWCs nurtured members of the new phylum Candidatus Coralsanbacteria, those of the order Candidatus Coralgsqaceae, and several of the suborder Candidatus Coralgongineae (Figs 3. & 4.; Gong et al. 2023).

Remarkably, around 50 percent of the transcripts of WWC's originated from their Symbiodiniaceae, while merely 2 percent of messenger RNA (mRNA) was "algal" partner-derived in CWCs. However, this trend was reversed in the bacterial communities of CWCs where 70 percent of expression was prokaryotic, whereas less than 1 percent occurred in those of shallow-water ecosystems. The core metabolomes of zooxanthellae in corals from either habitat were preoccupied with translation, transcription, energy, carbohydrates, lipids, and amino acids, yet merely exceedingly low-level expression was evident in the "algal" partners of CWCs. Moreover, the metabolic profiles of each host were comparable to those of their phototrophic symbionts (Fig 5.).

The prokaryotes of CWCs mostly express genes encoding components of the anaerobic carbon-fixing Wood-Lijungdahl pathway, those related to the biosynthesis of short chain fatty acids (SCFAs), and the cycling of nitrogen and sulphur. Their organic carbon is likely shared with their heterotrophic host like photosynthetic glucose in WWCs, while these metabolic roles may be indispensable for survival at depth. Furthermore, these communities may retain rapid adaptation competencies like metagenomic and metabolomic plasticity, whilst CWCs appear to be armed with the enzymes to breakdown exogenous cellulose and starch, and therefore, much of their organic carbon may be acquired through heterotrophic feeding (Gong et al. 2023).

SCFAs are associated with the health of gut microbiota in humans, so may support host/bacterial eubiosis. The prokaryotic communities of CWCs also express genes implicated in de- and -nitrification, as well as the fixation and breakdown of organic nitrogen (Fig 7. B; Gong et al. 2023).

The amino acid taurine and sulphates are significant sources of oceanic sulphur (Gong et al. 2023), whilst numerous marine bacteria derive much carbon and sulphur from the abundant marine compound, dimethylsulphoniopropionate (DMSP). Yet most metabolise it differently (Wirth et al. 2020). The bacterial mutualists and commensals of CWCs retain an arsenal of organic and inorganic sulphur-degradative and -cycling enzymes, including numerous proteins associated with the fixation of organic sulphur like those that biosynthesise the amino acid L-cysteine (Gong et al. 2023), whose residues stabilise globular proteins by forming intramolecular disulphide bridges. The cysteine precursors homocysteine and serine are catalysed by cystathionine β-synthase (Cbs) or cystathionase (cystathionine γ-lyase; Cth) in several kinds of coral, albeit intriguingly, these enzymes are lacking in species of *Acropora* which cannot generate this indispensable amino acid (Shinzato et al. 2011). Notwithstanding one prominent prokaryotic pathway requiring anoxia (Fig 7. A), the studied CWCs do not erect the massive structures common to numerous shallow-water hermatypes that likely provide borderline anaerobia in their deep endolithic matrices, yet bacterial thiosulphate and assimilative detoxification may protect the host from dissolved hydrogen sulphide and ionic bisulphide (Fig 7. C).

Redolent of the functional but nominal phototrophy of CWCs' zooxanthellae, all analysed Symbiodiniaceae expressed genes associated with light-driven energy transformation, CO_2 fixation, glycolysis, and the citric acid cycle (Fig 6.; Gong et al. 2023).

References

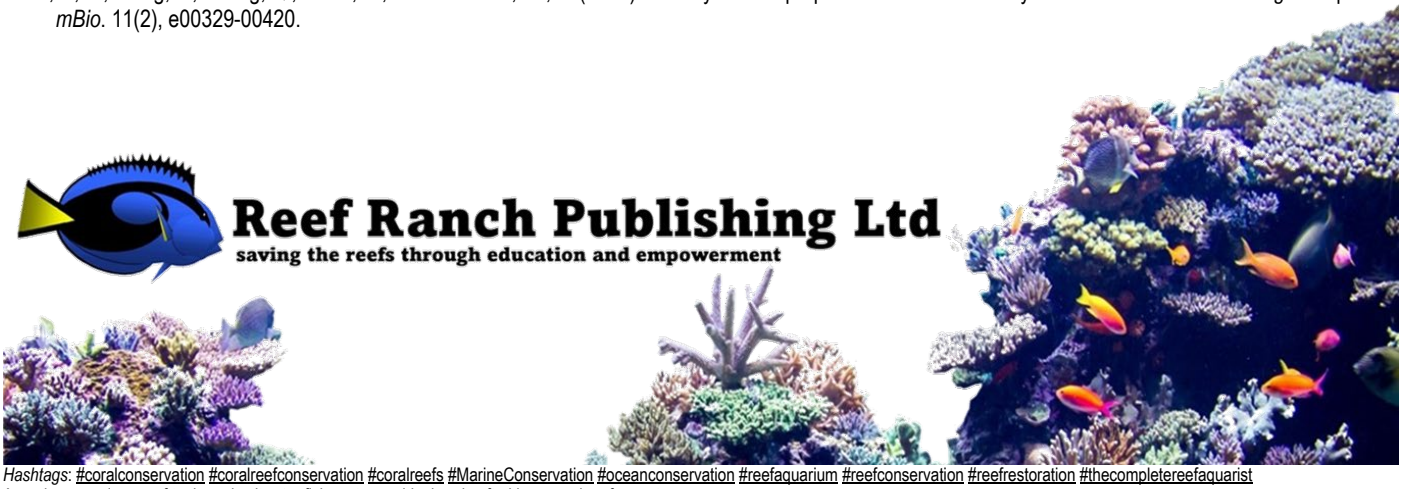
Gong, S., Liang, J., Jin, X., Xu, L., Zhao, M. & Yu, K. (2023) Unfolding the secrets of microbiome (Symbiodiniaceae and bacteria) in cold-water coral. Environmental Microbiology. 11(5). https://doi.org/10.1128/spectrum.01315-23

Konstantinou, D., Voultsiadou, E., Panteris, E. & Gkelis, S. (2021) Revealing new sponge-associated cyanobacterial diversity: novel genera and species. Mol Phylogenet Evol. 155, 106991. https://doi.org/10.1016/j.ympev.2020.106991

Rouzé, H., Galand, P., E., Medina, M., Bongaerts, P., Pichon, M., Pérez-Rosales, G., Torda, G. & Moya, A. (2021) Under The Pole Consortium. Symbiotic associations of the deepest recorded photosynthetic scleractinian coral (172 m depth). Raina, J-B. & Hédouin, L. (eds.). ISME J. 15, 1564-1568. https://doi.org/10.1038/s41396-020-00857-y

Shinzato, C., Shoguchi, E., Kawashima, T. et al. (2011) Using the Acropora digitifera genome to understand coral responses to environmental change. Nature. 476, 320-323.

Wirth, J., S., Wang, T., Huang, Q., White, R., H. & Whitman, W., B. (2020) Dimethylsulfoniopropionate Sulfur and Methyl Carbon Assimilation in *Ruegeria* Species. *mBio*. 11(2), e00329-00420.



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