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## Title
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## Permalink
https://escholarship.org/uc/item/7hm7d098

## Journal

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## Publication Date

## DOI

RESOURCE ARTICLE

MOLECULAR ECOLOGY
RESOURCES WILEY

# Comparative genomics of a vertically transmitted thiotrophic bacterial ectosymbiont and its close free-living relative

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## Abstract

Thiotrophic symbioses between sulphur-oxidizing bacteria and various unicellular and metazoan eukaryotes are widespread in reducing marine environments. The giant colonial ciliate *Zoothamnium niveum*, however, is the only host of thioautotrophic symbionts that has been cultivated along with its symbiont, the vertically transmitted ectosymbiont *Candidatus* Thiobius zoothamnicola (short Thiobius). Because theoretical predictions posit a smaller genome in vertically transmitted endosymbionts compared to free-living relatives, we investigated whether this is true also for an ectosymbiont. We used metagenomics to recover the high-quality draft genome of this bacterial symbiont. For comparison we have also sequenced a closely related free-living cultured but not formally described strain Milos ODIII6 (short ODIII6). We then performed comparative genomics to assess the functional capabilities at gene, metabolic pathway and trait level. 16S rRNA gene trees and average amino acid identity confirmed the close phylogenetic relationship of both bacteria. Indeed, Thiobius has about a third smaller genome than its free-living relative ODIII6, with reduced metabolic capabilities and fewer functional traits. The functional capabilities of Thiobius were a subset of those of the more versatile ODIII6, which possessed additional genes for oxygen, sulphur and hydrogen utilization and for the acquisition of phosphorus illustrating features that may be adaptive for the unstable environmental conditions at hydrothermal vents. In contrast, Thiobius possesses genes potentially enabling it to utilize lactate and acetate heterotrophically, compounds that may be provided as byproducts by the host. The present study illustrates the effect of strict host-dependence of a bacterial ectosymbiont on genome evolution and host adaptation.

**KEYWORDS**
ectosymbiosis, low-complexity metagenome, sulphur-oxidizing bacteria, thiotrophy, *Zoothamnium niveum*

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## 1 | INTRODUCTION

The beneficial associations between sulphur-oxidizing bacteria (SOBs) and diverse protist and invertebrate hosts (Cavanaugh et al., 2006; Dubilier et al., 2008; Ott et al., 2004; Sogin et al., 2021; Stewart et al., 2005) span the entire range of ectosymbiotic mutualism, from the highly diverse microbiomes of alvinocarid shrimps and the low-diversity microbiomes of alvinellid polychaetes at deep-sea hydrothermal vents (Cambon-Bonavita et al., 2021; Grzymski et al., 2008) to strictly single symbiont species such as those on stilbonematine nematodes from marine shallow-water sediments (Ott et al., 1991; Paredes et al., 2021; Petersen et al., 2010; Polz et al., 1994), or on an amphipod in freshwater caves (Dattagupta et al., 2009). Among ciliates, monolayered coats of single ectosymbiotic SOB species are known from the karyorelictid *Kentrophoros* (Faure-Fremiet, 1951; Fauré-Fremiet, 1950; Fenchel & Finlay, 1989; Finlay & Fenchel, 1989; Raikov, 1971, 1974; Seah et al., 2019) and the peritrichs *Pseudovorticella* (Grimonprez et al., 2018; Laurent et al., 2009; Maurin et al., 2010) and *Zoothamnium* (Bauer-Nebelsick et al., 1996a, 1996b; Hemprich & Ehrenberg, 1829, 1831; Rinke et al., 2006, 2009; Schuster & Bright, 2016). Some symbiotic SOBs form phylogenetic clusters with free-living relatives (Figure 1; Dubilier et al., 2008). Here, we provide genomic information on two closely related SOBs with different lifestyles, that is, an obligate vertically transmitted ectosymbiont and a free-living chemolithoautotrophic bacterium.

*Candidatus* Thiobius zoothamnicola of the Chromatiaceae (Chromatiales, Gammaproteobacteria) (Oren, 2017, short Thiobius) – originally introduced as *Candidatus* Thiobios zoothamnicoli (Rinke et al., 2006) – covers the surface of the giant colonial ciliate *Zoothamnium niveum* (short *Zoothamnium*) that can typically be found in shallow-water environments from tropical to temperate waters with decaying organic material (Bauer-Nebelsick et al., 1996a, 1996b, Rinke et al., 2006, 2009, reviewed in Bright et al., 2014). Thiobius is transmitted vertically (Bauer-Nebelsick et al., 1996a, 1996b; Rinke et al., 2006) and has not been found free-living despite extensive searches using general bacterial primers as well as a symbiont-specific primer and direct Sanger sequencing (Monika Bright personal observation, 2022). To our best knowledge, the giant ciliate mutualism is the only thiotrophic symbiosis that has been cultivated in the laboratory over several host generations (Rinke et al., 2007). In contrast, the facultative endosymbiont *Thiosocius teredinicola* of the shipworm *Kuphus polithalamius* is the only thiotrophic symbiont that has been cultivated without its host (Distel et al., 2017).

The host *Zoothamnium niveum* grows fast and reproduces asexually developing specialized macrozooids that leave the colony as ectosymbionts-covered propagules called swarmers to found new colonies upon settlement (Bauer-Nebelsick et al., 1996a, 1996b). Thiobius thriving on the host surface fixes inorganic carbon using sulphide and provides organic carbon to the host (Volland et al., 2018). In return, benefits to the symbiont come through the host's peculiar contraction and expansion behaviour that ensures the supply of oxygen and sulphide (Rinke et al., 2007). The ciliate colonies can show unspecific overgrowth on the stalk, which connects them to the substrate surface (Bauer-Nebelsick et al., 1996a).

Strain Milos ODIII6 (short ODIII6) is a cultured, but not yet formally described bacterium, that has been isolated from the shallow-water hydrothermal vents in Paliochori Bay (Milos, Greece; Kuever et al., 2002; Sievert, 1999). ODIII6 is mesophilic (optimal growth at 34°C) and oxidizes reduced sulphur compounds under aerobic conditions.

Evolutionary theory predicts that the genome of obligate microbial symbionts, transmitted vertically from one to the next host generation, will be reduced in size compared to free-living relatives and will contain a lower proportion of mobile elements than facultative symbionts (Newton & Bordenstein, 2011; Sachs et al., 2011). Being obligately host-associated, these bacteria experience bottlenecks in population size during each transmission event leading to genome reduction (Bobay & Ochman, 2017; McCutcheon & Moran, 2012; Moran, 1996; Moran et al., 2009; Toft et al., 2009; Toft & Andersson, 2010; Wernegreen, 2015). Trait function compensation by the host or other symbionts can lead to further gene loss and reduced genome size (Ellers et al., 2012). Since obligate symbiotic bacteria are often isolated in the host as endosymbionts, they experience limited possibilities for horizontal gene transfer and access to foreign DNA. Some even lack the machinery for uptake and incorporation of DNA (Medina & Sachs, 2010). In contrast, obligate ectosymbionts have access to novel gene pools (Newton & Bordenstein, 2011). Nevertheless, some thermophilic ectosymbiotic archaea undergo genome reduction. This so-called thermophilic streamlining was explained by having fewer and shorter genes in hot environments compared to archaea from other environments (Nicks & Rahn-Lee, 2017). Much less studied are vertically transmitted bacterial ectosymbionts located on the surface of eukaryotic hosts. Mostly, these ectosymbiotic microbiomes are composed of complex microbial communities. Only a few cases with a single microbial partner are known and even fewer genomic studies are available (Fokin & Serra, 2022; Husnik et al., 2021).

Here, we show that the ectosymbiont Thiobius has a smaller genome, fewer genes, reduced GC content, and a smaller mobilome than the free-living bacterium ODIII6, matching theoretical predictions. The analyses of metabolic capabilities revealed that the functional traits of Thiobius are largely a subset of the repertoire of ODIII6, which has a higher functional versatility to cope with broader environmental conditions as a free-living bacterium from a highly unstable, fluctuating hydrothermal vent environment. In contrast, Thiobius shows a potential genetic capability to grow heterotrophically as an adaptation to its host.

## 2 | MATERIALS AND METHODS

### 2.1 | Specimen collection and cultivation

Three *Zoothamnium niveum* colonies were collected from attached submerged mangrove roots and wood at 1 m depth in 2015 at Twin Cays (Belize, 6°50′3″ N, 88°6′14″ W; strains G42, G43 and G44), and one colony from a sunken wood at 70 cm depth in 2014

## 16S rRNA gene tree
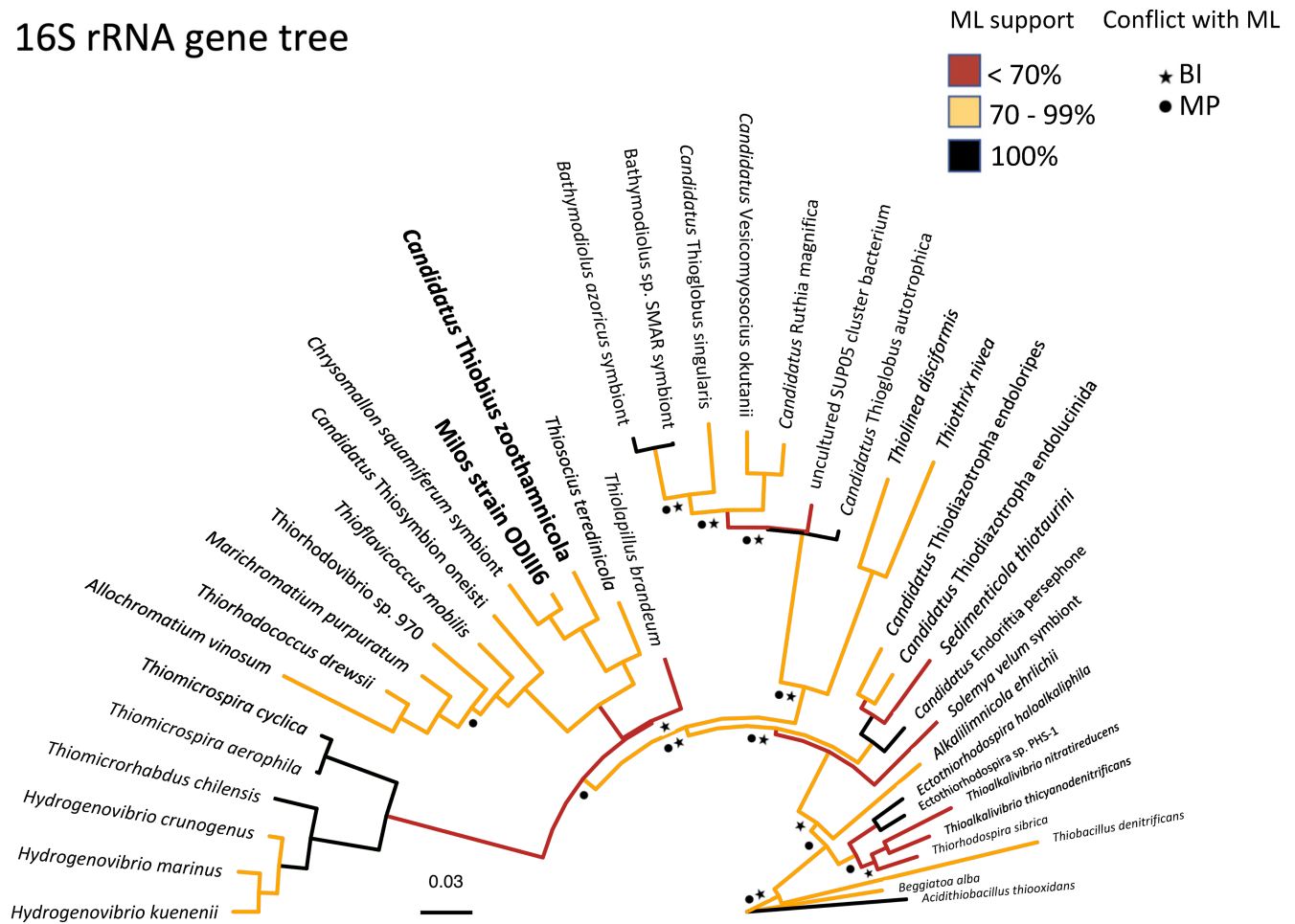


**FIGURE 1** Maximum likelihood (ML) tree based on the 16S rRNA gene. Bootstrap support of the internal nodes is indicated with the colour of the outgoing branches. Topology conflicts with two other techniques Maximum parsimony (MP, ⊛) and Bayesian inference (BI, ★) indicated with symbols in the affected internal nodes. The two microbes analysed in this study are highlighted in bold. The tree was generated with 306 sequences (Table S1), but only 40 organisms that have publicly released genomes are shown.

at Guadeloupe (16°16′38″ N, 61°33′27″ W; strain G4). The ODIII6 isolate was originally obtained from the $10^{-6}$ dilution of a sediment sample collected at a sediment depth of 8–13 mm and at 2 m distance from the centre of a gaseous hydrothermal vent at a water depth of 8 m in Paliochori Bay (Milos Island, Greece, 36°40′23″ N, 24°31′13″ E) (Kuever et al., 2002; Sievert, 1999; Sievert et al., 1999). A culture frozen at −80°C in dimethylsulphoxide was reactivated in 2015 and used to obtain DNA for genome sequencing.

### 2.2 | DNA preparation and sequencing

The lower parts of *Zoothamnium niveum* colonies are usually overgrown with diverse microbes (Bauer-Nebelsick et al., 1996a; Rinke et al., 2006), and were therefore cut off to minimize contamination using Schreiber micro scissors (Fridingen, Germany, European Union). The upper parts, covered by a monolayer of Thiobius, were homogenized with Tris-EDTA buffer by vortexing and DNA was extracted according to Zhou et al. (1996). The DNA extraction yielded up to 7 ng DNA per μL in ~30μL of final volume for G43. Nextera XT (Illumina)

DNA library preparation was used for multiplexing the *Zoothamnium* samples, and they were sequenced in a paired-end mode with 125 nucleotides of read length using the Illumina HiSeqV4 platform at the Vienna Biocenter Core Facility (https://www.viennabiocenter.org/vbcf/). The low amount of DNA yielded by a single *Zoothamnium* ciliate colony precluded the use of long-read sequencing technologies. The ODIII6 isolate was regrown from a frozen stock culture in 2015 and DNA was extracted with an UltraClean® microbial DNA isolation kit (MoBio laboratories). The DNA was sequenced with a MiSeq (Illumina) sequencer by a commercial provider, MR DNA (Shallowater, TX, https://www.mrdnalab.com/), using the 600 Cycles v3 Reagent Kit (Illumina). At MR DNA, the library of the sample was prepared with a Nextera DNA Sample Preparation Kit (Illumina).

### 2.3 | Quality refinement, de novo assembly and binning

The sequenced reads of the four *Zoothamnium* samples were quality filtered with the *bbduk* command of the suite *bbmap* v35.92

(BBMap–Bushnell B.–sourceforge.net/projects/bbmap/) under a more stringent quality threshold of 25 (Phred units). The filtered paired-end reads were assembled with *SPAdes* v3.7.1 in the metagenomic mode (Nurk et al., 2016). *MetaBAT* v0.26.3 (Kang et al., 2015) was then used for binning with default parameters. A nucleotide sequence homology *BLAST* search (Altschul et al., 1990) of the published 16S rRNA gene sequence of Thiobius (Rinke et al., 2006) identified the targeted bin. The genome of ODIII6 was de novo assembled by MR DNA using *NGEN* (DNAstar, https://www.dnastar.com). Completeness, heterogeneity and contamination of the ODIII6 and Thiobius assemblies were assessed with *CheckM* v1.0.5 (Parks et al., 2015). Presence of rRNAs and tRNAs for each amino acid was checked with *tRNAscan-SE* (Lowe & Eddy, 1997). Average amino acid identity (AAI; Konstantinidis & Tiedje, 2005) was calculated with *CompareM* (https://github.com/dparks1134/CompareM). The assembly of ODIII6 with *SPAdes* was performed to exclude contamination, using the same set of reads as the MR DNA assembly. The alignment between both assemblies was generated and visualized with *Mauve* (Darling et al., 2010, version snapshot_2015-02-25), establishing the correspondence between the contigs. The fastg file containing paired-end linkage information of the de Bruijn graph was visually inspected with *Bandage* v0.8.0 (Wick et al., 2015), allowing the assessment of physical connectivity between contigs of the bacterial chromosome.

## 2.4 | Synteny analysis and localized gap filling

The four Thiobius draft genome assemblies were aligned with *Mauve* (Darling et al., 2010) and the ordering of the contigs was partly reconciled. A less stringent quality threshold of 20 (Phred units) was applied for the quality filtering and trimming of the G43 reads with *bbduk* resulting in the recovery of the full 16S rRNA gene.

## 2.5 | Phylogenetic analyses

A total of 306 16S rRNA gene sequences were chosen following the taxa selection of Petersen et al. (2016) and Distel et al. (2017). 16S rRNA gene sequences were directly obtained from GenBank and RefSeq when available or extracted from publicly available genomes using *Metaxa* v2.2 (Bengtsson-Palme et al., 2015). The alignment was done with *MAFFT* v7 (Katoh & Standley, 2013) and *TrimAl* v1.2 (Capella-Gutierrez et al., 2009) was employed for trimming and filtering the alignments with default parameters. The obtained sequences were allocated to 292 putative species according to a 98.65% identity threshold (Kim et al., 2014; Table S1). Four species were excluded due to multiple divergent 16S rRNA gene copies (*Hydrogenovibrio halophilus*, *Lamprocystis purpurea*, *Thiofilum flexile* and *Thiothrix lacustris*). Phylogenetic analyses were performed using three methodological frameworks in order to assess the robustness of the tree inference: maximum parsimony (MP; Farris, 1970), maximum likelihood (ML; Felsenstein, 1981) and Bayesian inference (BI; Hastings, 1970). Packages *ape* v5.2 (Paradis

et al., 2004) and *phangorn* v2.4.0 (Schliep, 2011) were used for MP and ML, and BI was computed with *MrBayes* v3.2.7a (Huelsenbeck & Ronquist, 2001). The substitution model choice followed a Bayesian information criterion (Schwarz, 1978) as implemented in *phangorn*. Bootstrap support (Felsenstein, 1985) in ML and MP trees was calculated with 100 trees in each, and in BI posterior probability was used as metric of support. To facilitate visualization, 16S rRNA gene trees were reduced by pruning most of the branches (Rodriguez-Puente & Lazo-Cortes, 2013) retaining only the 40 organisms with genomes available. By comparing the tree topologies, shared internal nodes and conflicting topologies were manually identified. We used ML tree as the base of the representation to show the support of the three approaches.

## 2.6 | Functional inference of predicted genes and metabolic pathways

To predict and annotate genes the web version of *RASTtk* (https://rast.nmpdr.org, Brettin et al., 2015) was employed with default settings. Functional categories were assigned to the predicted genes by the use of *eggNOG mapper* version 2 (Huerta-Cepas et al., 2017, 2019). Metabolic pathways were obtained using the predicted and annotated genes as an input by two independent methods, followed by a manual curation: (a) the online KEGG tool (www.kegg.jp, Kanehisa & Goto, 2000, Moriya et al., 2007) interrogating pathway complete modules; and (b) the *PathoLogic* module from *Pathway Tools* (v23.5, Karp et al., 2002, 2010) of *MetaCyc* (Caspi et al., 2008). To these two levels of granularity (the fine grain of the genes and the coarser grain of the metabolic pathways; Vogt, 2010) we added the overarching level of functional traits (De Oliveira et al., 2022), defined as microbial characteristics that can be observed and are linked to fitness (Green et al., 2008). Additionally, secretion, motility and defence potential capabilities encoded in the genomes were assessed with *MacSyFinder* (Abby et al., 2016). The potential presence of traits was determined by putting together constituent genes following *MetaCyc* and *KEGG* when available, or *MacSyFinder* for motility and interaction traits (Abby et al., 2016). Some traits were not detected by *MetaCyc* and *KEGG*, and therefore, were manually inferred based on scientific literature (Table S2). In the manual curation logical operators (AND, OR) were employed to combine the gene presence/absence evidence into traits, as outlined in Karaoz and Brodie (2022) (Table S2).

## 2.7 | Orthology analysis

A total of 30 bacterial genomes, closest to Thiobius and ODIII6 based on the 16S rRNA gene tree phylogeny (Table S3), were selected excluding the genome of *Bathymodiolus* sp. SMAR symbiont due to poor quality of the assembly (52 scaffolds comprising 339 contigs with $N_{50}$ as low as 10,280 nt). Protein sequences were clustered into orthologous groups using *OrthoFinder* v2.5.4 (Emms & Kelly, 2019).

# 3 | RESULTS AND DISCUSSION

## 3.1 | Close phylogenetic relationship of Thiobius and ODIII6

In order to confirm the previously reported close phylogenetic placement of Thiobius and ODIII6 (Distel et al., 2017; Lenk et al., 2011; Nunoura et al., 2014; Rinke et al., 2006, 2009; Schuster & Bright, 2016), 16S rRNA gene ML, MP and BI phylogenies were constructed. A pruned visualization of the resulting ML tree with indication of the conflicts between the three approaches is shown in Figure 1 (full ML tree in Figure S1). While some of the shallower internal nodes (closer to the tips) showed good support (e.g. bootstrap values greater than 70%) and agreement between the three phylogenetic approaches, many of the deeper internal nodes were poorly supported and showed topology conflicts. While ODIII6 falls in a clade containing the endosymbionts of the hydrothermal vent snails *Chrysomallon squamiferum* and *Alviniconcha* sp. Lau Basin, and free-living bacteria, Thiobius can be assigned as sister taxon of this clade. The internal node connecting these clades obtained bootstrap supports of 70% (ML) and 63% (MP), while the posterior probability support of BI was 73%. The 16S rRNA gene sequences of Thiobius and ODIII6 were 95% identical (over 1396 aligned nucleotide positions). A relatively low average amino acid identity (AAI) of 67% was obtained for the whole genomes of Thiobius and ODIII6. Both 16S rRNA gene and AAI comparisons show that these two bacteria are related but ODIII6 is more closely related to both gastropod symbionts than to Thiobius (Distel et al., 2017, Lenk et al., 2011, Nunoura et al., 2014, Rinke et al., 2006, 2009, Schuster & Bright, 2016).

## 3.2 | High-quality draft genomes of Thiobius and ODIII6

The best Thiobius metagenome assembled genome (MAG) in terms of assembly completeness and contiguity was obtained from a single
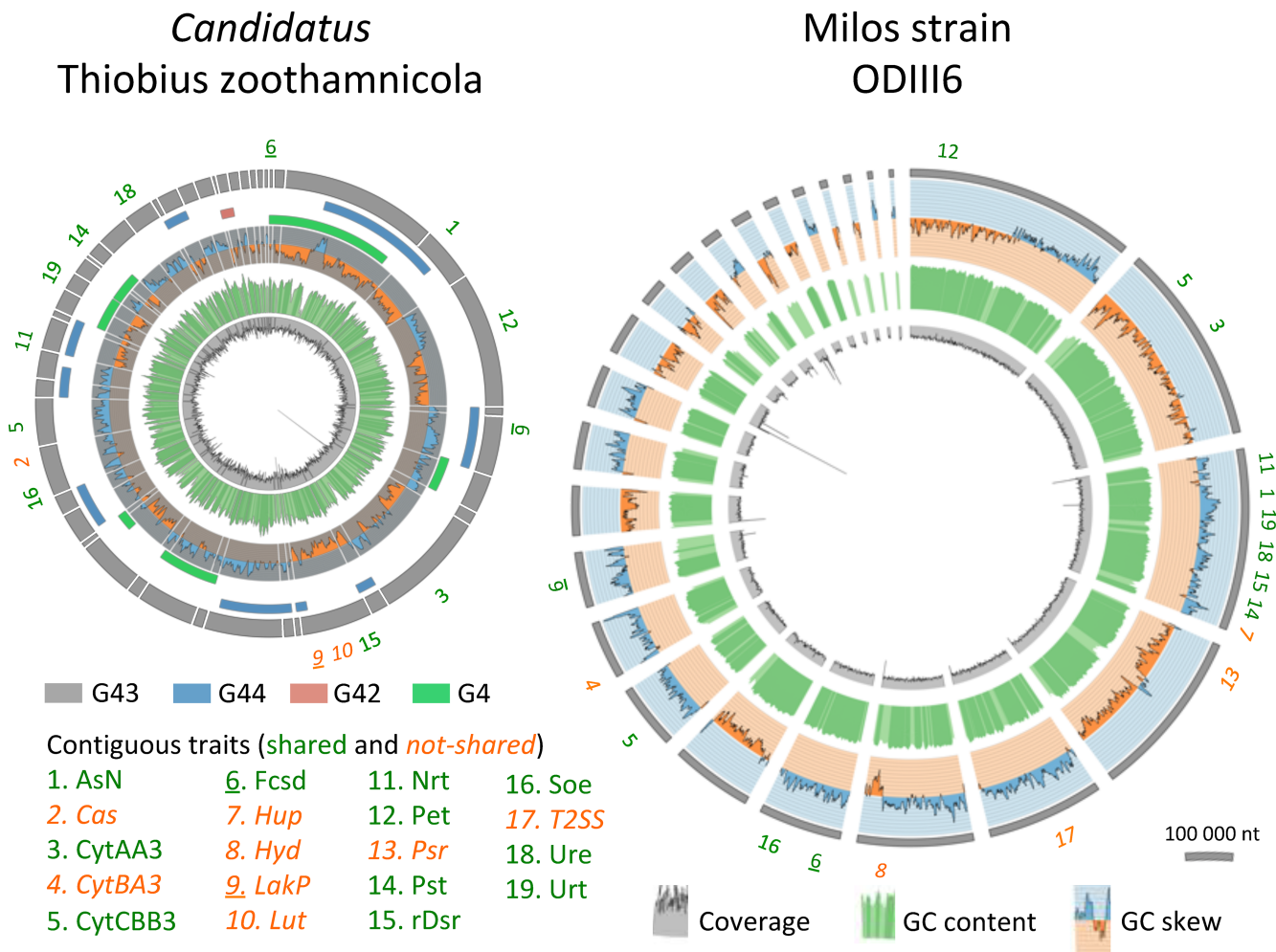
ciliate colony collected in Belize (labelled G43, Figure S2; Tables 1 and S4). A total of 8.8 million of the initial 13.5 million read pairs passed a more stringent quality threshold filtering (phred 25). *SPAdes* assembled these reads into 44,538 contigs. *MetaBAT* binning grouped these contigs into four bins, one of them containing two partial matches at contig ends to the published 16S rRNA gene of Thiobius according to a BLAST search (Rinke et al., 2006; Figure S3). A less stringent quality threshold filtering (phred 20) led to a very similar *SPAdes* assembly that recovered the full 16S rRNA gene. The corresponding long contig containing the full 16S rRNA gene was used to replace three contigs from the stringent filtering assembly that covered the same span (Figure S4, Table S5). Overall, Thiobius G43 assembly resulted in 46 contigs with a total length of 2.38 Mb, a $N_{50}$ value of 98,217, a coverage of 216× and a GC content of 49.4% (Figure 2). *CheckM* estimated its completeness as 96.0% and its contamination as 0.1%, and found no heterogeneity (Table 1), meeting the MIMAG standard for high-quality MAGs (Bowers et al., 2017). Synteny analysis with *Mauve* between the four Thiobius MAGs led to the identification of 26 clusters of contiguity that were utilized in the final ordering of the contigs (Figures 2 and S2, Table S5).

ODIII6's genome MR DNA assembly consisted of 26 contigs, and had no paired-end linkage associated information available (fastg file). Because *RASTtk* annotation revealed one contig with only phage related genes, we re-assembled the genome using *SPAdes* and found through the paired-end linkage information that the corresponding contig was circular and was thereby excluded (Figure S5). Accordingly, the final ODIII6 assembly resulted in 25 contigs, with a total of 3.53 Mb, a $N_{50}$ value of 245,984, a coverage of 103×, and a GC content of 61.9%. *CheckM* estimated its completeness as 99.6% and its contamination as 0.8%, and found no heterogeneity either (Table 1).

A comparison between the high-quality draft genomes of Thiobius and ODIII6 (both appropriate for general assessment of gene content, Chain et al., 2009), revealed a complete set of tRNAs for translation of all 20 amino acids in both bacteria. In agreement with theoretical

**TABLE 1** Statistics of the genomes assembled in this study.

| | Thiobius str. BelizeG43 | Thiobius str. BelizeG42 | Thiobius str. BelizeG44 | Thiobius str. GuadeloupeG4 | ODIII6 |
|---|---|---|---|---|---|
| Sequencing coverage | 216× | 296× | 176× | 131× | 103× |
| Assembly size (bp) | 2,381,364 | 2,379,254 | 2,311,386 | 2,369,374 | 3,528,654 |
| GC content | 49.4% | 49.4% | 49.6% | 49.6% | 61.9% |
| Number of contigs | 46 | 47 | 57 | 46 | 25 |
| N50 (bp) | 98,217 | 98,217 | 66,776 | 73,149 | 245,984 |
| Completeness | 96.0% | 96.0% | 94.4% | 96.0% | 99.6% |
| Contamination | 0.1% | 0.1% | 0.1% | 0.4% | 0.8% |
| Heterogeneity | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 16S recovered | Yes | Partially | Partially | Yes | Yes |
| Number of extracted tRNAs | 36 | 34 | 34 | 37 | 41 |
| Amino acids with tRNA | 20 | 19 | 19 | 20 | 20 |
| Missing tRNA amino acid | – | Ile | Ile | – | – |

*Candidatus* Thiobius zoothamnicola

Milos strain ODIII6

G43    G44    G42    G4

Contiguous traits (shared and *not-shared*)
| | | | |
|---|---|---|---|
| 1. AsN | 6. Fcsd | 11. Nrt | 16. Soe |
| *2. Cas* | *7. Hup* | 12. Pet | *17. T2SS* |
| 3. CytAA3 | *8. Hyd* | *13. Psr* | 18. Ure |
| *4. CytBA3* | *9. LakP* | 14. Pst | 19. Urt |
| 5. CytCBB3 | *10. Lut* | 15. rDsr | |

Coverage    GC content    GC skew

100 000 nt

**FIGURE 2** Representation of the draft genomes of Thiobius strain G43 (left) and ODIII6 (right). The most outer grey solid arcs represent the contigs. ODIII6 contigs are ordered by decreasing length. In Thiobius, information of three additional MAGs was employed to retrieve putative clusters of contiguity that are presented in decreasing length order. The bridging contigs are shown as solid arcs in colours, the rest of their contigs are omitted. GC skew typically reflects the attribution of the contig to the leading or to the lagging DNA strand. The correct ordering of the contigs has not been fully resolved, and this is reflected in the inconsistent GC skew pattern between neighbouring contigs. GC content and coverage are shown in the most inside rings. The location of relevant traits that show contiguity of their constitutive genes is also shown.

predictions (Sachs et al., 2011), the genome of the vertically transmitted ectosymbiont Thiobius was 33% smaller than the one of the free-living bacterium ODIII6, and showed a reduced relative GC content, consistent with the significant positive correlation between GC content and genome size in bacterial genomes (Almpanis et al., 2018). Whether the reduced genome size of the vertically transmitted ectosymbiont is due to any of the mechanisms that are known to affect vertically transmitted endosymbionts, such as those of thiotrophic vesicomyid clams (Kuwahara et al., 2007; Newton et al., 2007) and of marine flatworms *Paracatenula* (Jäckle et al., 2019), remains to be studied.

## 3.3 | Thiobius encodes fewer genes, pathways and functional traits compared to ODIII6

To characterize the potential functional capabilities of Thiobius and ODIII6, their gene complements were predicted with *RASTtk* (Brettin

et al., 2015). The annotation of Thiobius yielded 2486 predicted protein-coding sequences, of which 1494 (60%) received a meaningful automatic functional prediction. In the manual curation process 21 hypothetical genes (18 non-redundant) obtained a putative annotation. ODIII6 had 3452 predicted protein-coding sequences including 2063 (60%) with meaningful functional predictions, and in the manual curation 21 hypothetical genes (19 non-redundant) were functionally annotated. Thiobius contains about 28% less protein-coding sequences than ODIII6. Accordingly, the lower number of protein-coding sequences in Thiobius compared to ODIII6 is consistent with their genome sizes.

We manually selected 46 relevant functional traits involved in key metabolism and additional functions like storage or interactions, of these two SOBs. These traits can range from a single gene to the combination of several metabolic pathways (Tables 2 and S2). Less than half of the traits (19) showed a compact localization of their constitutive genes within contigs (Figure 2). *PathwayTools* inferred

203 *MetaCyc* metabolic pathways for Thiobius, while 39 complete *KEGG* modules were identified. The annotation of ODIII6 resulted in 233 *MetaCyc* pathways and 53 complete *KEGG* modules. Thiobius obtained 31 traits and ODIII6 yielded 39, of which 24 traits were shared (Table S6). Accordingly, Thiobius showed less pathways and relevant traits compared to ODIII6, particularly in those related with oxygen, phosphorus and sulphur.

In order to assess the functional commonalities between Thiobius and ODIII6 we performed an orthology analysis over a background set of 28 additional genomes of species included in the phylogenetic analysis (Table S3). Thiobius and ODIII6 shared 1409 orthogroups (Figure 3). A higher proportion of functional pathways were shared between the two organisms (Figure 3). Most of the genes of both organisms presented an orthologous one-to-one relationship (84% of genes in Thiobius and 90% of genes in ODIII6; Table S7). Overall, the relatively high percentage of shared orthogroups, pathways, modules and traits in Thiobius indicates that its functional capabilities are mainly a subset of those present in ODIII6 (Figure S6).

To further investigate the distribution of functional categories, *eggNOG* was applied to determine the attribution of COG categories (Tatusov et al., 2000). In general, the gene counts of the COG categories followed the genome sizes (Table S8), but there were some exceptions. ODIII6 and Thiobius COG categories yielded high count similarities in the categories 'lipid metabolism' and 'secondary structure'. Nevertheless, this did not imply a fully identical sets of genes, for example, of the orthogroups involved in the 'lipid metabolism' category only a 52% were shared, indicating that different 'non-orthologous' gene sets were recruited to perform same biological tasks in the two different bacteria. The least similar categories were 'cell motility' and 'signal transduction', for which the respective proportions were much lower in Thiobius in relation to its genome size (Table S8). Therefore, the COG functional categories composition reflects the free-living lifestyle of ODIII6, with increased motility representation and a more complex gene regulation that match the requirements of a free-living lifestyle.

## 3.4 | Potential for a mixotrophic lifestyle as an adaptation to the ciliate host

Both Thiobius and ODIII6 show the genomic potential to fix inorganic carbon through the Calvin-Benson-Bassham cycle (Cbb) with the capability to form carboxysomes to concentrate ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO; Badger & Bek, 2008), the key enzyme of the Calvin–Benson cycle responsible for $CO_2$-assimilation. According to the neighbouring genes the RuBisCO type in both Thiobius and ODIII6 is form IAc with a potential functional niche of low $CO_2$ and low to high oxygen concentration (Badger & Bek, 2008). This is in line with previous studies in Thiobius, in which RuBisCO was histochemically detected, carboxysomes were identified by TEM (Bauer-Nebelsick et al., 1996b) and a type IA RuBisCO large subunit sequence was retrieved (Rinke et al., 2009). In addition, carbon fixation in Thiobius was confirmed through tissue

autoradiography and NanoSIMS (Volland et al., 2018). Further, the contraction and expansion behaviour of the host creates a continuously changing abiotic environment for Thiobius ranging from oxygen rich to sulphidic, anoxic conditions (Bright et al., 2014). ODIII6 shows two putative bicarbonate transporter-encoding genes downstream of the carboxysome structural genes operon, consistent with previous reports on other chemoautotrophs (Axen et al., 2014; Scott et al., 2020). ODIII6 further possesses a gene for a beta class carbonic anhydrase not integrated in the carboxysome operon but elsewhere in the genome, which converts bicarbonate to $CO_2$ for carbon fixation (Supuran & Capasso, 2017).

Organic carbon is stored differently in Thiobius compared to ODIII6. While Thiobius has the genetic potential for using polyhydroxyalkanoates (Pha), genes for glycogen (Glg) and cyanophycin synthesis (Cph; storing carbon and nitrogen) were found in ODIII6. In both organisms, the organic carbon is oxidized through the TCA cycle (TcaC), although. Thiobius encodes additionally genes for the glyoxylate cycle pathway (GlC; Cozzone & El-Mansi, 2005). Putative genes encoding for transporters to import acetate (ActP) and lactate (LakP) along with genes encoding for all three components of L-lactate dehydrogenase for lactate utilization (Lut) were also present. Both acetate and lactate could potentially be metabolized by the glyoxylate cycle (El-Mansi et al., 1986; Serafini et al., 2019). While in many organisms (including *Bacillus subtilis*) *lutABC* belongs to the same operon with a lactate permease (Chai et al., 2009), Thiobius possesses a different DctP–TRAP-like transporter (LakP), similar to the one described for *Thermus thermophilus* (Fischer et al., 2010). The presence of these transporters indicates a potential for heterotrophic metabolism in Thiobius. As the ciliate host may potentially be able to switch to an anaerobic metabolism under sulphidic conditions and produce lactate and acetate, similarly to the rumen ciliate *Entodinium caudatum* (Park et al., 2021), it is tempting to speculate that these fermentation products may then be released from the host and taken up by the symbiont, but these anaerobic processes have yet to be studied in this mutualism. No equivalent capabilities were found in the genome of ODIII6 in line with its obligate autotrophic metabolism.

## 3.5 | Oxygen is the only electron acceptor in both bacteria, but ODIII6 is more versatile than Thiobius

According to the genomic potential of their draft genomes, oxygen is the only electron acceptor that Thiobius and ODIII6 can utilize. Genes for nitrate respiration, known in many free-living and symbiotic thiotrophic bacteria living at oxic–anoxic interfaces (De Oliveira et al., 2022; Flood et al., 2015; König et al., 2016; Nunoura et al., 2014; Paredes et al., 2021), were not found. This indicates that both bacteria strongly rely on oxygen both for respiration and for the oxidation of reduced sulphur species to generate energy. Indeed, both genomes encode for a cytochrome $bc_1$ electron transport complex (Pet), and two cytochrome terminal oxidases, that is, $cbb_3$ (CytCBB3), which was shown to have a high affinity for oxygen
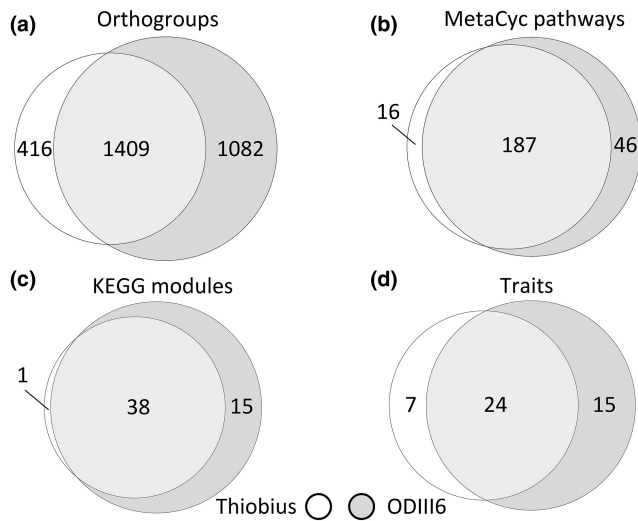
**TABLE 2** Selection of relevant functional traits.

| Id | TraitGroup | TraitName | ShortTraitName | GeneTraitComposition | Thiobius | ODIII6 |
|---|---|---|---|---|---|---|
| 1 | Carbon | Acetate transporter | ActP | +actP | Present | Absent |
| 2 | Carbon | Calvin-Benson-Bassham cycle | Cbb | +((+cbbS+cbbL),cbbL_formII)+pgk+gapA+tpiA+fbaA+fbp+tkt+rpe+prk+sir+rplA+PPi_PFK | Present | Present |
| 3 | Carbon | Glycogen biosynthesis | Glg | +glgC+glgA | Absent | Present |
| 4 | Carbon | Glyoxylate cycle | Glc | +mdh+CS+(acnA,acnB)+icl+aceB | Present | Absent |
| 5 | Carbon | Lactate transporter | LakP | +lakP+dctQ+dctM | Present | Absent |
| 6 | Carbon | Lactate utilization | Lut | +lutA+lutB+lutC | Present | Absent |
| 7 | Carbon | Polyhydroxyalkanoate synthesis | Pha | +pha | Present | Absent |
| 8 | Carbon | TCA cycle | TcaC | +CS+(acnA,acnB)+icd+((+sucA+sucB+lpd),(+korA+korB))+sucD+sucC+sdhA+sdhB+sdhC+sdhD+(fumA,fumC)+(mdh,mqo) | Present | Present |
| 9 | Carbon (and Nitrogen) | Cyanophycin synthesis | Cph | +cph | Absent | Present |
| 10 | Hydrogen | NiFe hydrogenase-based hydrogen oxidation | Hup | +hupS+hupL | Absent | Present |
| 11 | Nitrogen | Ammonium transporter | AmtB | +amtB | Present | Present |
| 12 | Nitrogen | Assimilatory nitrate reduction | AsN | +narB+nirB+nirD | Present | Present |
| 13 | Nitrogen | Incomplete urea cycle | iUcy | +carA+carB+argF+argG+argH | Present | Present |
| 14 | Nitrogen | Nitrate transporter | Nrt | +nrtA+nrtB+nrtC | Present | Present |
| 15 | Nitrogen | Urea transporter | Urt | +urtA+urtB+urtC+urtD+urtE | Present | Present |
| 16 | Nitrogen | Urease mediated urea degradation | Ure | +ureA+ureB+ureC | Present | Present |
| 17 | Oxygen | Cytochrome aa3 based oxygen respiration | CytAA3 | +coxA+coxB+coxC | Present | Present |
| 18 | Oxygen | Cytochrome ba3 based oxygen respiration | CytBA3 | +cbaA+cbaB | Absent | Present |
| 19 | Oxygen | Cytochrome bc1 complex mediated electron transport | Pet | +petA+petB+petC | Present | Present |
| 20 | Oxygen | Cytochrome bd based oxygen respiration | CytBD | +ndhA_to_ndhN+shdA+sdhB+sdhC+cydA+cydB | Absent | Present |
| 21 | Oxygen | Cytochrome cbb3 based oxygen respiration | CytCBB3 | +ccoN+ccoO+ccoP+ccoQ | Present | Present |
| 22 | Phosphorous | High-affinity Na+/Pi symporter | Hnp | +hnp | Absent | Present |
| 23 | Phosphorous | High-affinity phosphate transporter | Pst | +pstA+pstB+pstC+pstS | Present | Present |
| 24 | Phosphorous | Low-affinity phosphate transporter | Pit | +pit | Absent | Present |
| 25 | Phosphorous | Polyphosphate usage | PHK and PHX | PHK, PHX | Present | Present |
| 26 | Sulphur | APR and SAT mediated sulphite oxidation | AprSat | +sat+aprB+aprA-aprM | Present | Present |
| 27 | Sulphur | Reverse dissimilatory sulphate reductase mediated thiosulphate oxidation to sulphite | rDsr | +dsrA+dsrB+dsrC+dsrE+dsrF+dsrK+dsrH+tusA+rhd | Present | Present |

**TABLE 2** (Continued)

| Id | TraitGroup | TraitName | ShortTraitName | GeneTraitComposition | Thiobius | ODIII6 |
|---|---|---|---|---|---|---|
| 28 | Sulphur | Rhodanese mediated thiosulphate disproportionation | Tst | +tst | Absent | Present |
| 29 | Sulphur | Sulphate permease | SulP | +sulP | Absent | Present |
| 30 | Sulphur | Sulphate transporter | CysZ | +cysZ | Present | Present |
| 31 | Sulphur | Sulphydrogenase mediated sulphur reduction | Hyd | +hydA+hydB+hydC+hydD | Absent | Present |
| 32 | Sulphur | Polysulphide reductase | Psr | +psrA+psrB+psrC | Absent | Present |
| 33 | Sulphur | Sulphide dehydrogenase (flavocytochrome C) mediated sulphur oxidation | Fcsd | +fccA+fccB | Present | Present |
| 34 | Sulphur | Sulphide:quinone oxidoreductase mediated sulphur oxidation | Sqr | +sqr | Present | Present |
| 35 | Sulphur | Sulphite-oxidizing enzyme mediated sulphite oxidation | Soe | +soeA+soeB+soeC | Present | Present |
| 36 | Sulphur | Sulphur globules proteins | Sgp | sgpCV1,sgpCV2,sgpA,sgpB,sgpC,sgpD | Present | Present |
| 37 | Sulphur | Sulphur oxygenase reductase mediated sulphur disproportionation | Sor | +sor | Absent | Present |
| 38 | Sulphur | Thiosulphate transporter | YeeE | +yeeE | Absent | Present |
| 39 | Sulphur | Truncated SOX mediated sulphur oxidation from thiosulphate to sulphate | tSox | +soxA+soxB+soxX+soxL | Present | Present |
| 40 | Interactions | CRISPR associated proteins | Cas | +cas1+cas2+((cas3,cas7),cas9,cas10)-cas4-cas5-cas6-cas8-cse1-cse2-csb1-csb2-cmr4-cmr5-cmr6-csy1-csy2-csy3 | Present | Absent |
| 41 | Interactions | Type I secretion system | T1SS | +lapB+lapC+tolC | Absent | Present |
| 42 | Interactions | Type II secretion system | T2SS | -gspC+gspD+gspE+gspF+gspG-gspH+gspI+gspJ+gspK-gspL+gspM-gspN | Present | Present |
| 43 | Interactions | Type V secretion system | T5SS | apeE,fhaC,yadA | Present | Present |
| 44 | Interactions | Type VI secretion system | T6SS | -evpJ+tssA+tssB+tssC+tssD+tssE+tssF+tssG+tssH+tssI+tssJ+tssK+tssL+tssM | Present | Absent |
| 45 | Motility | Flagellum | Fla | +filF+filI+filN+filP+filQ+filR+flhB+flhA+flgB+flgC+filE | Absent | Present |
| 46 | Motility | Type IV pilus | T4P | +pilE+pilV+pilB+pilC+pilO+pilQ+pilM+pilN+pilP+pilT-pilD | Present | Present |

Note: '+' indicates that the following gene is required; '-' indicates that the following gene is not required, ',' indicates interchangeability; brackets are used for grouping; 'ndhA_to_ndhN' stands for 14 genes.

**(a)** Orthogroups

416 | 1409 | 1082

**(b)** MetaCyc pathways

16 | 187 | 46

**(c)** KEGG modules

1 | 38 | 15

**(d)** Traits

7 | 24 | 15

Thiobius ○ ● ODIII6

**FIGURE 3** Euler diagrams of different levels of granularity showing shared and unique genetic potential capabilities of Thiobius and ODIII6. The finer granularity level is the genes from the orthogroups (a). Two metabolic pathway databases were employed: MetaCyc (b), and KEGG modules (c). The coarser granularity level are the traits (d).

in *Bradyrhizobium japonicum* (Pitcher & Watmough, 2004), and aa₃ (CytAA3), which belongs to the Class A of oxidases with lower apparent oxygen affinities (Han et al., 2011). This indicates the ability to utilize oxygen at various concentrations. Interestingly, the genome of ODIII6 contains two copies of the genes encoding cbb₃, which is unusual and could be an additional adaptation to optimize oxygen utilization under varying concentrations, as was shown for *Pseudomonas aeruginosa* (Comolli & Donohue, 2004). Further, ODIII6 possesses two more high-affinity terminal oxidases, cytochrome bd (CytBD)- and ba₃ (CytBA3)-encoding genes, which points to a higher versatility under a broader range of oxygen regimes than Thiobius. In contrast, Thiobius lives on a ciliate host that can position itself

in microhabitats with optimal oxygen concentrations, which potentially renders the ability to express multiple terminal oxidases unnecessary.

## 3.6 | Both organisms use the oxidation of reduced sulphur compounds to generate energy

The energy fuelling processes for carbon fixation in Thiobius and ODIII6 comes from the oxidation of reduced sulphur species using oxygen as terminal electron acceptor. Sulphide oxidation to elemental sulphur occurs in both bacteria through two possible pathways, sulphide dehydrogenase (Fcsd; flavocytochrome C, Sorokin et al., 1998), and sulphide:quinone oxidoreductase (Sqr). ODIII6 has a type VI Sqr and Thiobius has type I and type VI Sqr (Dahl, 2017). The elemental sulphur formed by sulphide oxidation is stored in sulphur globules that are enveloped by proteins (Dahl, 2017) identified in both genomes (Sgp). Indeed, transmission electron micrographs (Bauer-Nebelsick et al., 1996b) and Raman microspectrometry previously revealed membrane bound elemental sulphur vesicles in Thiobius (Maurin et al., 2010) used to store sulphur under sulphidic conditions and oxidize it further during oxic conditions (Volland et al., 2018). In ODIII6 cultures, the formation of sulphur globules attached to the cell could also be observed if thiosulphate was provided (Stefan Sievert personal observation, 2022).

Thiosulphate is potentially oxidized in both organisms to sulphate and elemental sulphur through the truncated Sox pathway (tSox, Welte et al., 2009, Dahl, 2020), with the possible involvement of *SoxL* (Weissgerber et al., 2011). For both organisms, elemental sulphur is oxidized to sulphite in the cytoplasm by the reverse dissimilatory sulphate reductase pathway (rDsr; Dahl, 2015, Gregersen et al., 2011, Hensen et al., 2006), and sulphite is oxidized to sulphate either by the adenylylsulphate reductase and the sulphate adenylyltransferase (AprSat), or by the sulphite-oxidizing enzyme (Soe;

**FIGURE 4** Major metabolic capabilities found in the draft genomes of (a) *Candidatus* Thiobius zoothamnicola strain BelizeG43, and (b) strain Milos ODIII6. Chosen relevant potential functional capabilities are shown (for simplicity not all transporters are depicted in the figure). Predicted and functionally annotated genes are grouped in metabolic pathways. Functional traits are features such as single metabolic pathways or composites of them that affect the organism fitness. Structural features such as transporters or secretion systems, and storage capabilities are also considered as traits. Trait labels are generally in white fonts and in vertical orientation, except for the motility and interactions on the left. Compound labels are horizontally oriented. Storage compartments are indicated with ellipses. ActP, acetate transporter; AmtB, ammonium transporter; AprSat, sulphate adenylyltransferase and adenylylsulphate reductase sulphite oxidation; AsN, assimilatory nitrate reduction; Cbb, Calvin-Benson-Basham cycle; Cph, cyanophycin biosynthesis; CysZ, sulphate transporter; CytAA3, cytochrome aa₃ based oxygen respiration; CytBA3, cytochrome ba₃ based oxygen respiration; CytBD, cytochrome bd based oxygen respiration; CytCBB3, cytochrome cbb₃ based oxygen respiration; Fcsd, flavocytochrome c sulphide dehydrogenase sulphide oxidation; GlC, glyoxylate cycle; Glg, glycogen biosynthesis; Hnp, high-affinity sodium-phosphate symporter; Hup, putative hydrogen oxidation; Hyd, sulphydrogenase elemental sulphur oxidation; iUcy, incomplete urea cycle lacking last step arginase gene; LakP, lactate transporter; Lut, lactate utilization; Nrt, nitrate transporter; Pet, cytochrome bc₁ complex mediated electron transport chain; Pha; polyhydroxyalkanoate synthesis; PHK, polyphosphate kinase; PHX, exopolyphosphatase; Pit, low-affinity phosphate transporter; Psr, polysulphide reductase; Pst, high-affinity phosphate transporter; rDsr, reverse dissimilatory sulphate reductase mediated sulphur oxidation; Sgp, sulphur globule proteins; Soe, sulphite-oxidation enzyme sulphite oxidation; Sor, Sulphur oxygenase reductase mediated sulphur oxidation; Sqr, sulphide:quinone oxidoreductase sulphide oxidation; SulP, sulphate permease; T1SS, type I secretion system; T2SS, type II secretion system; T5SS, type V secretion system; T6SS, type VI secretion system; T4P, type IV pilus; TcaC, TCA cycle; tSox, truncated Sox mediated sulphur oxidation; Tst, thiosulphate disproportionation; Ure, Urease mediated urea degradation; Urt, urea transporter; YeeE, thiosulphate transporter. The hexagon represents a carboxysome.
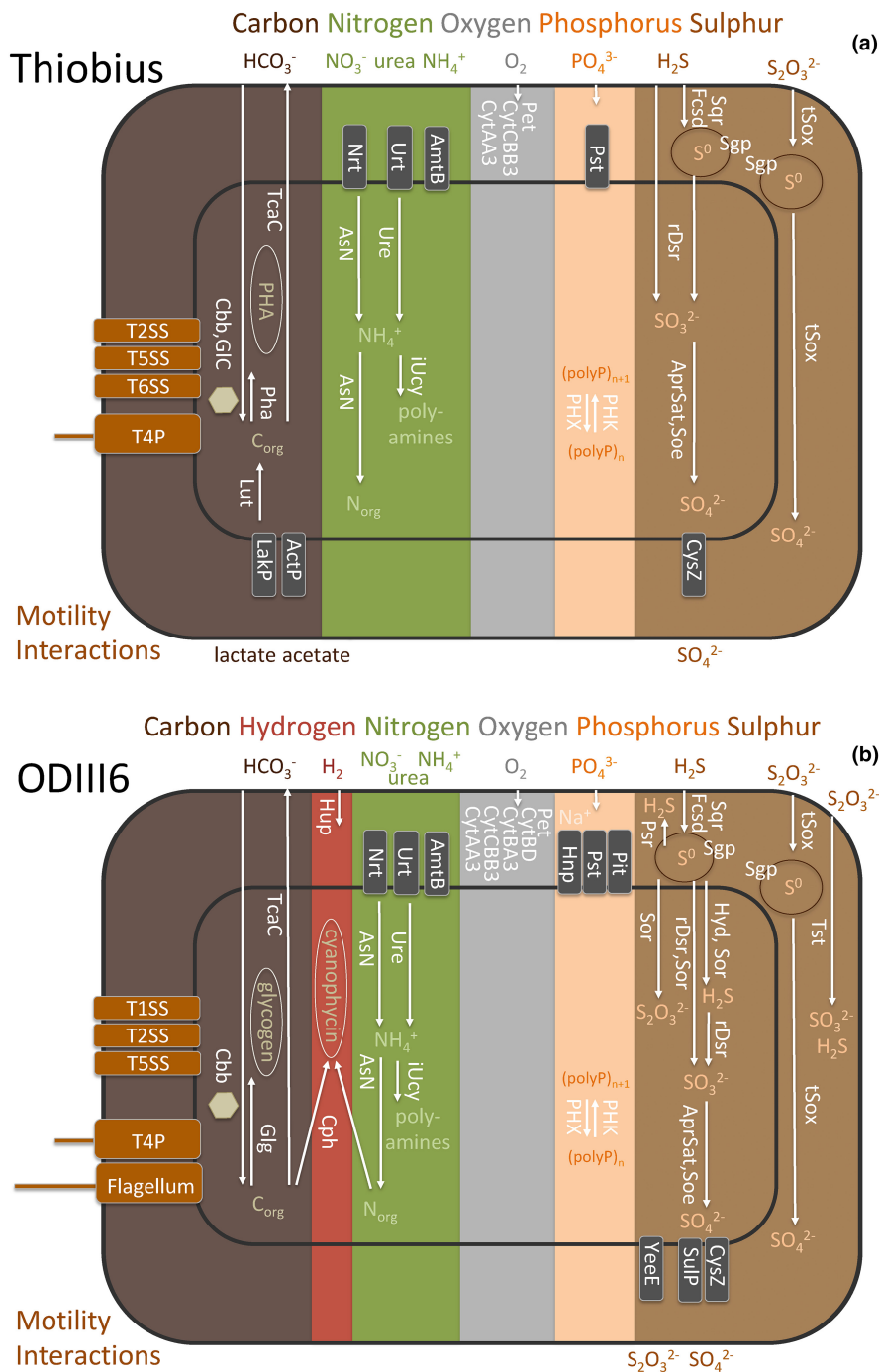
Dahl, 2017). Gene sequences for the alpha and beta subunits of the reverse-type dissimilatory sulphite reductase (*dsrAB*) and for the alpha subunit of the adenylylsulphate reductase (*aprA*) were previously reported in Thiobius (Rinke et al., 2009). Finally, the putative sulphate transporter CysZ exports the sulphate to the periplasm (Figure 4a; Hryniewicz et al., 1990).

In contrast to Thiobius, ODIII6 has additional sulphur-metabolizing capabilities: the periplasmic disproportionation of thiosulphate by a rhodanese-like sulphurtransferase to sulphide and sulphite (Tst; Deckert et al., 1998); elemental sulphur reduction to sulphide in the periplasm by a putative polysulphide reductase (Psr; De Oliveira et al., 2022), that might also be involved in oxidation as

suggested for *Allochromatium vinosum* (Weissgerber et al., 2013); cytoplasmic disproportionation of elemental sulphur to thiosulphate, sulphite and sulphide by the sulphur oxygenase reductase (Sor; Janosch et al., 2015), as suggested for *Thioalkalivibrio paradoxus* (Rühl et al., 2017); reduction of elemental sulphur to sulphide coupled to the oxidation of hydrogen by the sulphydrogenase (Hyd; Ng et al., 2000); and sulphate exportation to the periplasm by the sulphate permease SulP (Figure 4b; Aguilar-Barajas et al., 2011). A thiosulphate transporter gene (YeeE; Tanaka et al., 2020) is also found in the genome of ODIII6.

Although many sulphur oxidation traits are shared, we observe a higher versatility in ODIII6 than in Thiobius. In addition, the presence

of [NiFe] hygrogenase Hup genes (Vignais et al., 2001) in ODIII6 may indicate the potential of hydrogen oxidation. However, it was recently found in *Candidatus* Endoriftia persephone that *hup* genes present in its genome were not involved in hydrogen oxidation but may instead facilitate intracellular redox homeostasis (Mitchell et al., 2019). The two subunits L and S in ODIII6 show 75% and 71% amino acid identity with their *Candidatus* Endoriftia persephone orthologues.

## 3.7 | Similarities in nitrogen and phosphorous metabolism

Both organisms show similar potential capabilities in nitrogen metabolism. Nitrate is imported into the cytoplasm through the nitrate transporter Nrt (Maeda et al., 2019), and is reduced to ammonium in the first step of assimilatory nitrate reduction, and from there assimilated as organic nitrogen in form of biomolecules (AsN; Takai, 2019). In addition, the ammonium can be imported into the cytoplasm by the ammonium transporter AmtB (Wang et al., 2012) further fuelling assimilation. Urea is acquired by the urea transporter Urt and oxidized to ammonium by the urease (Ure; Bossé et al., 2001). An incomplete urea cycle lacking arginase is also present (iUcy; De Oliveira et al., 2022). Overall, urea as well as ammonium are well-known nitrogen waste products of ciliates (Caron & Goldman, 1990), and may serve as byproducts the host provides to the symbiont. Urea was shown to be a source of incorporated nitrogen for bacteria in intertidal sediments (Veuger & Middelburg, 2007). Whether urea is utilized by ODIII6 in its natural environment remains to be studied.

Phosphorus is a limiting nutrient in many marine environments, present in its inorganic dissolved fraction generally as orthophosphate (Paytan & McLaughlin, 2007). Both organisms encode the genes for a high-affinity phosphate transporter (Pst), and additionally ODIII6 possesses genes for two other transporters: a high-affinity $Na^+/P_i$ symporter (Hnp) and a low-affinity phosphate transporter (Pit). Once phosphate is incorporated into the cells, both organisms also show the potential for polymerizing it into polyphosphate and hydrolysing back to inorganic phosphates (PHKandPHX), using it as energy storage (Achbergerová & Nahálka, 2011).

## 3.8 | Interaction and motility in both organisms

Both Thiobius and ODIII6 have a repertoire of genes to interact with other organisms and with the environment. They share genes for the type II (T2SS) and type V (T5SS) secretion systems. Thiobius additionally has genes for the type VI secretion system (T6SS; Kapitein & Mogk, 2013) known to function in host interaction (Hachani et al., 2016) and CRISPR-Cas proteins (Cas), while ODIII6 has genes for the type I secretion system (T1SS). It remains to be studied how both bacteria use these traits in their natural environment and whether they may help Thiobius to interact with its host.

Both organisms further possess genes for type IV pilus potentially involved in twitching motility (T4P; Ayers et al., 2010), and

additionally ODIII6 genome contains several loci for the biosynthetic genes encoding for a flagellum (Fla). Indeed, ODIII6 was observed to be motile in cultures, however, loses its motility after a longer period of cultivation (Sievert pers. obs.). This suggests that the flagellum may no longer be expressed in ODIII6 if constant, favourable conditions render motility unnecessary. ODIII6 additionally shows genes for photolyase DNA protection against UV radiation, and repair (Sancar et al., 1987).

## 3.9 | Mobile genetic elements are less abundant in Thiobius than in ODIII6

Bacteria can experience horizontal gene transfer through mobile genetic elements such as phages, plasmids, transposons and insertion sequences, also referred to as the mobilome (Frost et al., 2005). Free-living bacteria like ODIII6 are more likely to be exposed to novel gene pools than symbionts (Newton & Bordenstein, 2011). In addition, vertically transmitted symbionts, such as Thiobius experience population bottlenecks as each swarmer is covered with relatively few symbionts that grow to cover the new colony (Bauer-Nebelsick et al., 1996a, 1996b). Accordingly, we hypothesized a smaller mobilome in the obligate symbiont Thiobius than in the free-living ODIII6. Indeed, *RASTtk* annotation of Thiobius revealed three genes attributed to phages and 18 genes attributed to other mobile genetic elements, while ODIII6 has two genes attributed to phages and 58 genes attributed to other mobile genetic elements (Table S9). Roughly 60% of these mobile elements were located at the extremes of the contigs, consistent with their disruptive effect on the assembly processes. These results point to a smaller mobilome in the host-associated Thiobius than in the free-living ODIII6.

## 4 | CONCLUSIONS

The phylogenetic relationship of the ectosymbiont Thiobius and the strain Milos ODIII6 is confirmed through 16S rRNA phylogeny and average amino acid identity and serves as baseline to compare the genomes of these two bacteria with very different lifestyles. In agreement with theoretical predictions, but hardly studied in ectosymbiotic bacteria, Thiobius' genome is smaller than that of its free-living relative ODIII6. The characterization at the levels of genes, metabolic pathways and traits reveals that Thiobius and ODIII6 share a large proportion of their genetic repertoire and metabolic capabilities. The lower number of lineage-specific metabolic pathways and relevant traits in Thiobius compared to ODIII6 may point to a more stable environment provided by the host, requiring less versatility. This may have led to a loss of genetic potential in Thiobius and/or gain in ODIII6. In comparison with Thiobius, ODIII6 shows a larger functional repertoire, in particular for its energy metabolism regarding the utilization of sulphur, oxygen and hydrogen, consistent with the requirements for a free-living bacterium to live under the fluctuating conditions of the hydrothermal vent environment. Thiobius, however, shows potential

for heterotrophic metabolism, which may be fuelled by byproducts from the host and thus might represent a remarkable adaptation to the life style of its protist host. In contrast to reduced genomes of vertically transmitted, thiotrophic endosymbionts like those of vesicomyid clams or catenulid plathylhelmints that experience no microbial competition and little potential for horizontal gene transfer inside host organs and cells, Thiobius, as an ectosymbiont, faces potential competitive interactions and viral attacks similar to free-living bacteria such as ODIII6, which is reflected in its capacity to interact with the environment. In the future, transcriptome evidence and other omics analyses in conjunction with physiological experiments can elaborate on the intricacy of these functional capabilities.

## AUTHOR CONTRIBUTIONS

S.E.-H., C.K., S.M.S. and M.B. designed the research. S.E.-H. performed the DNA extraction and preparation, and synteny analyses (together with T.W.) for Thiobius, the final versions of genome annotation for Thiobius and ODIII6, the phylogenies, the orthology analyses (together with A.L.de.O.) and reassembled ODIII6 with SPAdes. F.S. and L.S. performed the assembly and binning of Thiobius. M.H. provided valuable input for DNA extraction, assembly, binning and annotation of Thiobius, S.E.-H., A.S. and M.B. manually curated the functional inference results. S.M.S. isolated ODIII6 from the environment, helped C.K. to grow ODIII6 for DNA extraction, and provided input on gene annotation and pathway inference. C.K. extracted DNA of ODIII6, carried out an initial comparison of the two genomes and their genome contents using PathwayTools and KEGG modules, and provided a summary as part of a MSc thesis. S.E.-H. wrote the initial draft of the manuscript and C.K., S.M.S. and M.B contributed considerably to the writing. All authors commented and approved the final version of the manuscript.

## ACKNOWLEDGEMENTS

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Thiobius and ODIII6 Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank under the BioProjects PRJNA906600 (Thiobius) and PRJNA910104 (ODIII6), with the following accessions (G43) JAPQLC000000000; (G42) JAPUCE000000000; (G44) JAPUCF000000000; (G4) JAPUCG000000000 and (ODIII6) JAPTHR000000000. The Illumina reads are also accessible in the SRA associated entries. All other data and code files are available from the Dryad repository (https://doi.org/10.5061/dryad.wh70rxwrq).

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## REFERENCES

Abby, S. S., Cury, J., Guglielmini, J., Néron, B., Touchon, M., & Rocha, E. P. C. (2016). Identification of protein secretion systems in bacterial genomes. *Scientific Reports*, 6, 23080. https://doi.org/10.1038/srep23080

Achbergerová, L., & Nahálka, J. (2011). Polyphosphate–An ancient energy source and active metabolic regulator. *Microbial Cell Factories*, 10(1), 1–14. https://doi.org/10.1186/1475-2859-10-63

Aguilar-Barajas, E., Díaz-Pérez, C., Ramírez-Díaz, M. I., Riveros-Rosas, H., & Cervantes, C. (2011). Bacterial transport of sulfate, molybdate, and related oxyanions. *Biometals*, 24, 687–707. https://doi.org/10.1007/s10534-011-9421-x

Almpanis, A., Swain, M., Gatherer, D., & McEwan, N. (2018). Correlation between bacterial G+C content, genome size and the G+C content of associated plasmids and bacteriophages. *Microbial Genomics*, 4(4), e000168. https://doi.org/10.1099/mgen.0.000168

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Axen, S. D., Erbilgin, O., & Kerfeld, C. A. (2014). A taxonomy of bacterial microcompartment loci constructed by a novel scoring method. *PLoS Computational Biology*, 10(10), e1003898. https://doi.org/10.1371/journal.pcbi.1003898

Ayers, M., Howell, P. L., & Burrows, L. L. (2010). Architecture of the type II secretion and type IV pilus machineries. *Future Microbiology*, 5(8), 1203–1218. https://doi.org/10.2217/fmb.10.76

Badger, M. R., & Bek, E. J. (2008). Multiple RuBisCO forms in proteobacteria: Their functional significance in relation to $CO_2$ acquisition by the CBB cycle. *Journal of Experimental Botany*, 59(7), 1525–1541. https://doi.org/10.1093/jxb/erm297

Bauer-Nebelsick, M., Bardele, C. F., & Ott, J. A. (1996a). Redescription of *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg, 1838 (Oligohymenophora, Peritrichida), a ciliate with ectosymbiotic, chemoautotrophic bacteria. *European Journal of Protistology*, 32, 18–30. https://doi.org/10.1016/S0932-4739(96)80036-8

Bauer-Nebelsick, M., Bardele, C. F., & Ott, J. A. (1996b). Electron microscopic studies on *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg 1838 (Oligohymenophora, Peritrichida), a ciliate with ectosymbiotic, chemoautotrophic bacteria. *European Journal of Protistology*, 32, 202–215. https://doi.org/10.1016/S0932-4739(96)80020-4

Bengtsson-Palme, J., Hartmann, M., Eriksson, K. M., Pal, C., Thorell, K., Larsson, S. G. J., & Nilsson, R. H. (2015). Metaxa2: Improved identification and taxonomic classification of small and large subunit

rRNA in metagenomic data. *Molecular Ecology Resources*, *15*, 1403–1414. https://doi.org/10.1111/1755-0998.12399

Bobay, L. M., & Ochman, H. (2017). The evolution of bacterial genome architecture. *Frontiers in Genetics*, *8*, 72. https://doi.org/10.3389/fgene.2017.00072

Bossé, J. T., Gilmour, H. D., & MacInnes, J. I. (2001). Novel genes affecting urease acivity in *Actinobacillus pleuropneumoniae*. *Journal of Bacteriology*, *183*(4), 1242–1247. https://doi.org/10.1128/JB.183.4.1242-1247.2001

Bowers, R. M., Kyrpides, N. C., Stepanauskas, R., Harmon-Smith, M., Doud, D., Reddy, T. B. K., Schulz, F., Jarett, J., Rivers, A. R., Eloe-Fadrosh, E. A., Tringe, S. G., Ivanova, N. N., Copeland, A., Clum, A., Becraft, E. D., Malmstrom, R. R., Birren, B., Podar, M., Bork, P., ... Woyke, T. (2017). Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nature Biotechnology*, *35*(8), 725–731. https://doi.org/10.1038/nbt.3893

Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., Olson, R., Overbeek, R., Parrello, B., Pusch, G. D., Shukla, M., Thomason, I. I. I. J. A., Stevens, R., Vonstein, V., Wattam, A. R., & Xia, F. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*, *5*, 8365. https://doi.org/10.1038/srep08365

Bright, M., Espada-Hinojosa, S., Lagkouvardos, I., & Volland, J.-M. (2014). The giant ciliate *Zoothamnium niveum* and its thiotrophic epibiont *Candidatus* Thiobios zoothamnicoli: A model system to study inter-species cooperation. *Frontiers in Microbiology*, *5*, 1–13. https://doi.org/10.3389/fmicb.2014.00145

Cambon-Bonavita, M. A., Aubé, J., Cueff-Gauchard, V., & Reveillaud, J. (2021). Niche partitioning in the *Rimicaris exoculata* holobiont: The case of the first symbiotic Zetaproteobacteria. *Microbiome*, *9*(1), 1–16. https://doi.org/10.1186/s40168-021-01045-6

Capella-Gutierrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). triMAl: A tool for automated alignment trimming in large phylogenetic analyses. *Bioinformatics*, *25*(15), 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Caron, D. A., & Goldman, J. C. (1990). Protozoan nutrient regeneration. In G. M. Capriulo (Ed.), *Ecology of marine protozoa* (pp. 283–306). Oxford University Press.

Caspi, R., Foerster, H., Fulcher, C. A., Kaipa, P., Krummenacker, M., Latendresse, M., Mueller, L. A., Ong, Q., Paley, S., Subhraveti, P., Weaver, D. S., & Karp, P. D. (2008). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Research*, *36*(Database issue), D471–D480. https://doi.org/10.1093/nar/gkv1164

Cavanaugh, C. M., McKiness, Z. P., Newton, I. L. G., & Stewart, F. J. (2006). Marine chemosynthetic symbioses. In *The prokaryotes* (Vol. 1, pp. 475–507). Springer.

Chai, Y., Kolter, R., & Losick, R. (2009). A widely conserved gene cluster required for lactate utilization in *Bacillus subtilis* and its involvement in biofilm formation. *Journal of Bacteriology*, *191*(8), 2423–2430. https://doi.org/10.1128/JB.01464-08

Chain, P. S. G., Grafham, D. V., Fulton, R. S., Fitzgerald, M. G., Hostetler, J., Muzny, D., Ali, J., Birren, B., Bruce, D. C., Buhay, C., Cole, J. R., Ding, Y., Dugan, S., Field, D., Garrity, G. M., Gibbs, R., Graves, T., Han, C. S., Harrison, S. H., ... Detter, J. C. (2009). Genome project standards in a new era of sequencing. *Science*, *326*, 236–237. https://doi.org/10.1126/science.1180614

Comolli, J. C., & Donohue, T. J. (2004). Differences in two *Pseudomonas aeruginosa* Cbb3 cytochrome oxidases: Two distinct *P. Aeruginosa* Cbb3 oxidases. *Molecular Microbiology*, *51*(4), 1193–1203. https://doi.org/10.1046/j.1365-2958.2003.03904.x

Cozzone, A. J., & El-Mansi, M. (2005). Control of isocitrate dehydrogenase catalytic activity by protein phosphorylation in *Escherichia coli*. *Journal of Molecular Microbiology and Biotechnology*, *9*, 132–146. https://doi.org/10.1159/000089642

Dahl, C. (2015). Cytoplasmic sulfur trafficking in sulfur-oxidizing prokaryotes. *IUBMB Life*, *67*, 268–274. https://doi.org/10.1002/iub.1371

Dahl, C. (2017). Sulfur metabolism in phototrophic bacteria. In P. C. Hallenbeck (Ed.), *Modern topics in the phototrophic prokaryotes: Metabolism, bioenergetics, and omics* (pp. 27–66). Springer International Publishing.

Dahl, C. (2020). A biochemical view on the biological sulfur cycle. In N. L. Piet (Ed.), *Environmental technologies to treat Sulphur pollution: Principles and engineering* (pp. 55–96). IWA Publishing. https://doi.org/10.2166/9781789060966_0055

Darling, A. E., Mau, B., & Perna, N. T. (2010). ProgressiveMauve: Multiple genome alignment with gene gain, loss and rearrangement. *PLoS One*, *5*, e11147. https://doi.org/10.1371/journal.pone.0011147

Dattagupta, S., Schaperdoth, I., Montanari, A., Mariani, S., Kita, N., Valley, J. W., & Macalady, J. L. (2009). A novel symbiosis between chemoautotrophic bacteria and a freshwater cave amphipod. *The ISME Journal*, *3*, 935–943. https://doi.org/10.1038/ismej.2009.34

De Oliveira, A.-L., Srivastava, A., Espada-Hinojosa, S., & Bright, M. (2022). The complete and closed genome of the facultative generalist *Candidatus* Endoriftia persephone from deep-sea hydrothermal vents. *Molecular Ecology Resources*, *22*(8), 3106–3123. https://doi.org/10.1111/1755-0998.13668

Deckert, G., Warren, P. V., Gaasterland, T., Young, W. G., Lenox, A. L., Graham, D. E., Overbeek, R., Snead, M., Keller, M., Aujay, M., Huberk, R., Feldman, R., Short, J., Olsen, G., & Swanson, R. V. (1998). The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. *Nature*, *392*(6674), 353–358. https://doi.org/10.1038/32831

Distel, D., Altamia, M. A., Lin, Z., & Haygood, M. G. (2017). Discovery of chemoautotrophic symbiosis in the giant shipworm *Kuphus polythalamia* (Bivalvia: Teredinidae) extends wooden-steps theory. *Proceedings of the National Academy of Sciences*, *114*, E3652–E3658. https://doi.org/10.1073/pnas.1620470114

Dubilier, N., Bergin, C., & Lott, C. (2008). Symbiotic diversity in marine animals: The art of harnessing chemosynthesis. *Nature Reviews Microbiology*, *6*, 725–740. https://doi.org/10.1038/nrmicro1992

Ellers, J., Kiers, E. T., Currie, C. R., McDonald, B. R., & Visser, B. (2012). Ecological interactions drive evolutionary loss of traits. *Ecology Letters*, *15*(10), 1071–1082. https://doi.org/10.1111/j.1461-0248.2012.01830.x

El-Mansi, E. M. T., Nimmo, H. G., & Holms, W. H. (1986). Pyruvate metabolism and the phosphorylation state of isocitrate dehydrogenase in *Escherichia coli*. *Journal of General Microbiology*, *132*(3), 797–806. https://doi.org/10.1099/00221287-132-3-797

Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, *20*, 238. https://doi.org/10.1186/s13059-019-1832-y

Farris, J. S. (1970). Methods for computing Wagner trees. *Systematic Biology*, *19*(1), 83–92. https://doi.org/10.1093/sysbio/19.1.83

Fauré-Fremiet, E. (1950). Caulobactéries épizoiques associées aux Centrophorella (ciliés holotriches). *Bulletin de la Société Zoologique de France*, *75*, 134–137.

Faure-Fremiet, E. (1951). The marine sand-dwelling ciliates of Cape Cod. *Biological Bulletin*, *100*, 59–70. https://doi.org/10.2307/1538541

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, *17*, 368–376. https://doi.org/10.1007/bf01734359

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*, 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fenchel, T., & Finlay, B. J. (1989). Kentrophoros: A mouthless ciliate with a symbiotic kitchen garden. *Ophelia*, *30*(2), 75–93.

Finlay, B., & Fenchel, T. (1989). Everlasting picnic for protozoa. *New Scientist*, 123(1671), 66–69.

Fischer, M., Zhang, Q. Y., Hubbard, R. E., & Thomas, G. H. (2010). Caught in a TRAP: Substrate-binding proteins in secondary transport. *Trends in Microbiology*, 18(10), 471–478. https://doi.org/10.1016/j.tim.2010.06.009

Flood, B. E., Jones, D. S., & Bailey, J. V. (2015). Complete genome sequence of *Sedimenticola thiotaurini* strain SIP-G1, a polyphosphate- and poly-hydroxyalkanoate-accumulating sulfur-oxidizing gammaproteobacterium isolated from salt marsh sediments. *Genome Announcements*, 3(3), e00671. https://doi.org/10.1128/genomeA.00671-15

Fokin, S. I., & Serra, V. (2022). Bacterial symbiosis in ciliates (Alveolata, Ciliophora): Roads traveled and those still to be taken. *Journal of Eukaryotic Microbiology*, 69(5), e12886. https://doi.org/10.1111/jeu.12886

Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: The agents of open source evolution. *Nature Reviews Microbiology*, 3(9), 722–732. https://doi.org/10.1038/nrmicro1235

Green, J. L., Bohannan, B. J. M., & Whitaker, R. J. (2008). Microbial biogeography: From taxonomy to traits. *Science*, 320, 1039–1043. https://doi.org/10.1126/science.1153475

Gregersen, L. H., Bryant, D. A., & Frigaard, N.-U. (2011). Mechanisms and evolution of oxidative sulfur metabolism in green sulfur bacteria. *Frontiers in Microbiology*, 2, 116. https://doi.org/10.3389/fmicb.2011.00116

Grimonprez, A., Molza, A., Laurent, M. C., Mansot, J. L., & Gros, O. (2018). Thioautotrophic ectosymbiosis in Pseudovorticella sp., a peritrich ciliate species colonizing wood falls in marine mangrove. *European Journal of Protistology*, 62, 43–55. https://doi.org/10.1016/j.ejop.2017.11.002

Grzymski, J. J., Murray, A. E., Campbell, B. J., Kaplarevic, M., Gao, G. R., Lee, C., Daniel, R., Ghadiri, A., Feldman, R. A., & Cary, S. C. (2008). Metagenome analysis of an extreme microbial symbiosis reveals eurythermal adaptation and metabolic flexibility. *Proceedings of the National Academy of Sciences*, 105(45), 17516–17521. https://doi.org/10.1073/pnas.0802782105

Hachani, A., Wood, T. E., & Filloux, A. (2016). Type VI secretion and anti-host effectors. *Current Opinion in Microbiology*, 29, 81–93. https://doi.org/10.1016/j.mib.2015.11.006

Han, H., Hemp, J., Pace, L. A., Ouyang, H., Ganesan, K., Roh, J. H., Daldal, F., Blanke, S. R., & Gennis, R. B. (2011). Adaptation of aerobic respiration to low $O_2$ environments. *Proceedings of the National Academy of Sciences*, 108(34), 14109–14114. https://doi.org/10.1073/pnas.1018958108

Hastings, W. K. (1970). Monte Carlo sampling methods using Markov chains and their applications. *Biometrika*, 57(1), 97–109. https://doi.org/10.2307/2334940

Hemprich, F. W., & Ehrenberg, C. G. (1829). *Symbolae Physicae. Evertebrata. I. Protozoa*. Abhandlungen der Akademie der Wissenchaften zu Berlin.

Hemprich, F. W., & Ehrenberg, C. G. (1831). *Evertebrata I. Phytozoa. Simbolae physicae*. Abhandlungen der Akademie der Wissenchaften zu Berlin.

Hensen, D., Sperling, D., Trüper, H. G., Brune, D. C., & Dahl, C. (2006). Thiosulphate oxidation in the phototrophic Sulphur bacterium *Allochromatium vinosum*. *Molecular Microbiology*, 62, 794–810. https://doi.org/10.1111/j.1365-2958.2006.05408.x

Hryniewicz, M., Sirko, A., Pałucha, A., Böck, A., & Hulanicka, D. (1990). Sulfate and thiosulfate transport in *Escherichia coli* K-12: Identification of a gene encoding a novel protein involved in thiosulfate binding. *Journal of Bacteriology*, 172(6), 3358–3366. https://doi.org/10.1128/jb.172.6.3358-3366.1990

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., von Mering, C., & Bork, P. (2017). Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Molecular Biology and Evolution*, 34(8), 2115–2122. https://doi.org/10.1093/molbev/msx148

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., Mende, D. R., Letunic, I., Rattei, T., Jensen, L. J., von Mering, C., & Bork, P. (2019). eggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research*, 47(D1), D309–D314. https://doi.org/10.1093/nar/gky1085

Husnik, F., Tashyreva, D., Boscaro, V., George, E. E., Lukeš, J., & Keeling, P. J. (2021). Bacterial and archaeal symbioses with protists. *Current Biology*, 31(13), R862–R877. https://doi.org/10.1016/j.cub.2021.05.049

Jäckle, O., Seah, B., Tietjen, M., Leisch, N., Liebeke, M., Kleiner, M., Berg, J. S., & Gruber-Vodicka, H. R. (2019). Chemosynthetic symbiont with a drastically reduced genome serves as primary energy storage in the marine flatworm Paracatenula. *Proceedings of the National Academy of Sciences*, 116, 8505–8514. https://doi.org/10.1073/pnas.1818995116

Janosch, C., Remonsellez, F., Sand, W., & Vera, M. (2015). Sulfur oxygenase reductase (sor) in the moderately thermoacidophilic leaching bacteria: Studies in *Sulfobacillus thermosulfidooxidans* and *Acidithiobacillus caldus*. *Microorganisms*, 3, 707–724. https://doi.org/10.3390/microorganisms3040707

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 28, 27–30. https://doi.org/10.1093/nar/28.1.27

Kang, D. D., Froula, J., Egan, R., & Wang, Z. (2015). MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. *PeerJ*, 3, e1165. https://doi.org/10.7717/peerj.1165

Kapitein, N., & Mogk, A. (2013). Deadly syringes: Type VI secretion system activities in pathogenicity and interbacterial competition. *Current Opinion in Microbiology*, 16, 52–58. https://doi.org/10.1016/j.mib.2012.11.009

Karaoz, U., & Brodie, E. L. (2022). MicroTrait: A toolset for a trait-based representation of microbial genomes. *Frontiers in Bioinformatics*, 2, 918853. https://doi.org/10.3389/fbinf.2022.918853

Karp, P. D., Paley, S., & Romero, P. (2002). The pathway tools software. *Bioinformatics*, 18(suppl 1), S225–S232. https://doi.org/10.1093/bioinformatics/18.suppl_1.s225

Karp, P. D., Paley, S. M., Krummenacker, M., Latendresse, M., Dale, J. M., Lee, T. J., Kaipa, P., Gilham, F., Spaulding, A., Popescu, L., Altman, T., Paulsen, I., Keseler, I. M., & Caspi, R. (2010). Pathway tools version 13.0: Integrated software for pathway/genome informatics and systems biology. *Briefings in Bioinformatics*, 11, 40–79. https://doi.org/10.1093/bib/bbp043

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kim, M., Oh, H.-S., Park, S.-C., & Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 64(2), 346–351. https://doi.org/10.1099/ijs.0.059774-0

König, S., Gros, O., Heiden, S. E., Hinzke, T., Thürmer, A., Poehlein, A., Meyer, S., Vatin, M., Mbéguié-A-Mbéguié, D., Tocny, J., Ponnudurai, R., Daniel, R., Becher, D., Schweder, T., & Markert, S. (2016). Nitrogen fixation in a chemoautotrophic lucinid symbiosis. *Nature Microbiology*, 2(1), 16193. https://doi.org/10.1038/nmicrobiol.2016.193

Konstantinidis, K. T., & Tiedje, J. M. (2005). Towards a genome-based taxonomy for prokaryotes. *Journal of Bacteriology*, 187(18), 6258–6264. https://doi.org/10.1128/jb.187.18.6258-6264.2005

Kuever, J., Sievert, S. M., Stevens, H., Brinkhoff, T., & Muyzer, G. (2002). Microorganisms of the oxidative and reductive part of the Sulphur cycle at a shallow-water hydrothermal vent in the Aegean Sea (Milos, Greece). *Cahiers de Biologie Marine*, 43, 413–416.

Kuwahara, H., Yoshida, T., Takaki, Y., Shimamura, S., Nishi, S., Harada, M., Matsuyama, K., Takishita, K., Kawato, M., Uematsu, K., Fujiwara, Y., Sato, T., Kato, C., Kitagawa, M., Kato, I., & Maruyama, T. (2007). Reduced genome of the thioautotrophic intracellular symbiont in a deep-sea clam, *Calyptogena okutanii*. *Current Biology*, 17(10), 881–886. https://doi.org/10.1016/j.cub.2007.04.039

Laurent, M. C. Z., Gros, O., Brulport, J. P., Gaill, F., & Bris, N. L. (2009). Sunken wood habitat for thiotrophic symbiosis in mangrove swamps. *Marine Environmental Research*, 67, 83–88. https://doi.org/10.1016/j.marenvres.2008.11.006

Lenk, S., Arnds, J., Zerjatke, K., Musat, N., Amann, R., & Mußmann, M. (2011). Novel groups of Gammaproteobacteria catalyse sulfur oxidation and carbon fixation in a coastal, intertidal sediment. *Environmental Microbiology*, 13(3), 758–774. https://doi.org/10.1111/j.1462-2920.2010.02380.x

Lowe, T. M., & Eddy, S. R. (1997). tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research*, 25(5), 955–964. https://doi.org/10.1093/nar/25.5.955

Maeda, S., Aoba, R., Nishino, Y., & Omata, T. (2019). A novel bacterial nitrate transporter composed of small transmembrane proteins. *Plant and Cell Physiology*, 60(10), 2180–2192. https://doi.org/10.1093/pcp/pcz112

Maurin, L. C., Himmel, D., Mansot, J., & Gros, O. (2010). Raman microspectrometry as a powerful tool for a quick screening of thio- trophy: An application on mangrove swamp meiofauna of Guadeloupe (F.W.I.). *Marine Environmental Research*, 69, 382–389. https://doi.org/10.1016/j.marenvres.2010.02.001

McCutcheon, J. P., & Moran, N. A. (2012). Extreme genome reduction in symbiotic bacteria. *Nature Reviews Microbiology*, 10, 13–26. https://doi.org/10.1038/nrmicro2670

Medina, M., & Sachs, J. L. (2010). Symbiont genomics, our new tangled bank. *Genomics*, 95(3), 129–137. https://doi.org/10.1016/j.ygeno.2009.12.004

Mitchell, J. H., Leonard, J. M., Delaney, J., Girguis, P. R., & Scott, K. M. (2019). Hydrogen does not appear to be a major electron donor for symbiosis with the deep-sea hydrothermal vent tubeworm *Riftia pachyptila*. *Applied and Environmental Microbiology*, 86(1), e01522-19. https://doi.org/10.1128/AEM.01522-19

Moran, N. A. (1996). Accelerated evolution and Muller's rachet in endosymbiotic bacteria. *Proceedings of the National Academy of Sciences*, 93(7), 2873–2878. https://doi.org/10.1073/pnas.93.7.2873

Moran, N. A., McLaughlin, H. J., & Sorek, R. (2009). The dynamics and time scale of ongoing genomic erosion in symbiotic bacteria. *Science*, 323, 379–382. https://doi.org/10.1126/science.1167140

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., & Kanehisa, M. (2007). KAAS: An automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research*, 35(Web Server), W182–W185. https://doi.org/10.1093/nar/gkm321

Newton, I. L., & Bordenstein, S. R. (2011). Correlations between bacterial ecology and mobile DNA. *Current Microbiology*, 62(1), 198–208. https://doi.org/10.1007/s00284-010-9693-3

Newton, I. L. G., Woyke, T., Auchtung, T. A., Dilly, G. F., Dutton, R. J., Fisher, M. C., Fontanez, K. M., Lau, E., Stewart, F. J., Richardson, P. M., Barry, K. W., Saunders, E., Detter, J. C., Wu, D., Eisen, J. A., & Cavanaugh, C. M. (2007). The *Calyptogena magnifica* chemoautotrophic symbiont genome. *Science*, 315(5814), 998–1000. https://doi.org/10.1126/science.1138438

Ng, K. Y., Kamimura, K., & Sugio, T. (2000). Production of hydrogen sulfide from tetrathionate by the iron-oxidizing bacterium *Thiobacillus ferrooxidans* NASF-1. *Journal of Bioscience and Bioengineering*, 90(2), 193–198. https://doi.org/10.1016/s1389-1723(00)80109-7

Nicks, T., & Rahn-Lee, L. (2017). Inside out: Archaeal ectosymbionts suggest a second model of reduced-genome evolution. *Frontiers in Microbiology*, 8, 384. https://doi.org/10.3389/fmicb.2017.00384

Nunoura, T., Takaki, Y., Kazama, H., Kakuta, J., Shimamura, S., Makita, H., Hirai, M., Miyazaki, M., & Takai, K. (2014). Physiological and genomic features of a novel sulfur-oxidizing gammaproteobacterium belonging to a previously uncultivated symbiotic lineage isolated from a hydrothermal vent. *PLoS One*, 9(8), e104959. https://doi.org/10.1371/journal.pone.0104959

Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. (2016). metaSPAdes: a new versatile de novo metagenomic assembler. *Genome Research*, 27, 824–834. https://doi.org/10.1101/gr.213959.116

Oren, A. (2017). A plea for linguistic accuracy–Also for Candidatus taxa. *International Journal of Systematic and Evolutionary Microbiology*, 67(4), 1085–1094. https://doi.org/10.1099/ijsem.0.001715

Ott, J., Bright, M., & Bulgheresi, S. (2004). Marine microbial thiotrophic ectosymbioses. *Oceanography and Marine Biology: An Annual Review*, 42, 95–118. https://doi.org/10.1201/9780203507810.ch4

Ott, J. A., Novak, R., Schiemer, F., Hentschel, U., Nebelsick, M., & Polz, M. (1991). Tackling the sulfide gradient: A novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. *Marine Ecology*, 12, 261–279. https://doi.org/10.1111/j.1439-0485.1991.tb00258.x

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290. https://doi.org/10.1093/bioinformatics/btg412

Paredes, G. F., Viehboeck, T., Lee, R., Palatinszky, M., Mausz, M. A., Reipert, S., Schintlmeister, A., Maier, A., Volland, J.-M., Hirschfeld, C., Wagner, M., Berry, D., Markert, S., Bulgheresi, S., & König, L. (2021). Anaerobic sulfur oxidation underlies adaptation of a chemosynthetic symbiont to oxic-anoxic interfaces. *mSystems*, 6(3), e01186-20. https://doi.org/10.1128/mSystems.01186-20

Park, T., Wijeratne, S., Meulia, T., Firkins, J. L., & Yu, Z. (2021). The macronuclear genome of anaerobic ciliate *Entodinium caudatum* reveals its biological features adapted to the distinct rumen environment. *Genomics*, 113(3), 1416–1427. https://doi.org/10.1016/j.ygeno.2021.03.014

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research*, 25, 1043–1055. https://doi.org/10.1101/gr.186072.114

Paytan, A., & McLaughlin, K. (2007). The oceanic phosphorus cycle. *Chemical Reviews*, 107(2), 563–576. https://doi.org/10.1021/cr0503613

Petersen, J. M., Kemper, A., Gruber-Vodicka, H., Cardini, U., van der Geest, M., Kleiner, M., Bulgheresi, S., Mußmann, M., Herbold, C., Seah, B. K., Antony, C. P., Liu, D., Belitz, A., & Weber, M. (2016). Chemosynthetic symbionts of marine invertebrate animals are capable of nitrogen fixation. *Nature Microbiology*, 2, 16195. https://doi.org/10.1038/nmicrobiol.2016.195

Petersen, J. M., Ramette, A., Lott, C., Cambon-Bonavita, M. A., Zbinden, M., & Dubilier, N. (2010). Dual symbiosis of the vent shrimp *Rimicaris exoculata* with filamentous gamma- and epsilonproteobacteria at four mid-Atlantic ridge hydrothermal vent fields. *Environmental Microbiology*, 12(8), 2204–2218. https://doi.org/10.1111/j.1462-2920.2009.02129.x

Pitcher, R. S., & Watmough, N. J. (2004). The bacterial cytochrome cbb3 oxidases. *Biochimica et Biophysica acta (BBA)–Bioenergetics*, 1655, 388–399. https://doi.org/10.1016/j.bbabio.2003.09.017

Polz, M. F., Distel, D. L., Zarda, B., Amann, R., Felbeck, H., Ott, J. A., & Cavanaugh, C. M. (1994). Phylogenetic analysis of a highly specific association between ectosymbiotic, sulfur-oxidizing bacteria and a marine nematode. *Applied and Environmental Microbiology*, 60(12), 4461–4467. https://doi.org/10.1128/aem.60.12.4461-4467.1994

Raikov, I. B. (1971). Bactéries épizoiques et mode de nutrition du cilié psammophile *Kentrophoros fistulosum* Fauré-Fremiet (étude au microscope électronique). *Protistologica*, 7, 365–378. https://doi.org/10.1016/S0074-7696(08)60584-7

Raikov, I. B. (1974). Étude ultrastructurale des bactéries épizoiques et endozoiques de *Kentrophoros latum* Raikov, cilié holotriche mésopsammique. *Cahiers de Biologie Marine*, 15, 379–393.

Rinke, C., Lee, R., Katz, S., & Bright, M. (2007). The effects of sulphide on growth and behaviour of the thiotrophic *Zoothamnium niveum* symbiosis. *Proceedings–Royal Society of London. Biological Sciences*, 274, 2259–2269. https://doi.org/10.1098/rspb.2007.0631

Rinke, C., Schmitz-Esser, S., Loy, A., Horn, M., Wagner, M., & Bright, M. (2009). High genetic similarity between two geographically distinct strains of the sulfur-oxidizing symbiont 'Candidatus Thiobios zoothamnicoli'. *FEMS Microbiology Ecology*, 67, 229–241. https://doi.org/10.1111/j.1574-6941.2008.00628.x

Rinke, C., Schmitz-Esser, S., Stoecker, K., Nussbaumer, A. D., Molnár, D. A., Vanura, K., Wagner, M., Horn, M., Ott, J. A., & Bright, M. (2006). "Candidatus Thiobios zoothamnicoli", an ectosymbiotic bacterium covering the giant marine ciliate *Zoothamnium niveum*. *Applied and Environmental Microbiology*, 72, 2014–2021. https://doi.org/10.1128/AEM.72.3.2014-2021.2006

Rodriguez-Puente, R., & Lazo-Cortes, M. S. (2013). Algorithm for shortest path search in geographic information systems by using reduced graphs. *Springerplus*, 2, 291. https://doi.org/10.1186/2193-1801-2-291

Rühl, P., Pöll, U., Braun, J., Klingl, A., & Kletzin, A. (2017). A sulfur oxygenase from the haloalkaliphilic bacterium *Thioalkalivibrio paradoxus* with atypically low reductase activity. *Journal of Bacteriology*, 199(4), e00675-16. https://doi.org/10.1128/JB.00675-16

Sachs, J. L., Essenberg, C. J., & Turcotte, M. M. (2011). New paradigms for the evolution of beneficial infections. *Trends in Ecology and Evolution*, 26(4), 202–209. https://doi.org/10.1016/j.tree.2011.01.010

Sancar, G. B., Smith, F. W., Reid, R., Payne, G., Levy, M., & Sancar, A. (1987). Action mechanism of *Escherichia coli* DNA photolyase. I. Formation of the enzyme-substrate complex. *Journal of Biological Chemistry*, 262(1), 478–485.

Schliep, K. P. (2011). Phangorn: Phylogenetic analysis in R. *Bioinformatics*, 27, 592–593. https://doi.org/10.1093/bioinformatics/btq706

Schuster, L., & Bright, M. (2016). A novel colonial ciliate *Zoothamnium ignavum* sp. nov.(Ciliophora, Oligohymenophorea) and its ectosymbiont *Candidatus* Navis piranensis gen. Nov., sp. nov. from shallow-water wood falls. *PLoS One*, 11(9), e0162834. https://doi.org/10.1371/journal.pone.0162834

Schwarz, G. (1978). Estimating the dimension of a model. *The Annals of Statistics*, 6(2), 461–464. https://doi.org/10.1214/aos/1176344136

Scott, K. M., Harmer, T. L., Gemmell, B. J., Kramer, A. M., Sutter, M., Kerfeld, C. A., Barber, K. S., Bari, S., Boling, J. W., Campbell, C. P., Gallard-Gongora, J. F., Jackson, J. K., Lobos, A., Mounger, J. M., Radulovic, P. W., Sanson, J. M., Schmid, S., Takieddine, C., Warlick, K. F., & Whittaker, R. (2020). Ubiquity and functional uniformity in $CO_2$ concentrating mechanisms in multiple phyla of bacteria is suggested by a diversity and prevalence of genes encoding candidate dissolved inorganic carbon transporters. *FEMS Microbiology Letters*, 367(13), fnaa106. https://doi.org/10.1093/femsle/fnaa106

Seah, B. K. B., Antony, C. P., Huettel, B., Zarzycki, J., Schada von Borzyskowski, L., Erb, T. J., Kouris, A., Kleiner, M., Liebeke, M., Dubilier, N., & Gruber-Vodicka, H. R. (2019). Sulfur-oxidizing symbionts without canonical genes for autotrophic $CO_2$ fixation. *mBio*, 10, e01112–e01119. https://doi.org/10.1128/mBio.01112-19

Serafini, A., Tan, L., Horswell, S., Howell, S., Greenwood, D. J., Hunt, D. M., Phan, M. D., Schembri, M., Monteleone, M., Montague, C. R., Britton, W., Garza-Garcia, A., Snijders, A. P., VanderVen, B., Gutierrez, M. G., West, N. P., & de Carvalho, L. P. S. (2019). *Mycobacterium tuberculosis* requires glyoxylate shunt and reverse methylcitrate cycle for

lactate and pyruvate metabolism. *Molecular Microbiology*, 112(4), 1284–1307. https://doi.org/10.1111/mmi.14362

Sievert, S. M. (1999). *Microbial communities at a shallow submarine hydrothermal vent in the Aegean Sea (Milos, Greece)*. PhD Dissertation. Universität Bremen. Chapter 6. https://pure.mpg.de/rest/items/item_3254112/component/file_3254113/content

Sievert, S. M., Brinkhoff, T., Muyzer, G., Ziebis, W., & Kuever, J. (1999). Spatial heterogeneity of bacterial populations along an environmental gradient at a shallow submarine hydrothermal vent near Milos Island (Greece). *Applied and Environmental Microbiology*, 65, 3834–3842. https://doi.org/10.1128/AEM.65.9.3834-3842.1999

Sogin, E. M., Kleiner, M., Borowski, C., Gruber-Vodicka, H.-R., & Dubilier, N. (2021). Life in the dark: Phylogenetic and physiological diversity of chemosynthetic symbioses. *Annual Review of Microbiology*, 75(1), 695–718. https://doi.org/10.1146/annurev-micro-051021-123130

Sorokin, D. Y., de Jong, G. A., Robertson, L. A., & Kuenen, G. J. (1998). Purification and characterization of sulfide dehydrogenase from alkaliphilic chemolithoautotrophic sulfur-oxidizing bacteria. *FEBS Letters*, 427, 11–14. https://doi.org/10.1016/s0014-5793(98)00379-2

Stewart, F. J., Newton, I. L. G., & Cavanaugh, C. M. (2005). Chemosynthetic endosymbioses: Adaptations to oxic-anoxic interfaces. *Trends in Microbiology*, 13(9), 439–448. https://doi.org/10.1016/j.tim.2005.07.007

Supuran, C. T., & Capasso, C. (2017). An overview of the bacterial carbonic anhydrases. *Metabolites*, 7(4), 56. https://doi.org/10.3390/metabo7040056

Takai, K. (2019). The nitrogen cycle: A large, fast, and mystifying cycle. *Microbes and Environments*, 34(3), 223–225. https://doi.org/10.1264/jsme2.ME3403rh

Tanaka, Y., Yoshikaie, K., Takeuchi, A., Ichikawa, M., Mori, T., Uchino, S., Sugano, Y., Hakoshima, T., Takagi, H., Nonaka, G., & Tsukazaki, T. (2020). Crystal structure of a YeeE/YedE family protein engaged in thiosulfate uptake. *Science Advances*, 6(35), p.eaba7637. https://doi.org/10.1126/sciadv.aba7637

Tatusov, R. L., Galperin, M. Y., Natale, D. A., & Koonin, E. V. (2000). The COG database: A tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Research*, 28(1), 33–36. https://doi.org/10.1093/nar/28.1.33

Toft, C., & Andersson, S. G. (2010). Evolutionary microbial genomics: Insights into bacterial host adaptation. *Nature Reviews Genetics*, 11(7), 465–475. https://doi.org/10.1038/nrg2798

Toft, C., Williams, T. A., & Fares, M. A. (2009). Genome-wide functional divergence after the symbiosis of proteobacteria with insects unraveled through a novel computational approach. *PLoS Computational Biology*, 5, e1000344. https://doi.org/10.1371/journal.pcbi.1000344

Veuger, B., & Middelburg, J. J. (2007). Incorporation of nitrogen from amino acids and urea by benthic microbes: Role of bacteria versus algae and coupled incorporation of carbon. *Aquatic Microbial Ecology*, 48(1), 35–46.

Vignais, P., Billoud, B., & Meyer, J. (2001). Classification and phylogeny of hydrogenases. *FEMS Microbiology Reviews*, 25, 455–501. https://doi.org/10.1111/j.1574-6976.2001.tb00587.x

Vogt, L. (2010). Spatio-structural granularity of biological material entities. *BMC Bioinformatics*, 11(1), 289. https://doi.org/10.1186/1471-2105-11-289

Volland, J.-M., Schintlmeister, A., Zambalos, H., Reipert, S., Mozetič, P., Espada-Hinojosa, S., Turk, V., Wagner, M., & Bright, M. (2018). NanoSIMS and tissue autoradiography reveal symbiont carbon fixation and organic carbon transfer to giant ciliate host. *ISME Journal*, 12, 714–727. https://doi.org/10.1038/s41396-018-0069-1

Wang, S., Orabi, E. A., Baday, S., Bernèche, S., & Lamoureux, G. (2012). Ammonium transporters achieve charge transfer by fragmenting their substrate. *Journal of the American Chemical Society*, 134(25), 10419–10427. https://doi.org/10.1021/ja300129x

Weissgerber, T., Dobler, N., Polen, T., Latus, J., Stockdreher, Y., & Dahl, C. (2013). Genome-wide transcriptional profiling of the purple sulfur bacterium *Allochromatium vinosum* DSM 180[T] during growth on different reduced sulfur compounds. *Journal of Bacteriology, 195*(18), 4231–4245. https://doi.org/10.1128/JB.00154-13

Weissgerber, T., Zigann, R., Bruce, D., Chang, Y. J., Detter, J. C., Han, C., Hauser, L., Jeffries, C. D., Land, M., Munk, A. C., Tapia, R., & Dahl, C. (2011). Complete genome sequence of *Allochromatium vinosum* DSM 180[T]. *Standards in Genomic Sciences, 5*, 311–330. https://doi.org/10.4056/sigs.2334270

Welte, C., Hafner, S., Krätzer, C., Quentmeier, A., Friedrich, C. G., & Dahl, C. (2009). Interaction between sox proteins of two physiologically distinct bacteria and a new protein involved in thiosulfate oxidation. *FEBS Letters, 583*(8), 1281–1286. https://doi.org/10.1016/j.febslet.2009.03.020

Wernegreen, J. J. (2015). Endosymbiont evolution: Predictions from theory and surprises from genomes. *Annals of the new York Academy of Sciences, 1360*(1), 16–35. https://doi.org/10.1111/nyas.12740

Wick, R. R., Schultz, M. B., Zobel, J., & Holt, K. E. (2015). Bandage: Interactive visualisation of de novo genome assemblies. *Bioinformatics, 31*(20), 3350–3352. https://doi.org/10.1093/bioinformatics/btv383

Zhou, J., Bruns, M. A., & Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. *Applied and Environmental Microbiology, 62*, 316–322. https://doi.org/10.1128/aem.62.2.316-322.1996

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**How to cite this article:** Espada-Hinojosa, S., Karthäuser, C., Srivastava, A., Schuster, L., Winter, T., de Oliveira, A. L., Schulz, F., Horn, M., Sievert, S., & Bright, M. (2024). Comparative genomics of a vertically transmitted thiotrophic bacterial ectosymbiont and its close free-living relative. *Molecular Ecology Resources, 24*, e13889. https://doi.org/10.1111/1755-0998.13889