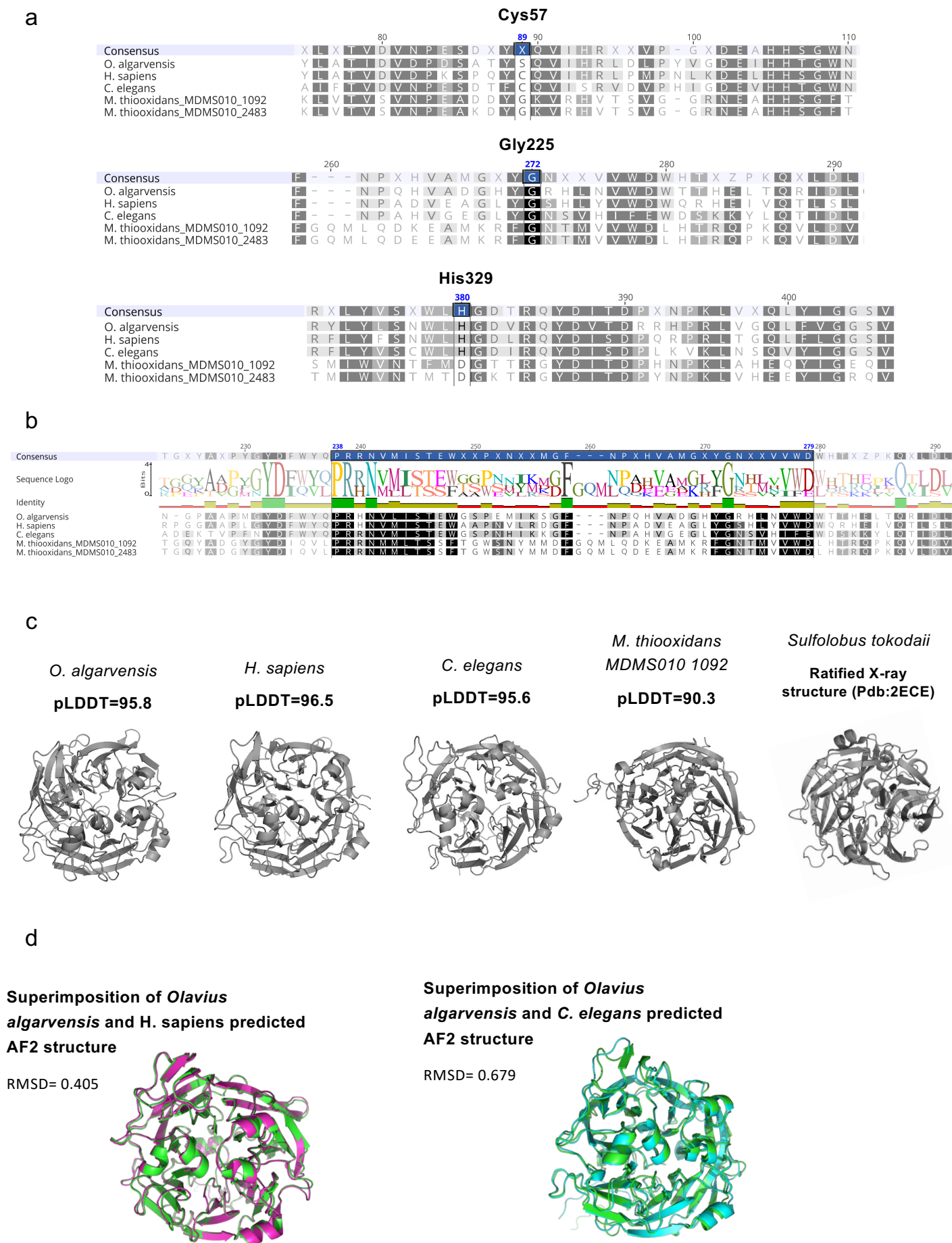




Supplementary Fig. S1. Conservation and predicted structure of *O. algarvensis* MtoX compared with bacterial and animal homologs. (a) Amino acid alignments of *O. algarvensis* MtoX with *H. sapiens* SELENBP1, *C. elegans* MTOX, and *Methylophaga thiooxidans* MtoX show conservation of two catalytic residues (G272 and H380, blue) known to coordinate the catalytic copper center, while H89 is replaced by serine. (b) Sequence logo highlighting conservation of the WD40-like β -propeller motif characteristic of the MtoX/SELENBP1 family. (c) AlphaFold2-predicted tertiary structures for all homologs display the canonical eight-bladed β -propeller fold. (d) Structural overlays of *O. algarvensis* MtoX with *H. sapiens* SELENBP1 (left) and *M. thiooxidans* MtoX (right) show strong overall structural conservation despite the active-site substitution, suggesting potential retention of methanethiol-oxidase-like function.

a

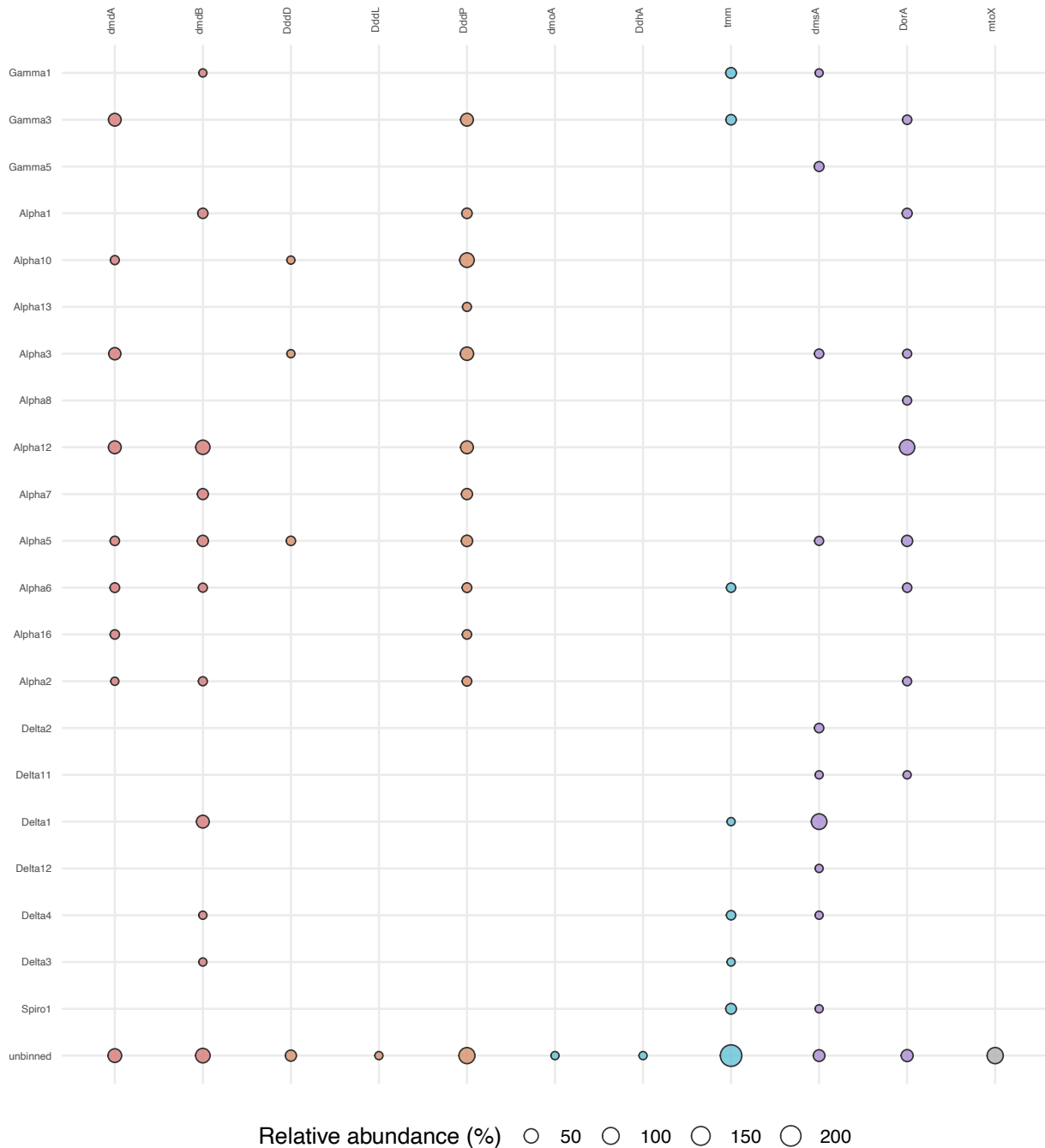
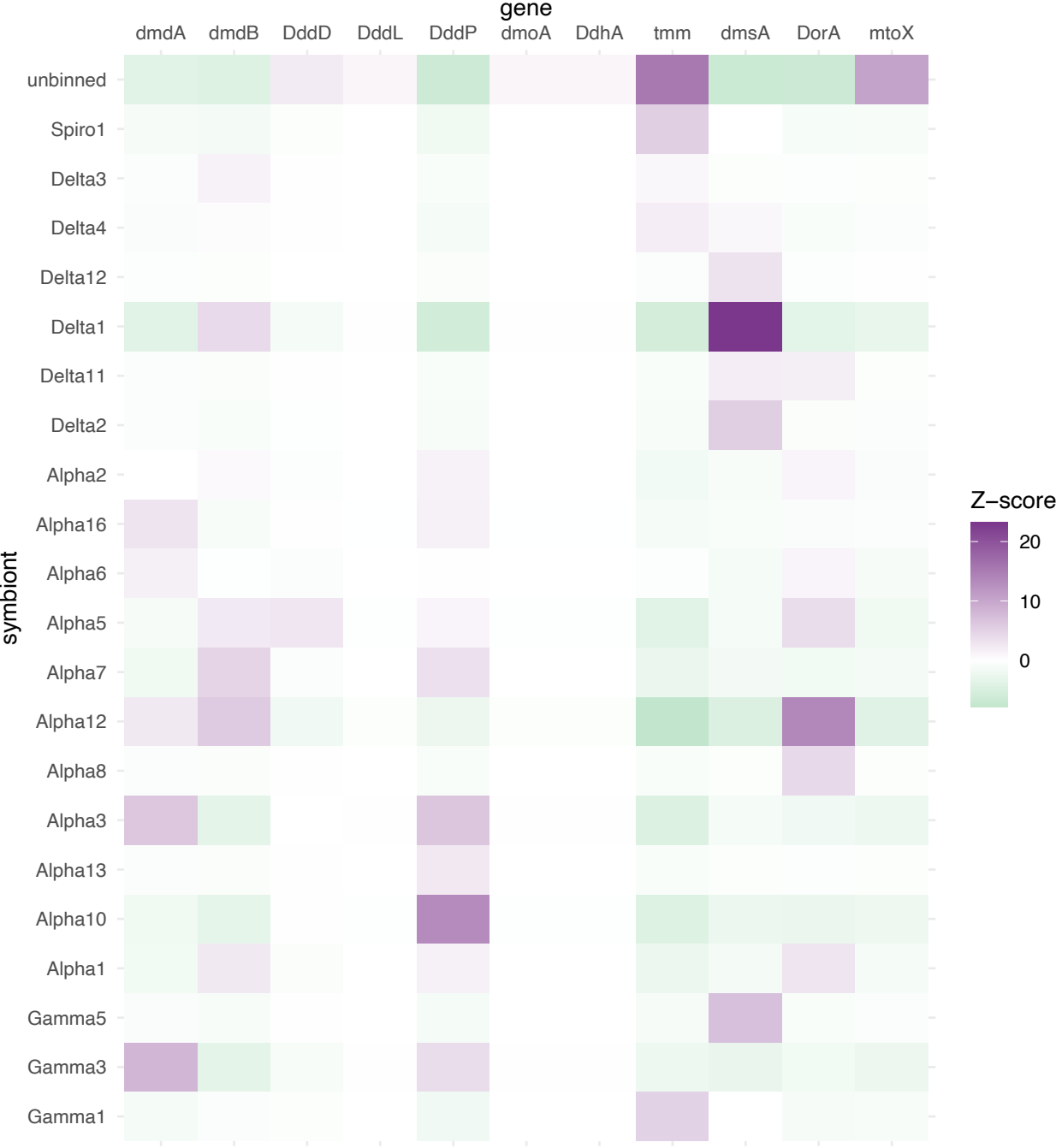
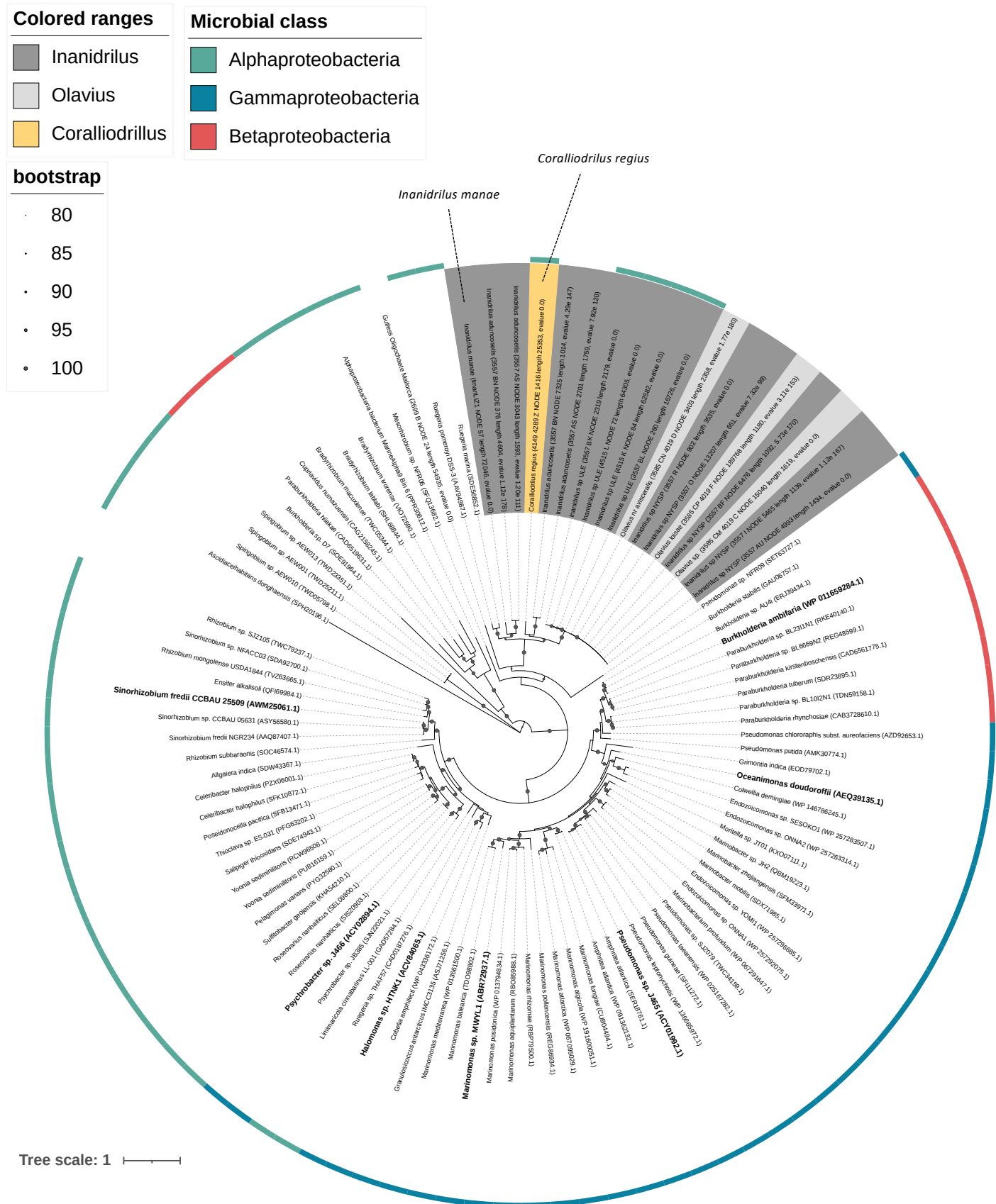


Figure S2. (a) Distribution of DMSP-, DMS-, and MeSH-cycling genes across symbiont lineages in gutless oligochaetes. Bubble plot showing the relative abundance of organosulfur metabolism genes detected on contigs assigned to major symbiont lineages (Gamma-, Delta-, Alpha-proteobacteria, Spirochaetes, and unbinned sequences) across 60 gutless oligochaete metagenomes. Bubble size corresponds to the relative abundance (%) of each gene per lineage. **(b) Symbiont-gene enrichment analysis based on Z-scores.** Z-scores were calculated from standardized residuals of a chi-squared test to assess whether specific symbiont lineages are enriched or depleted in particular organosulfur metabolism genes. Positive values (purple) indicate significant enrichment. Gamma3, several Deltaproteobacteria, and multiple Alphaproteobacteria lineages show strong enrichment for distinct gene sets, consistent with their specialized functional roles.

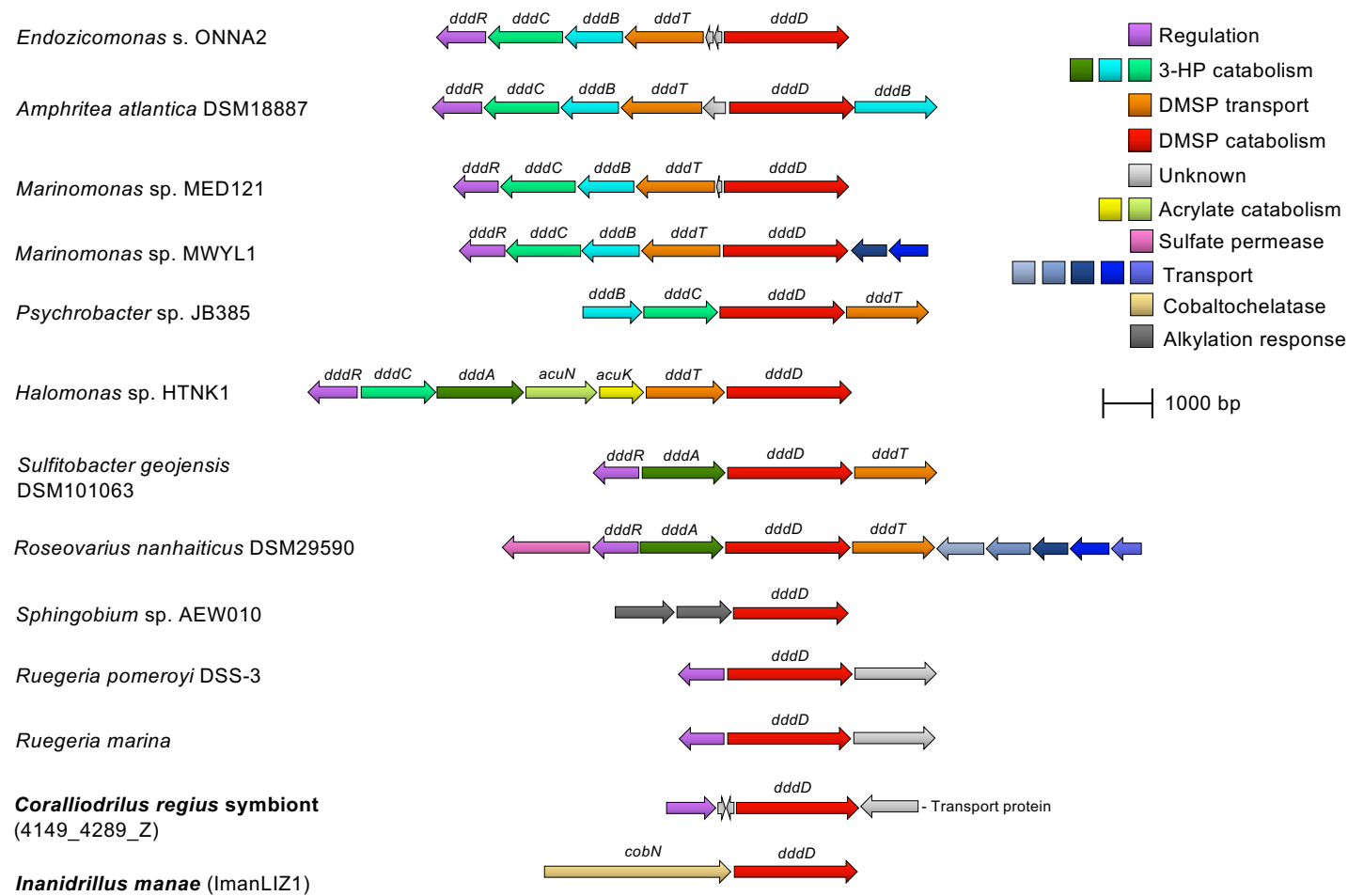
b



DMSP → DMS + Acryloyl-CoA



b



a

DMSP → DMS + Acrylate

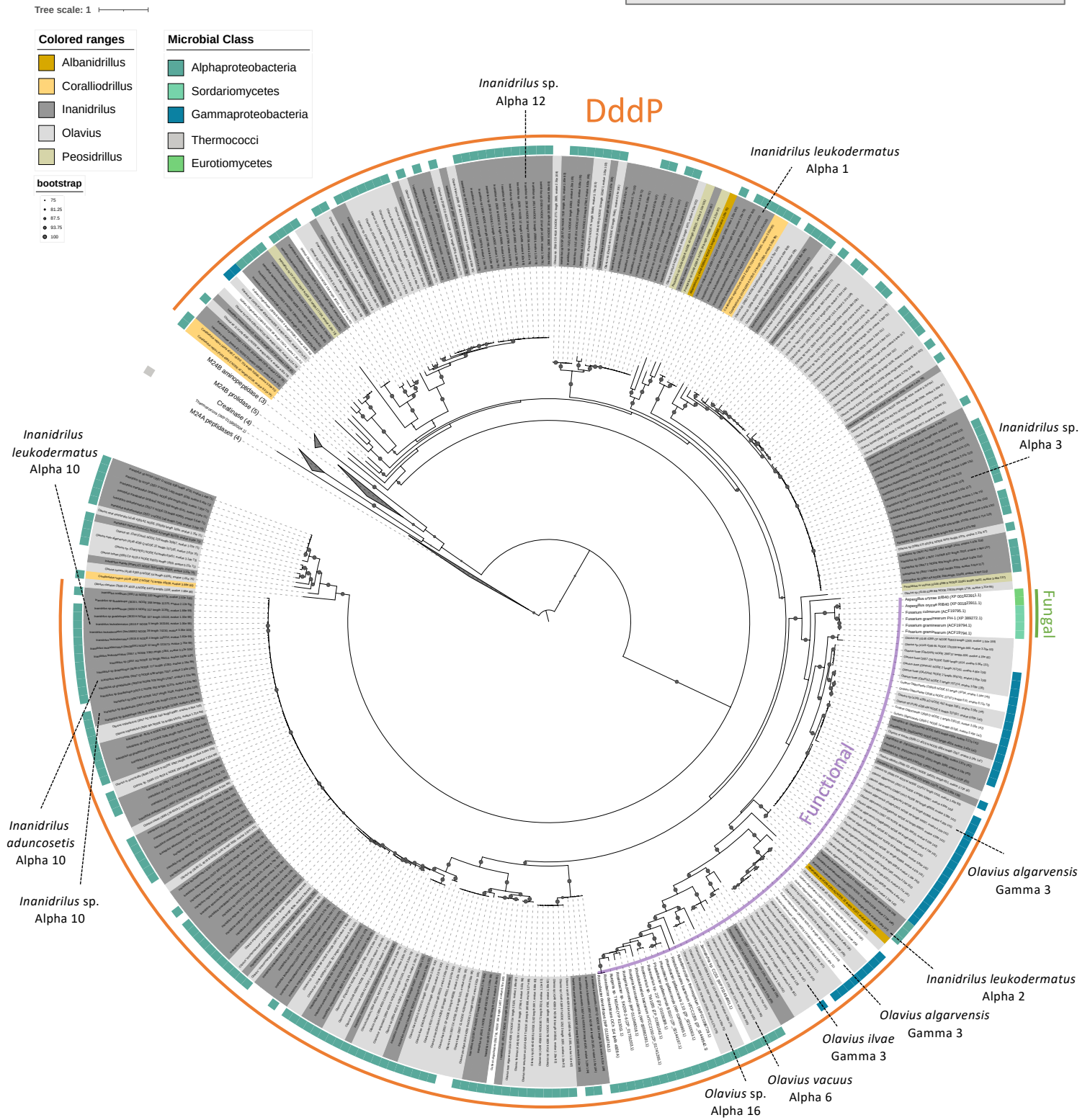
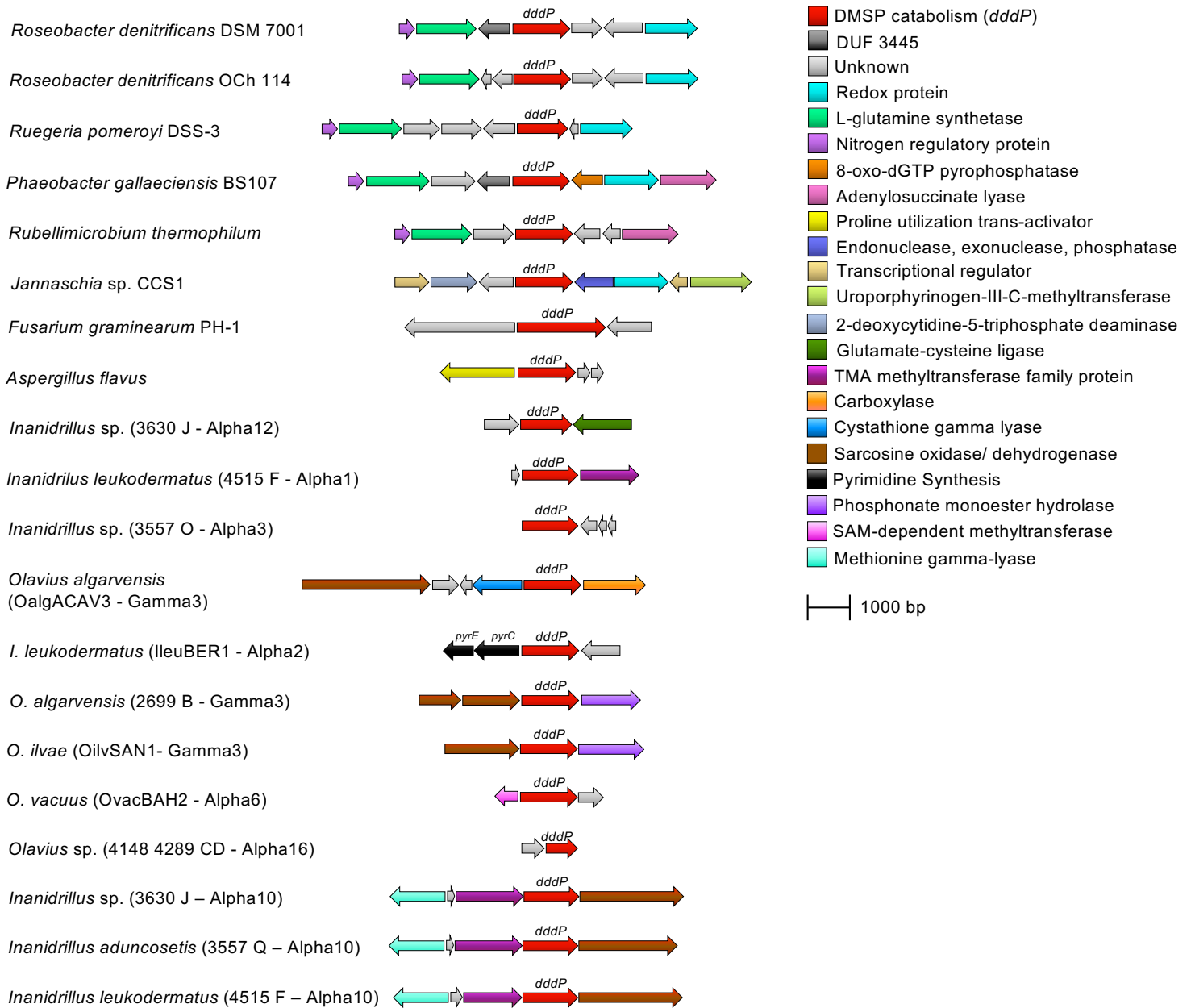
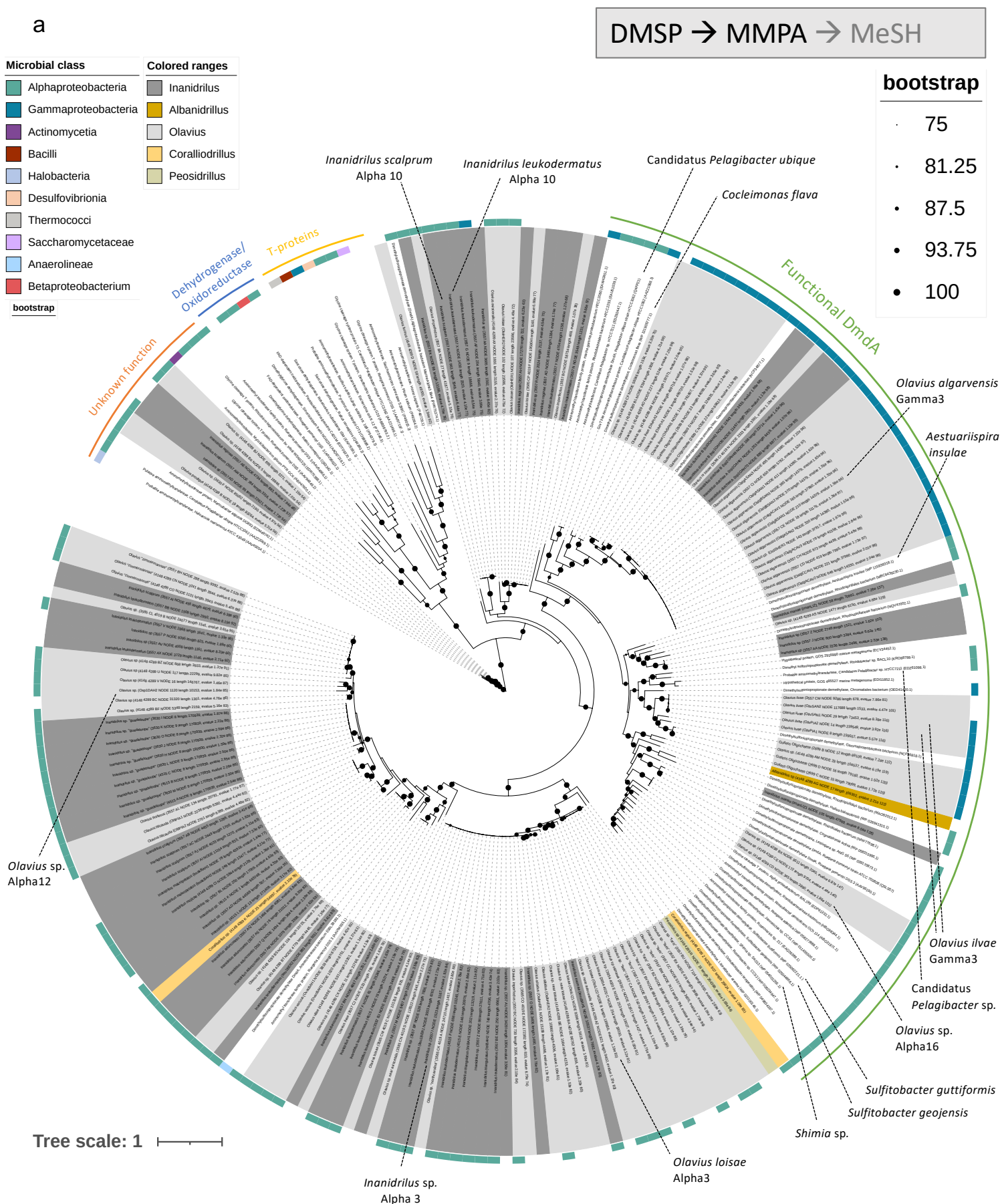


Figure S4. DMSP lyase *dddP* in gutless-oligochaete metagenomes.

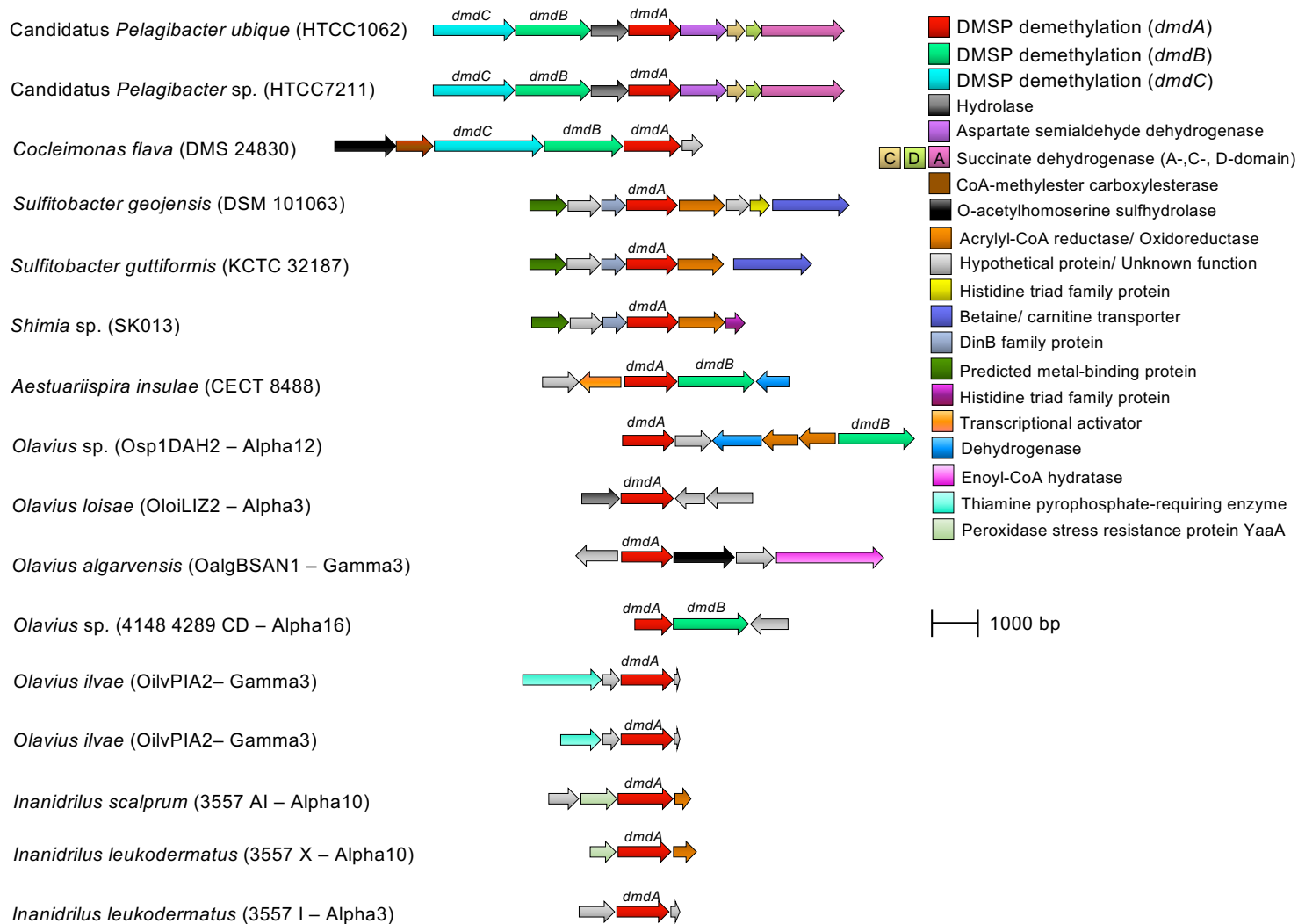
(a) Maximum-likelihood phylogeny of *DddP*. Assembly-derived *DddP* sequences from gutless-oligochaete metagenomes are placed among curated references. M24A peptidases (and representative M24B peptidase/prolidase/creatinase proteins) are included as outgroup/context. The clade that contains *DddP* homologs whose function has been confirmed biochemically is highlighted with a purple line. Fungal *DddP* homologs are indicated as a distinct clade (marked green). Light and dark grey shadings indicate metagenomes from gutless oligochaetes (*Olavius/Inanidrilus*) and yellow, brownish shadings indicate metagenomes from gut-bearing representatives. The outer ring denotes microbial class; tip labels end with accession numbers. Proteins were aligned with Clustal Omega (Geneious Prime v2021.0.3). The tree was inferred with IQ-TREE v1.6.12, ModelFinder model selection, and ultrafast bootstrap (UFBoot) = 1000. Support values are shown for nodes $\geq 75\%$; scale bar = substitutions per site. Reference sequences were used from Todd et al. (2009) and Wang et al. (2015). **(b) Gene neighbourhoods.** Representative *dddP*-positive contigs from gutless-oligochaete assemblies and selected references are shown (≤ 10 kb span; 1 kb scale). Arrows indicate gene orientation. (Neighbourhood gene calls are from this study's contig-level inspection.)

b





b



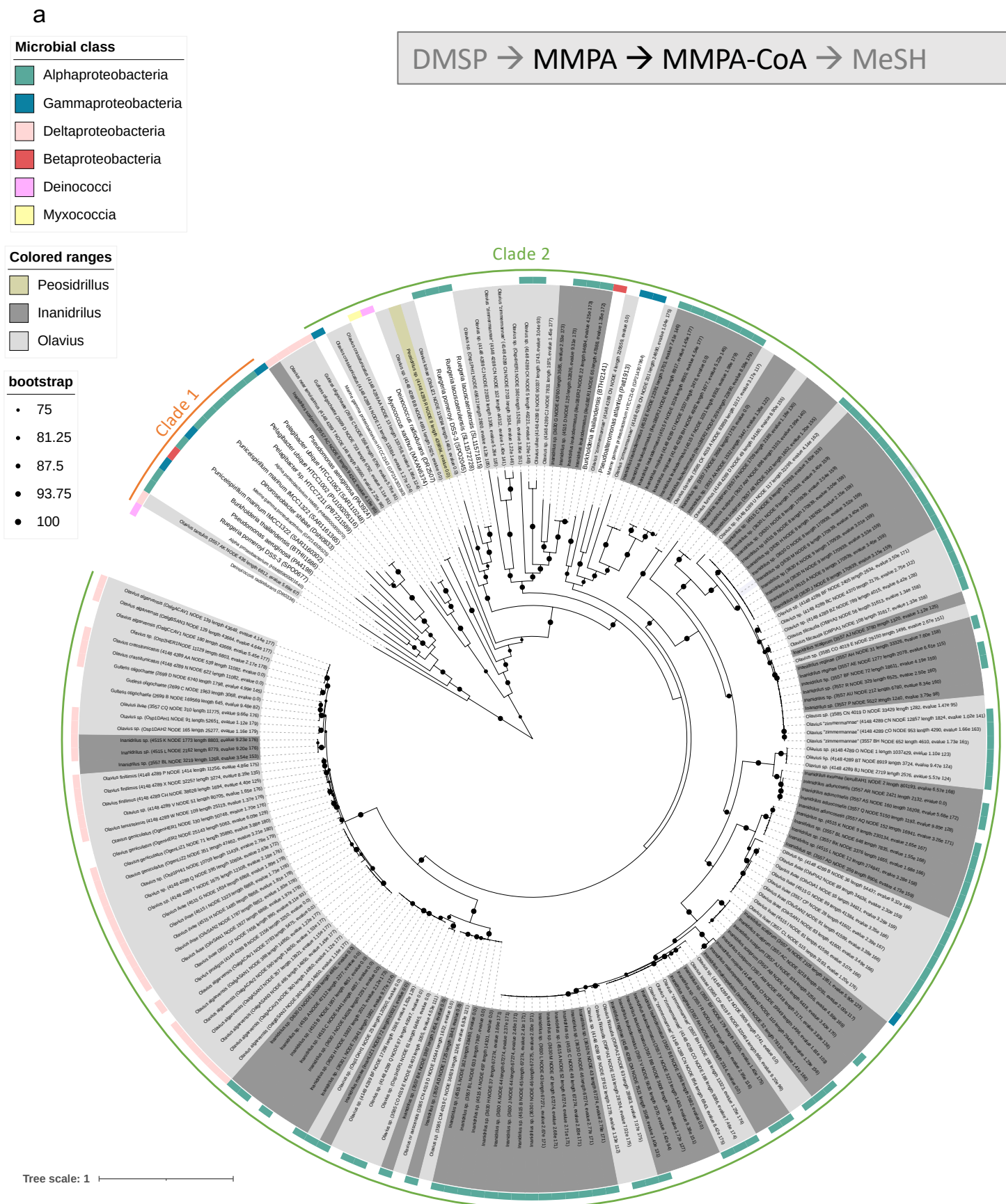


Figure S6. MMPA-CoA ligase *dmdB* in gutless-oligochaete metagenomes
Maximum-likelihood phylogeny of DmdB. Assembly-derived DmdB sequences from gutless-oligochaete metagenomes are shown alongside a curated reference set emphasizing the two canonical DmdB clades (clade1/clade2) reported previously (Bullock et al. 2014). Tip labels end with accession numbers, and the outer ring indicates microbial class. Proteins were aligned with Clustal Omega (Geneious Prime v2021.0.3); the tree was inferred with IQ-TREE v1.6.12 using ModelFinder and ultrafast bootstrap (UFBoot) = 1000; support values are shown for nodes $\geq 60\%$; scale bar = substitutions/site. Reference sequences were drawn from Bullock et al., 2014.

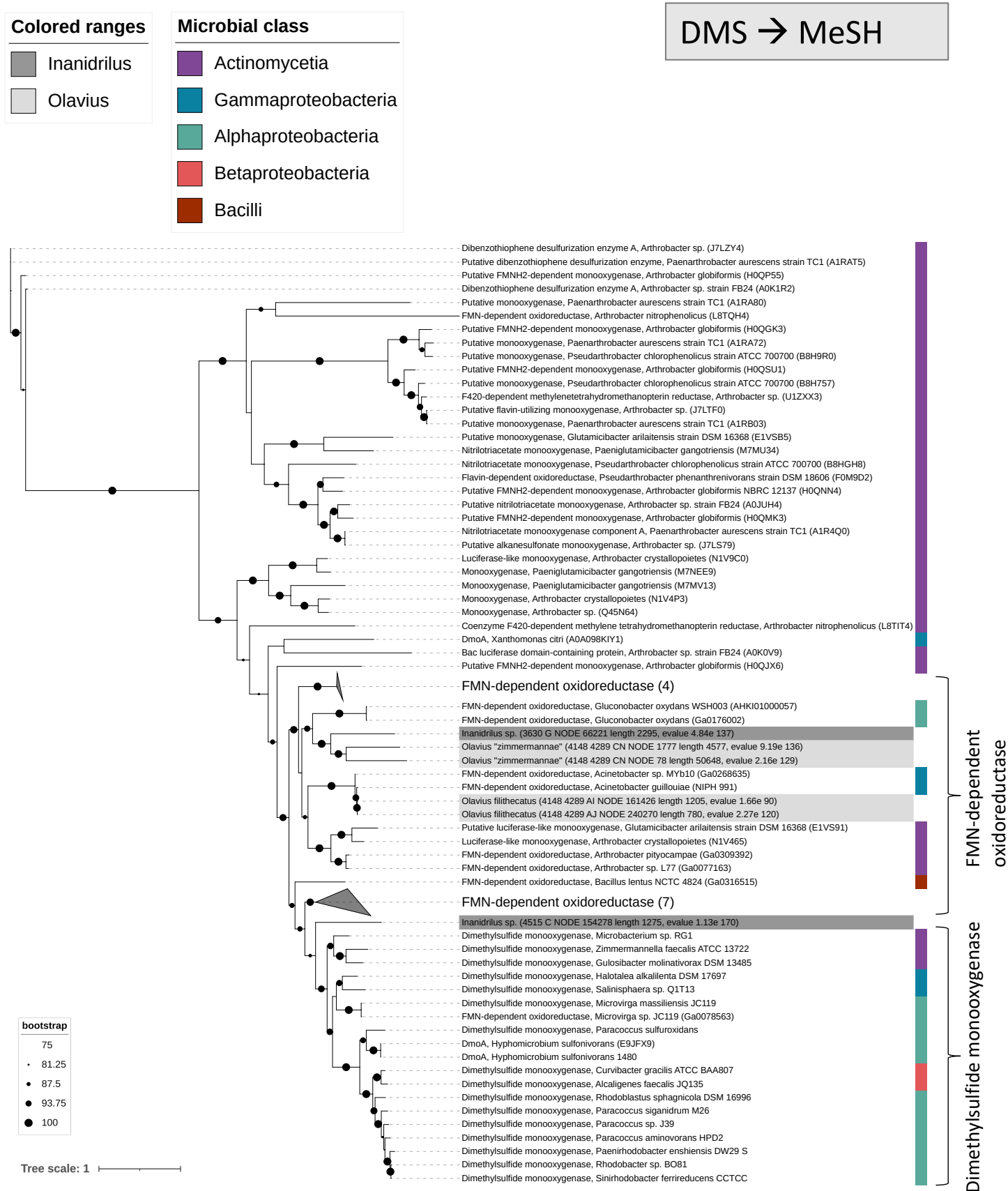


Figure S7. DMS monooxygenase A-subunit *dmoA* in gutless-oligochaete metagenomes.

Maximum-likelihood phylogeny of DmoA and related FMNH₂-dependent oxidoreductases. Assembly-derived candidates from gutless-oligochaete metagenomes were placed among curated references (including sequences from Hammers et al. 2020). Dibenzoanthropene desulfurization enzyme was used as an outgroup. Tip labels end with accession numbers. Proteins were aligned with Clustal Omega (Geneious Prime v2021.0.3); the tree was inferred with IQ-TREE v1.6.12 using ModelFinder; UFBoot = 1000; support values are shown for nodes ≥75%; scale bar indicates substitutions/site.

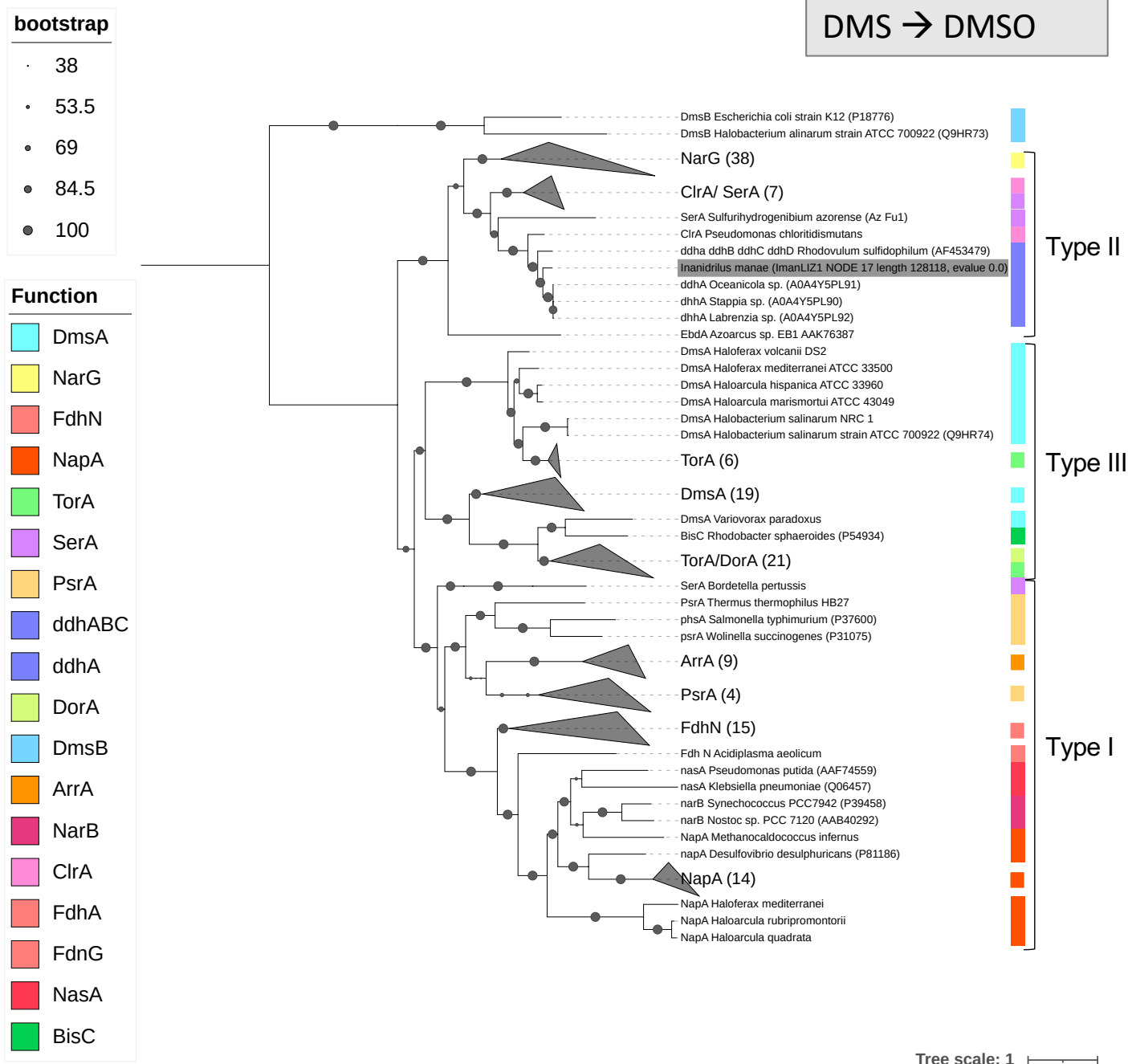


Figure S8. *ddhA* (DMS dehydrogenase, catalytic subunit) in gutless-oligochaete metagenomes. **Maximum-likelihood phylogeny of DdhA within the DMSO-reductase family.** Assembly-derived DdhA candidates from gutless-oligochaete metagenomes are placed among curated references from *Rhodovulum sulfidophilum* and other DMSO-reductase family members (NarG/NapA/DmsA/SerA/EbdA etc.) from Yamazaki et al. 2020 and McDevitt et al. 2002. The iron-sulfur subunit DmsB was included as an outgroup; additional DMSO-reductase family sequences were added as closely related context. Proteins were aligned with Clustal Omega (Geneious Prime v2021.0.3); tree was inferred with IQ-TREE v1.6.12 using ModelFinder; node support is UFBoot from 1000 replicates, shown for nodes $\geq 38\%$; scale bar = substitutions/site. Tip labels end with accession numbers; DMSO-reductase family members are shown as a bar on the right side. Abbreviations: DmsA/DmsB, periplasmic bacterial DMSO reductase; NarG, respiratory nitrate reductase; FdhA/FdhG/FdhN, formate dehydrogenase; NapA, periplasmic nitrate reductase; TorA, trimethylamine N-oxide (TMAO) reductase; SerA, selenate reductase; PsrA, Polysulfide reductase; DdhA/DdhABC, DMS dehydrogenase; DorA, DMSO reductase; ArrA, respiratory arsenate reductases; NarB/NasA, assimilatory nitrate reductase; ClrA, chlorate reductase; BisC, biotin sulfoxide reductase.

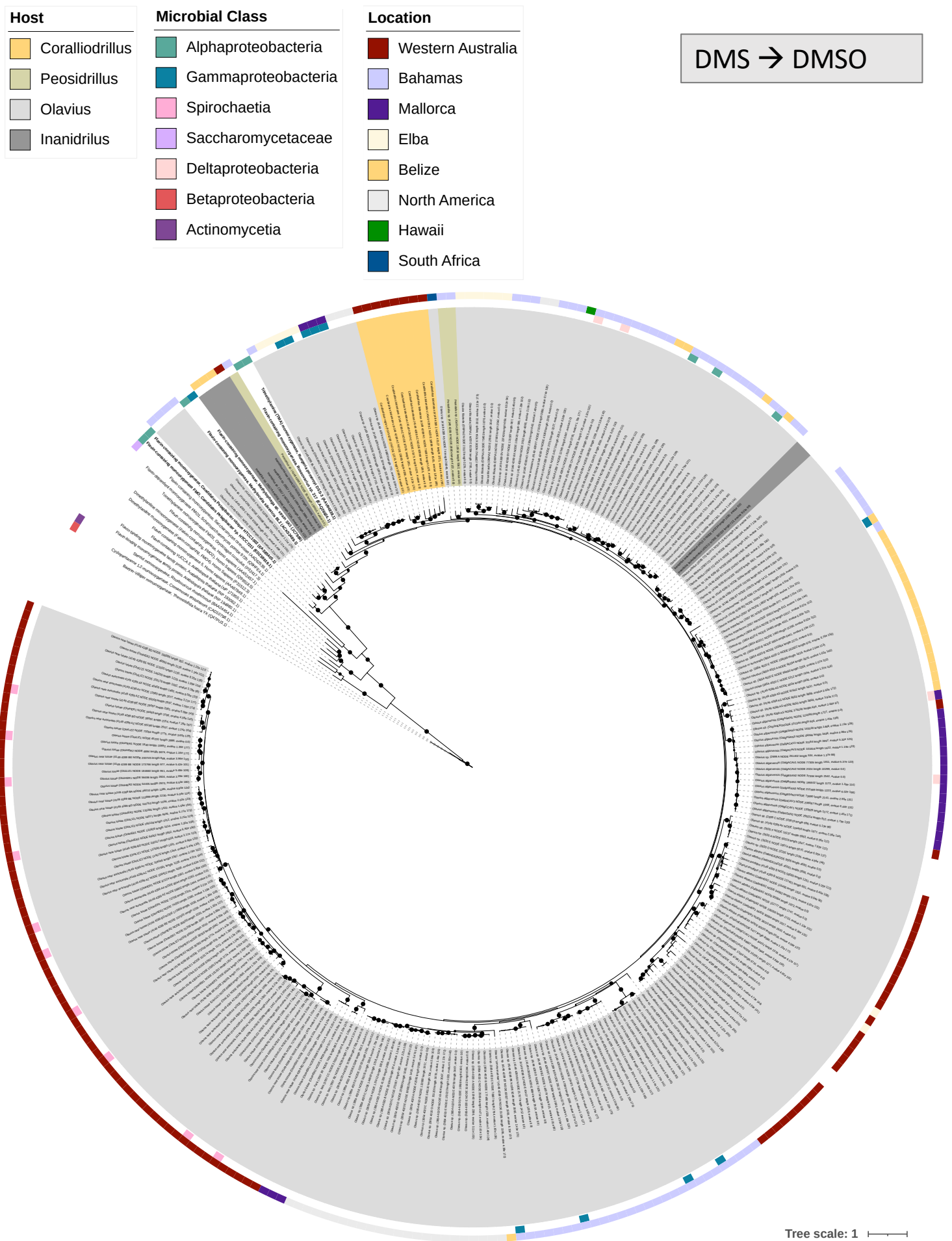


Figure S9. Maximum-likelihood phylogeny of Tmm and related FMOs.
 Assembly-derived Tmm candidates from gutless-oligochaete metagenomes were placed with curated references (including Chen et al. 2011) and Baeyer-Villiger monooxygenases (BVMO) as outgroup. Light and dark grey shadings indicate metagenomes from gutless oligochaetes (*Olavius*/*Inanidrillus*) and yellow, brownish shadings indicate metagenomes from gut-bearing representatives. Tip labels end with accession numbers. The inner one of the outer ring shows microbial class. The second outer ring shows sampling location of gutless oligochaete metagenomes. Alignment was done with Clustal Omega (Geneious Prime v2021.0.3); tree was inferred with IQ-TREE v1.6.12 with ModelFinder, UFBoot = 1000; support is shown for nodes $\geq 75\%$; scale bar = substitutions/site.

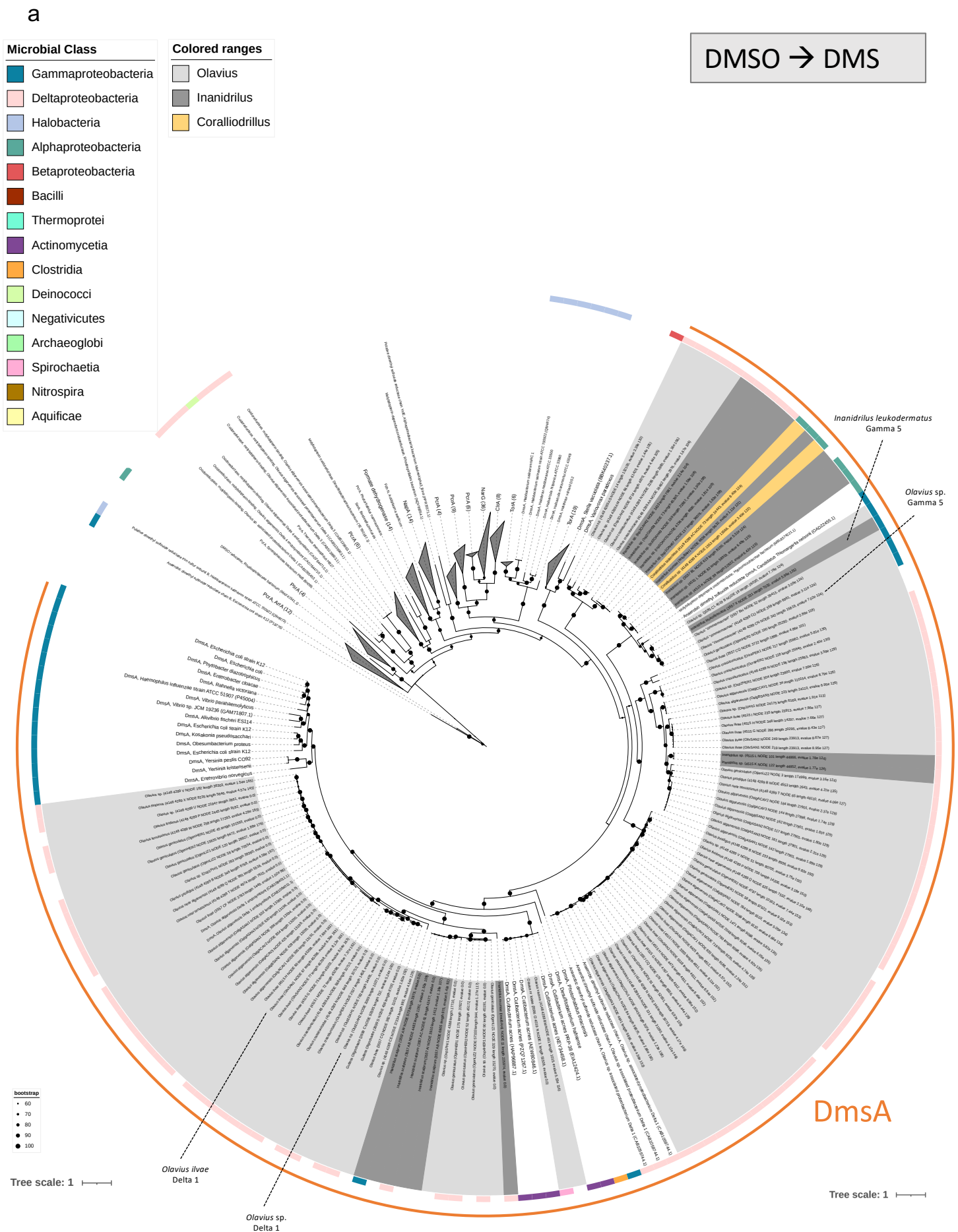
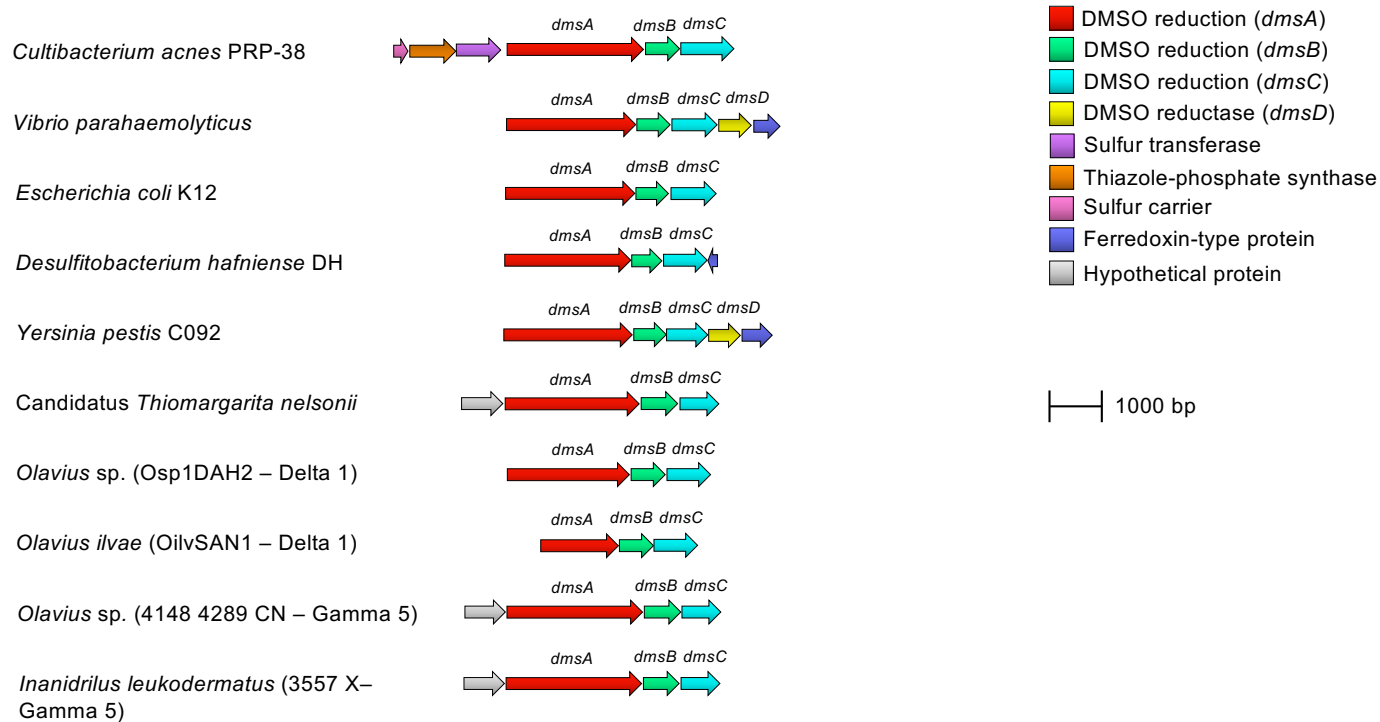


Figure S10. (a) Maximum-likelihood phylogeny of DmsA in gutless-oligochaete metagenomes within the DMSO-reductase family. Assembly-derived DmsA sequences from gutless-oligochaete metagenomes were placed among curated references representing the DMSO-reductase superfamily (e.g., NarG/NapA/FdhA/TorA/DorA) from Yamazaki et al. 2020 and McDevitt et al. 2002 used as close context. Light and dark grey shadings indicate metagenomes from gutless oligochaetes (*Olavius*/*Inanidrilus*) and yellow shadings from gut-bearing representatives. Proteins were aligned with Clustal Omega (Geneious Prime v2021.0.3); tree was inferred with IQ-TREE v1.6.12 using ModelFinder; node support is UFBoot from 1000 replicates and shown for nodes $\geq 60\%$; scale bar = substitutions per site. Tip labels end with accession numbers, and the outer ring indicates microbial class. Samples that were investigated in the gene neighborhood analysis are indicated with a line and the name of the host/symbiont. **(b) Gene neighbourhoods.** Representative *dmsA*-positive contigs from gutless-oligochaete assemblies and selected references (≤ 10 kb span; 1 kb scale) are shown. *dmsA* co-localized with *dmsB* (Fe-S subunit) and *dmsC* (membrane anchor). Windows ≤ 10 kb total span; scale bar = 1 kb. Arrows denote gene orientation.

b



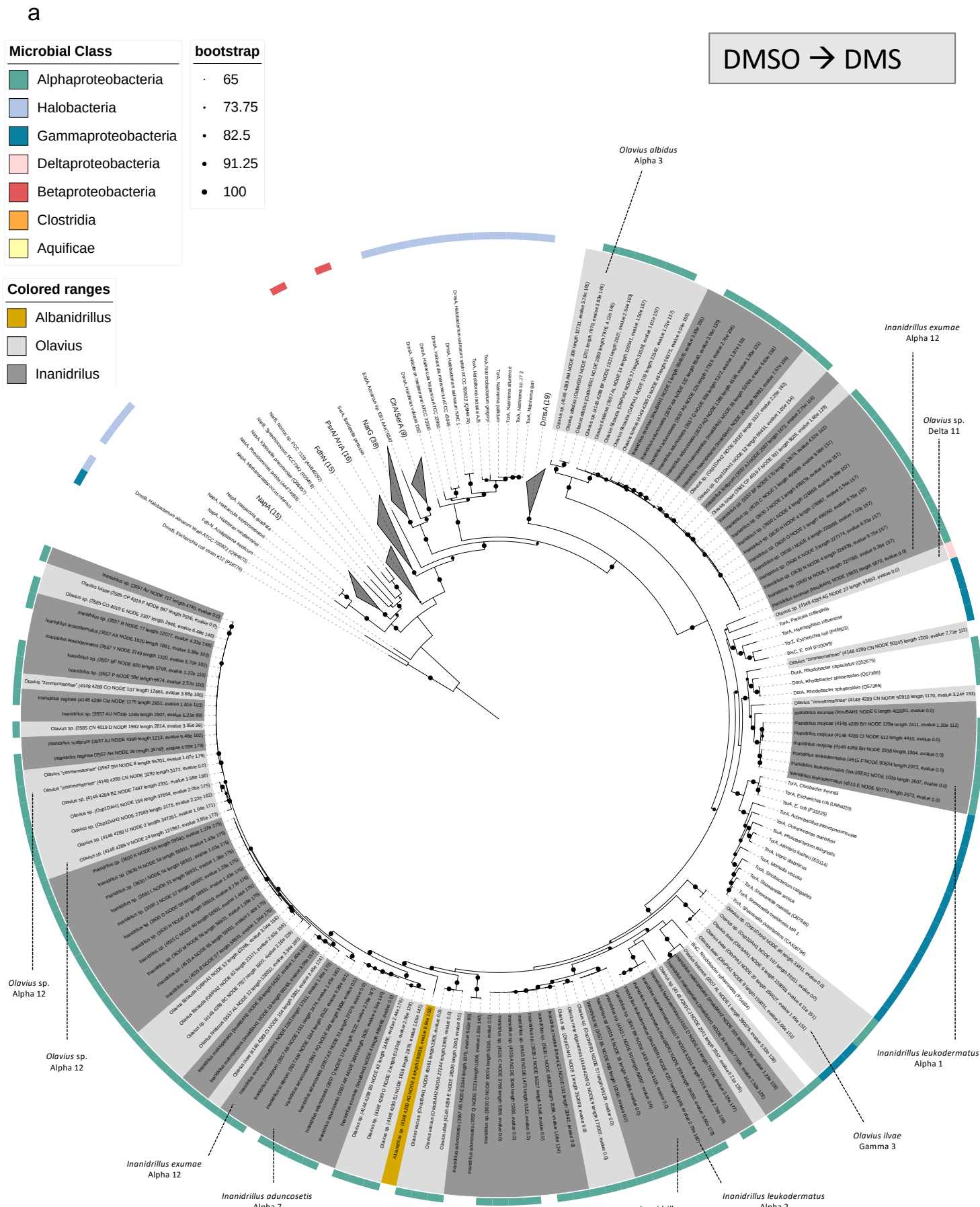
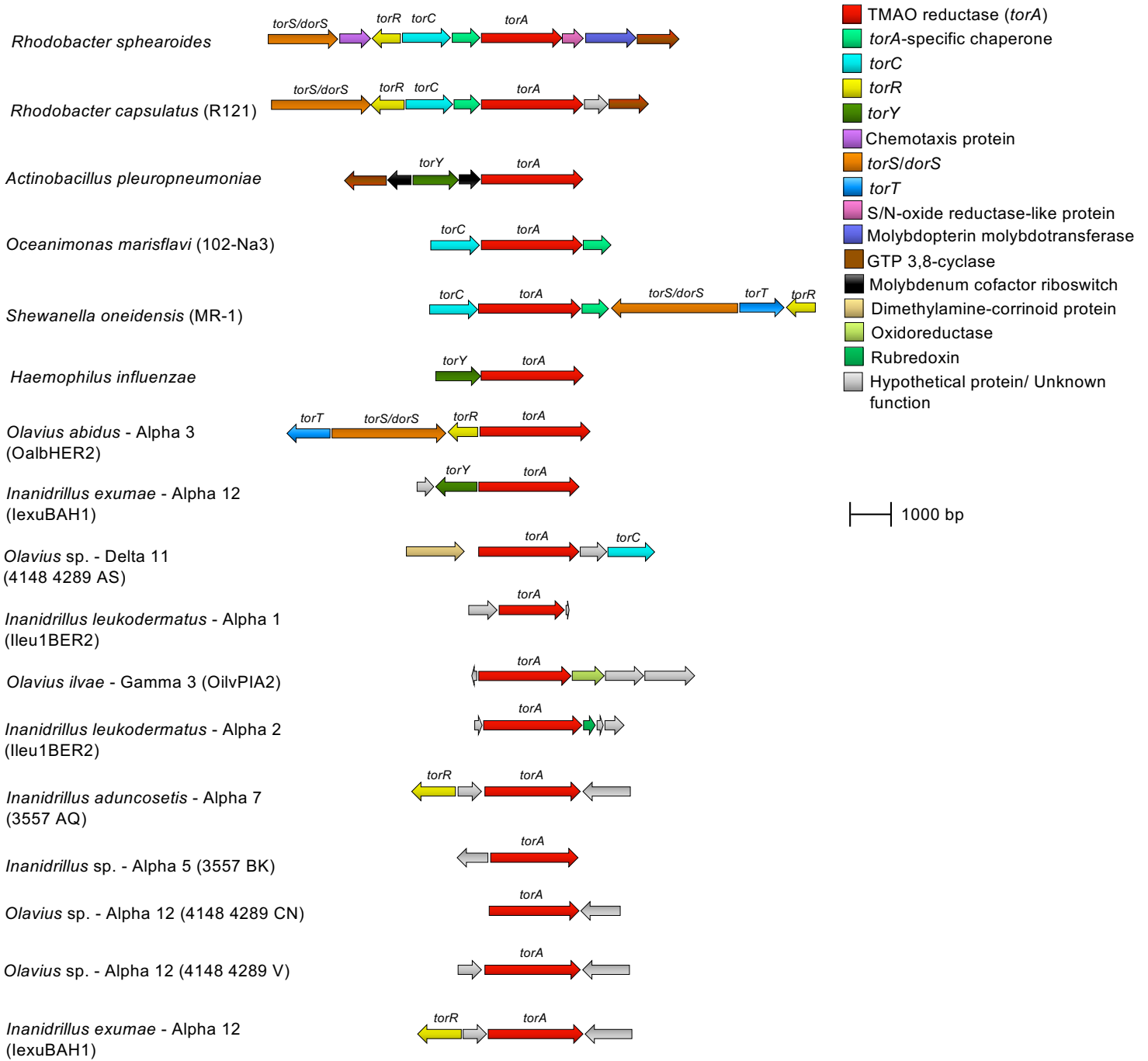
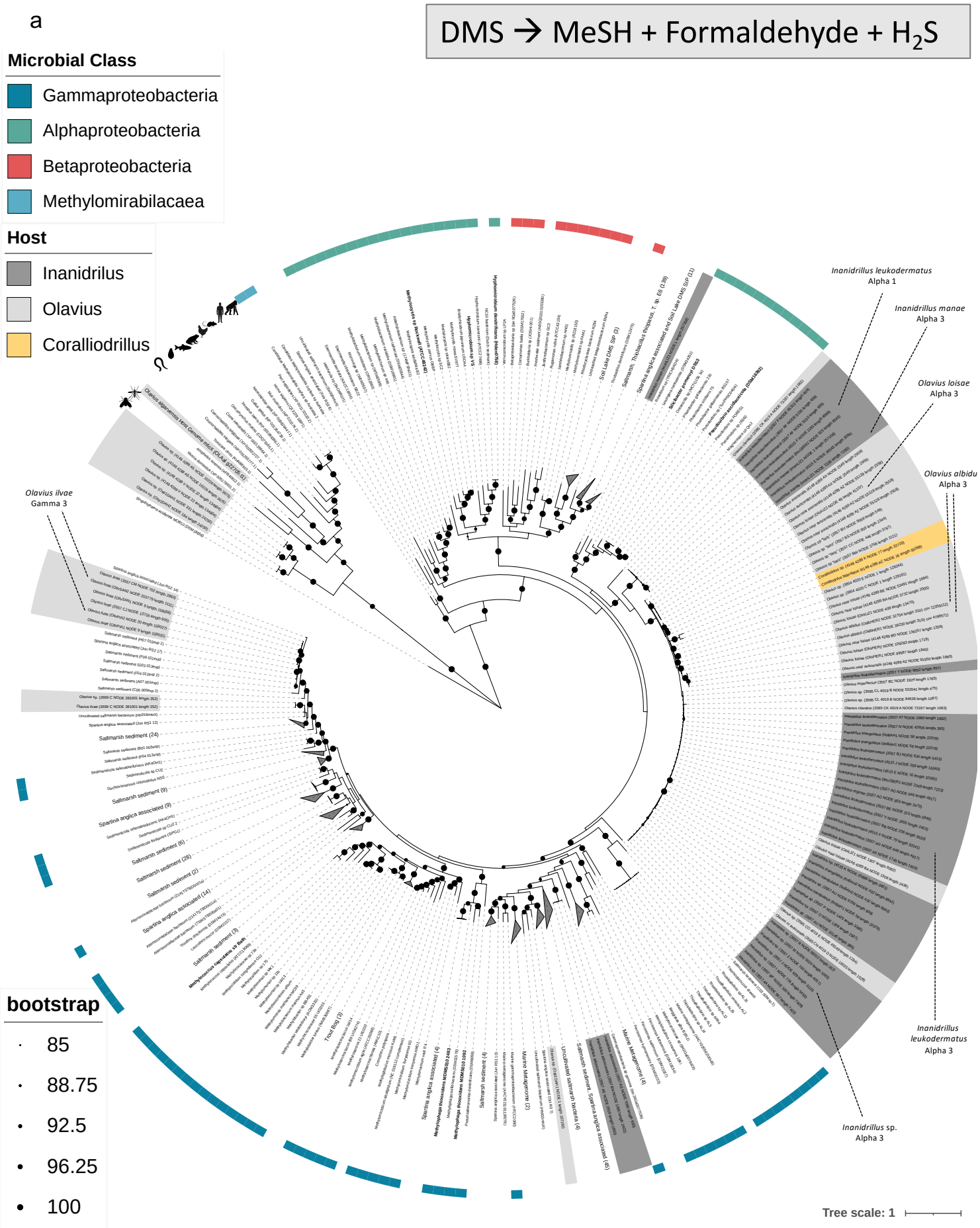


Figure S11. Periplasmic N-oxide/DMSO reductases (*torA/dorA*) in gutless-oligochaete metagenomes. (a) Maximum-likelihood phylogeny of *TorA/DorA* within the DMSO-reductase superfamily. Assembly-derived *TorA/DorA* candidates from gutless-oligochaete metagenomes were placed among close relatives from the DMSO-reductase family (*NarG/NapA/FdhA/DmsA*, etc.) for context. *DmsB* (Fe–S subunit of *DmsABC*) was used as the outgroup. Proteins were aligned with Clustal Omega (Geneious Prime v2021.0.3); the tree was inferred with IQ-TREE v1.6.12 using ModelFinder; UFBot = 1000; support values are shown for nodes $\geq 65\%$; scale bar = substitutions/site. Tip labels end with accession numbers; an outer ring indicates microbial class. Samples that were investigated in the gene neighborhood analysis are indicated with a line and the name of the host/symbiont. (b) Gene neighbourhoods. Representative *torA/dorA*-positive contigs and reference genomes showing *torA/dorA* flanked by canonical partner/regulatory genes (windows ≤ 10 kb; scale bar = 1 kb). Common cluster features: *torC/dorC* (c-type cytochrome), *torD/dorD* (maturation chaperone), *torS/torR* (regulators), *torT* (periplasmic TMAO sensor), occasional *torY/torZ*, and adjacent Molybdenum cofactor riboswitch. Arrows denote gene orientation.

b





b

