

## **A novel and specific association of bathymodiolin mussels with Epsilonproteobacteria that is widespread at deep-sea hydrothermal vents and cold seeps**

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Bathymodiolin mussels are well known for their associations with gammaproteobacterial endosymbionts that oxidize sulfide or methane for carbon fixation. We recently discovered epsilonproteobacterial epibionts that colonize the gills of bathymodiolin mussels from vents and seeps worldwide. The epsilonproteobacterial 16S rRNA sequences were closely related indicating that the association with bathymodiolins is highly specific. The sequences were at most 87.6% identical to the closest cultured relative, and 91.2% identical to the closest sequences in public databases, indicating that this clade represents a novel family within the Epsilonproteobacteria. We used metagenomics and metatranscriptomics to better understand the function of these epibionts. Their genomes revealed that these Epsilonproteobacteria are sulfur oxidizers. Remarkably, these epsilonproteobacterial epibionts lack genes for the reductive tricarboxylic acid cycle (rTCA), which all known epsilonproteobacterial autotrophs use to fix carbon. Instead, they have the genetic potential to fix inorganic carbon through the Calvin-Benson-Bassham (CBB) cycle. Transcriptomics confirmed that the key genes of the CBB cycle are expressed. Intriguingly, phylogenetic reconstruction of key CBB genes suggest they were acquired by horizontal gene transfer from Gammaproteobacteria closely related to the sulfur-oxidizing endosymbionts of bathymodiolin mussels. We hypothesize that the switch to a novel carbon fixation pathway, gained through horizontal gene transfer, was one of the key genomic mechanisms that allowed these Epsilonproteobacteria to establish a successful association with bathymodiolin mussels.