

Supplementary Materials for

Methanotrophic bacterial symbionts fuel dense populations of deep-sea feather duster worms (Sabellida, Annelida) and extend the spatial influence of methane seepage

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The PDF file includes:

Figs. S1 to S6
Tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/14/eaay8562/DC1)

Data file S1

Supplemental Figure Legends

Fig. S1 | Relative abundance of bacterial phylotypes, based on 16S rRNA. Bacterial community structure (to the phylotype level; 99% 16S rRNA sequence similarity) for crown radioles of the serpulid *Laminatubus* n. sp. and sabellid *Bispira* n. sp. from Jaco Scar and Mound 12 seeps, Costa Rica. “Non-seep” specimens were collected from inactive areas at 320-520 m depth. Each color on the graph represents a distinct genus-level phylotype or lowest level available. Phylotypes were grouped to 97% 16S rRNA sequence similarity. Dominant phylotypes are indicated in the key. Genera that were not putative aerobic methanotrophs are shown in gray or black. See SI Appendix Table 4-6 for raw and processed data, as well as representative sequences for all dominant hits.

Fig. S2 | Phylogenetic relationships of the dominant 16S rRNA and pmoA sequences recovered from two polychaete species featured in this study. Targeted PCR amplification of (A) Methylococcales-specific 16S rRNA sequences (663 bp final length sequence) recovered using the 27F/850R primer pair and (B) the particulate methane monooxygenase gene sequence (pmoA; 408 bp final length sequence), recovered using the 189f/661r primer pair, from the radioles of the serpulid *Laminatubus* n. sp. and *Bispira* n. sp., as well as surrounding seawater samples taken by CTD above the seep sites (all shown in bold). Close relatives were assigned using BLAST. Each tree is based on neighbor-joining analysis, constructed with a Jukes-Cantor distance model, and the resulting tree topology was evaluated by bootstrap analysis (* at nodes indicate bootstrap support >60, from 1000 resampled data sets). The scale bars represent % substitutions per site. HTV = hydrothermal vent.

Fig. S3 | Fluorescence Microscopy of *Bispira* sp. and *Laminatubus* sp. crown radioles. (A-B) Fluorescence microscopy of annelid radioles showing comparison between the general bacterial probe Eub338 I-III labelled with Cy3 (A, D) and probe MTC851 labelled with FITC, designed in this study to be an exact match to the MOX symbionts of both *Laminatubus* and *Bispira* from Jaco Scar, plus the overlay (C,F). *Bispira* n. sp. is shown in A-C. *Laminatubus* n. sp. is shown in D-F. DAPI-stained nuclei of host cells are shown in blue. All scale bars are 20 μm .

Fig. S4 | Microscopy of *Bispira* sp. and *Laminatubus* sp. crown radioles. Fluorescence microscopy of (A) a mannose-specific biofilm, via HHA staining (in red) surrounding the radiolar crown tip of *Bispira*. DNA of host cell nuclei and nearby bacterial symbionts shown via DAPI-staining in cyan. Fluorescence microscopy of (B-C) lipid-rich organelles, via FM46-4 staining (in red) surrounding the radiolar tips of both *Bispira* and *Laminatubus*, respectively. A Methylococcales/Marine Methylophilic Group 2 specific FISH probe (MTC851) is shown in green, and DNA is shown in blue/cyan via DAPI counterstain. Transmission electron microscopy of *Laminatubus* n. sp. (D-E) and *Bispira* n. sp. (F-G) radioles, showing MOX bacteria, with dense internal membranes (asterisks), completely engulfed by host cells (arrowheads). A-C scale bars are 10 μm , D-G scale bars are 500 nm.

Fig. S5 | Fluorescence Microscopy of *Bispira* sp. and *Laminatubus* sp. whole specimens. For both species, the digestive tract is a straight, ciliated tube, with a simple foregut, a stomach, intestine, and hindgut. The anterior portion of the gut is constricted by mesenteries, and expands into a series of spherical chambers. *Bispira* whole specimen (A), 3- μm section of specimen embedded in Steedman's resin (B). Letters C-E correspond to FISH images of various digestive tract regions, including the mouth (C), intestine (D), and hindgut (E). A Methylococcales/ Marine Methylophilic Group 2 specific FISH probe (MTC851) is shown in green (none are positive), and host nuclei are shown in blue via DAPI counterstain. *Laminatubus* whole specimen (F), 3- μm section of specimen embedded in Steedman's resin (G). Letters H-J correspond to FISH images of various body regions, including the radioles (H), mouth (I), and intestine (J). A general bacterial probe set Eub338 I-III labelled with Cy3 is shown in orange (only the radioles are positive), and host nuclei are shown in blue via DAPI counterstain. Inset in J shows non-bacterial autofluorescent objects within a region of the intestine. All scale bars are 100 μm .

Fig. S6 | ^{13}C -labelled dissolved inorganic carbon generated during shipboard experiments. The generation of substantial ^{13}C -labelled dissolved inorganic carbon in the surrounding seawater was measured within 15 hours of incubation for both annelid species (*Bispira* and *Laminatubus*), confirming CH_4 conversion to CO_2 by the MOX bacterial symbionts. ^{13}C -labelled methane ranged from 25 at% (*), to 50-55at%, to 100 at% (**). See SI Appendix Table 3 for more details.

Supp Table 1: Sample locations along the west coast of Costa Rica, along with dive information and sampling date.

Site	Geo location	Date	Dive # ¹	Depth (m)
Jaco Scar – Active Seep	9.11715°N / 84.84131°W	Oct 17-18, 2018 Nov 4, 2018	AD4971-72 AD4989	1824
JS 600m away – inactive	9.11730°N / 84.83961°W	Oct 17, 2018	AD4971	1796
JS 1400m away – inactive	9.11491°N / 84.83972°W	Oct 19, 2018	AD4973	1887
Mound 12 – Active Seep	8.93075°N / 84.3128°W	May 21, 2017 Oct 24, 2018	AD4906 AD4978	995 999
Seamount 6 - inactive	7.68025°N / 85.91171°W	Jan 22, 2019	SO227	527
Coco South - inactive	5.46692°N / 87.13112°W	Jan 19, 2019	SO224	321

¹ AD = DSRV *Alvin* dive number (Woods Hole Oceanographic Institute). SO = ROV *SubBastian* dive number (Schmidt Ocean Institute).

Supp Table 2: $\delta^{13}\text{C}$ (‰) of various body tissues for both species, including native worms and 16-month transplants (avg \pm 1 SD).

Tissue region	$\delta^{13}\text{C}$ (‰)		
	<i>Bispira</i> (native)	<i>Laminatubus</i> (native)	<i>Laminatubus</i> (transplanted)
crown	-50.5 \pm 1.3	-56.9 \pm 0.9	-50.5 \pm 0.7
body	-47.8 \pm 0.9	-56.9 \pm 0.5	-51.5 \pm 0.5
gut	-48.0 \pm 1.4 (n = 2)	-57.9 \pm 0.6 (n = 4)	-51.9 \pm 0.8 (n = 6)

Supp Table 3: Initial conditions of labeled $^{13}\text{CH}_4$ (concentration and the ^{13}C atom percent; atom%) at the start of each isotope incubation experiment.

Incub #	Time Incubated	Sample type	Vol SW (mL)	Methane Conc. (mM)	$^{13}\text{CH}_4$ At%
10854-1-5	24-37 h	<i>Bispira</i> without tubes	40	1.11	50
10854-6-8	69 h	<i>Laminatubus</i> without tubes	40	1.11	50
10854-A	80 h	<i>Laminatubus</i> on carbonate	1400	0.22	55
10861	105 h	<i>Laminatubus</i> on carbonate	450	0.37	25
10882	35 h	<i>Bispira</i> on carbonate	600	0.32	25
11669	24 h	<i>Bispira</i> on Lamellibrachia tubes	800	0.11	100

Data File 1: Excel file of raw MiSeq 16S rRNA sequence data collected in this study (sheet 1), normalized and trimmed MiSeq 16S rRNA sequence data collected in this study (sheet 2), and representative Methylococcales - Marine Methylotrophic Group 2 sequences associated with *Laminatubus*, *Bispira*, and water samples from Jaco Scar, Costa Rica (sheet 3). Attached as separate .csv file

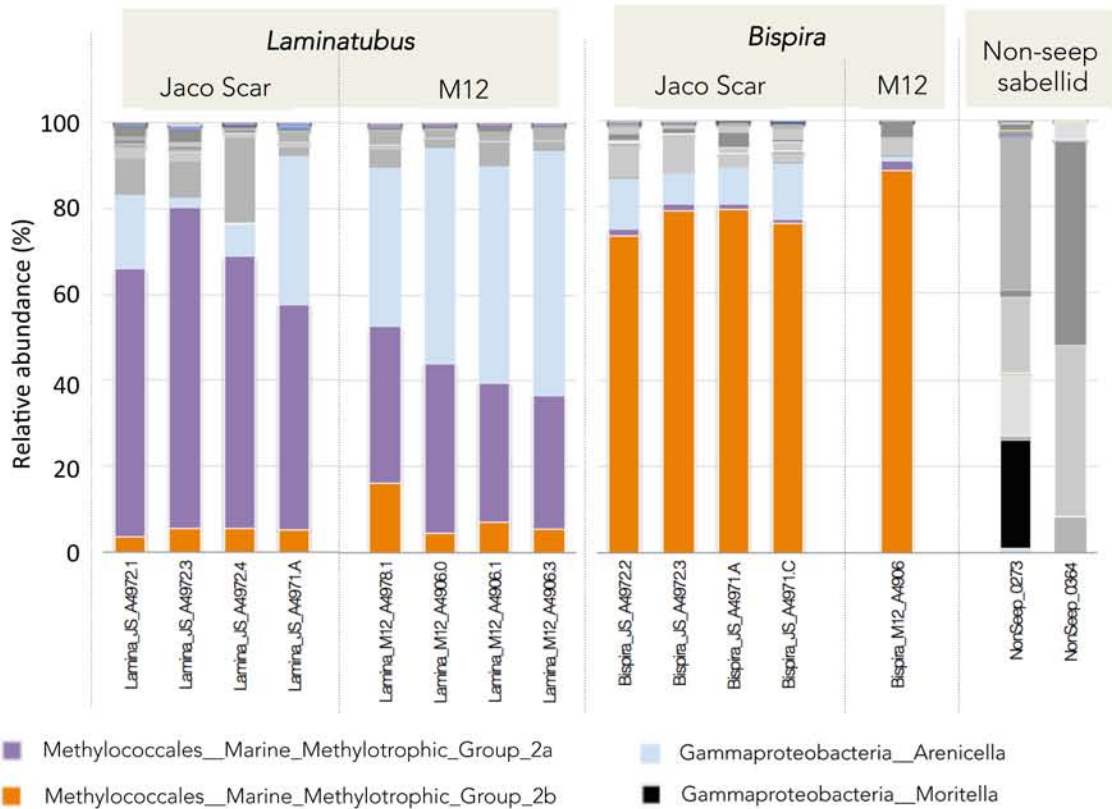


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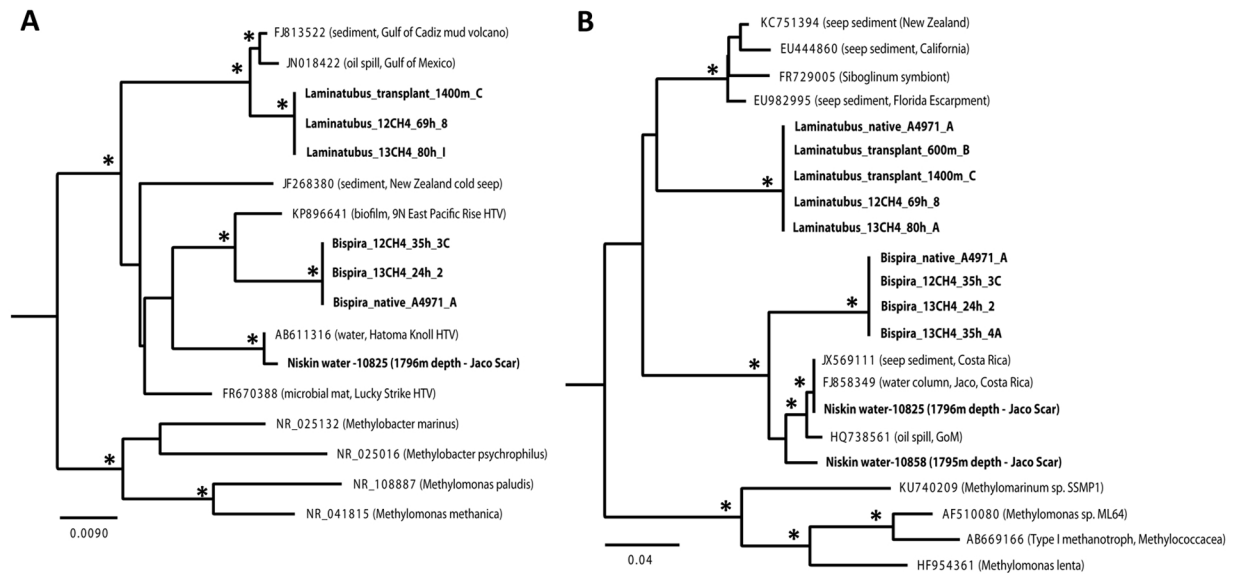


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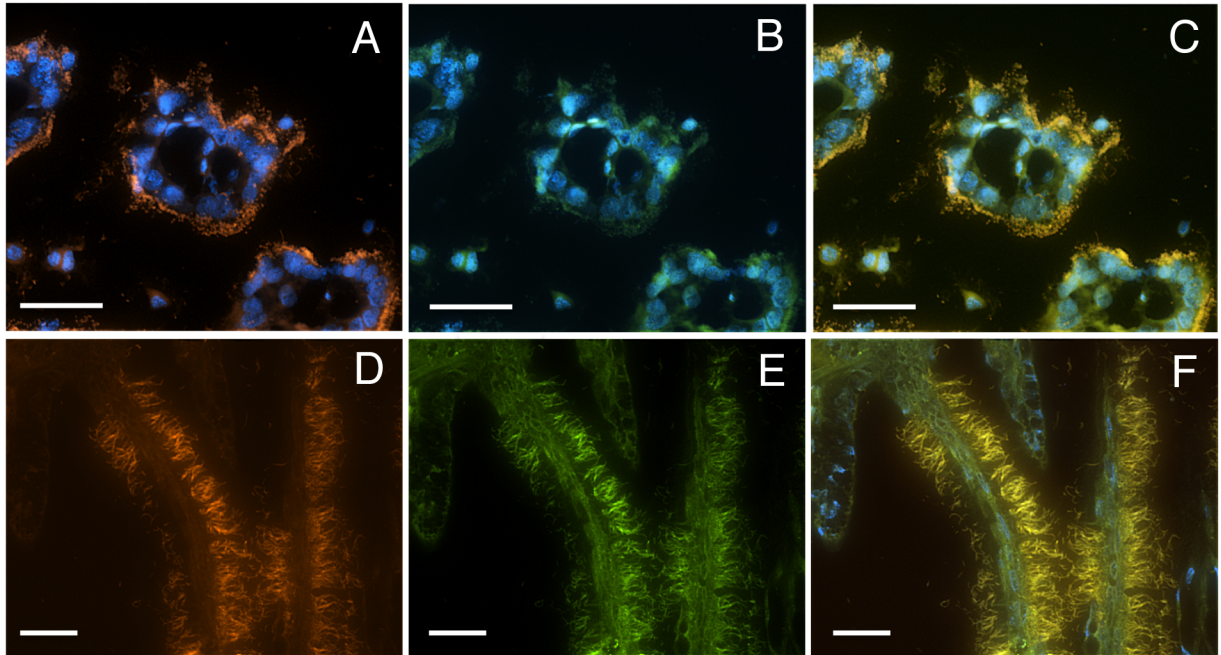


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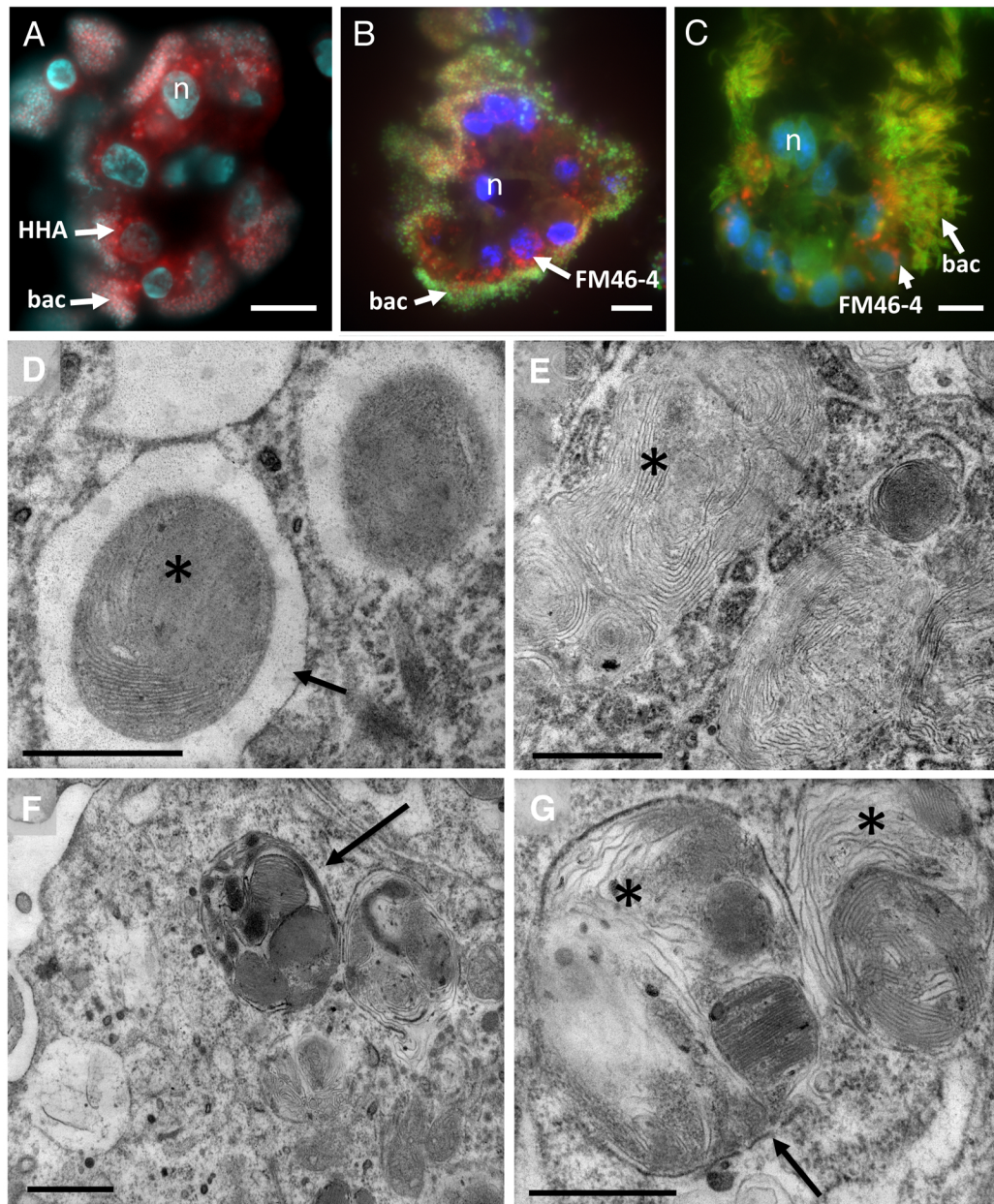


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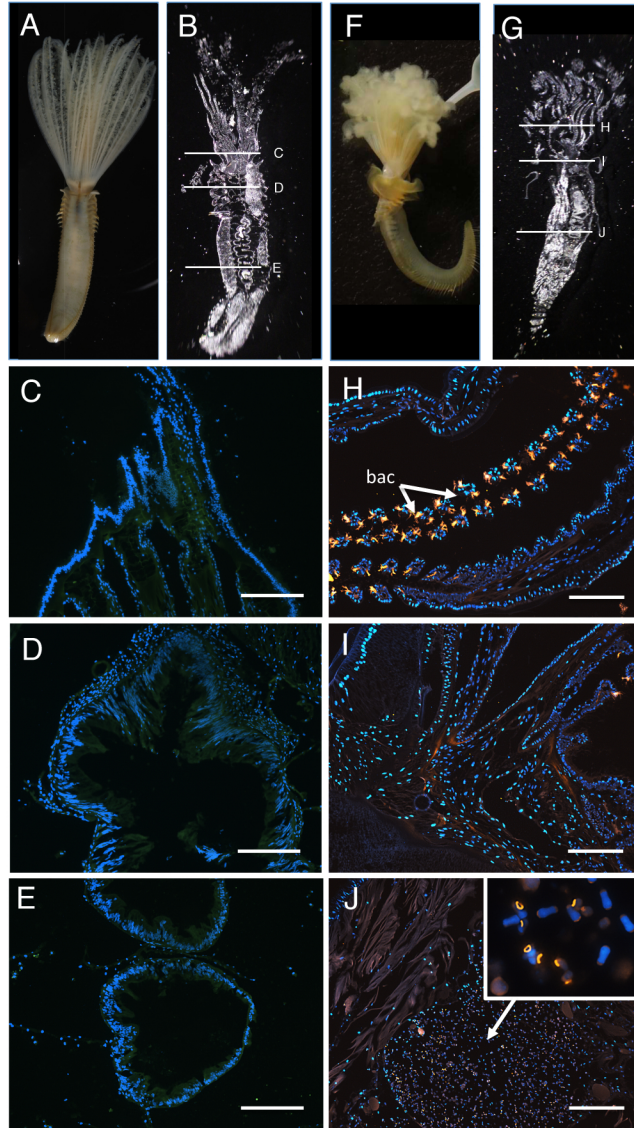


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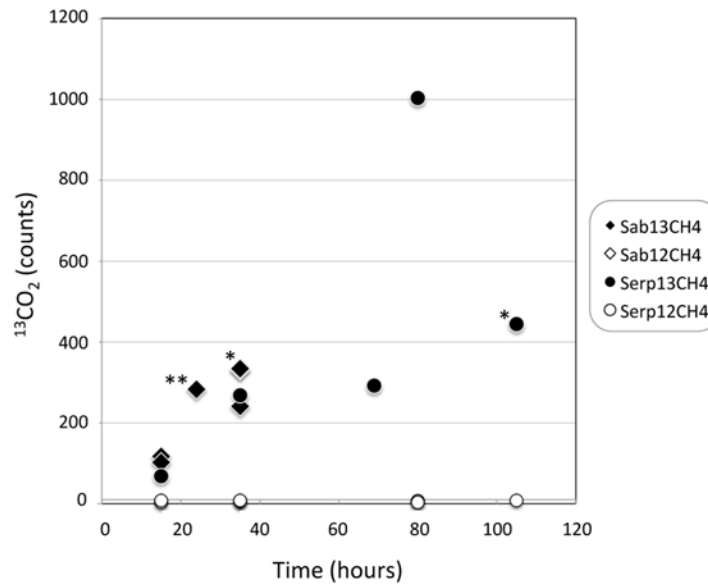


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