Polar Biology (2019) 42:2305–2312
https://doi.org/10.1007/s00300-019-02599-w

SHORT NOTE

# Bacterial endosymbiont of *Oligobrachia* sp. (Frenulata) from an active mud volcano in the Canadian Beaufort Sea

**Yung Mi Lee[1] · Hyun-Ju Noh[1,2] · Dong-Hun Lee[3] · Jung-Hyun Kim[1] · Young Keun Jin[1] · Charles Paull[4]**

Received: 19 March 2019 / Revised: 21 October 2019 / Accepted: 29 October 2019 / Published online: 13 November 2019


## Abstract

Siboglinid tubeworms of the genus *Oligobrachia* that thrive in obligatory association with endosymbionts have been predominantly observed in Arctic and high-latitude Atlantic cold seeps. Metabolic features of endosymbionts provide fundamental understanding for the survival strategy of tubeworms in cold seeps. However, no information on the bacterial endosymbionts of tubeworms from the Canadian Beaufort Sea has been available until now. In this study, we investigated the phylogeny and metabolic potential of a bacterial endosymbiont of siboglinid tubeworms from an active mud volcano in the Canadian Beaufort Sea using Illumina MiSeq sequencing of 16S rRNA gene amplicons. The siboglinid tubeworm shared 99.9% 18S rRNA gene sequence similarity with *Oligobrachia haakonmosbiensis* and 99.7–99.8% mitochondrial cytochrome C oxidase subunit I gene similarity with members of *Oligobrachia* sp. CPL-clade and was designated '*Oligobrachia* sp. BS1.' The endosymbiont of *Oligobrachia* sp. BS1, which belongs to the Gammaproteobacteria, was most closely related to endosymbionts of *Oligobrachia* sp. CPL-clade, revealing the close relationships between the endosymbionts and their hosts. The bacterial endosymbiont of *Oligobrachia* sp. BS1 contained the key gene required for sulfur oxidation, *aprA* gene encoding the α-subunit of adenosine 1,5-phosphosulfate reductase, suggesting that this endosymbiont is capable of using sulfide as an energy source. The bacterial endosymbiont of an *Oligobrachia* species from an active mud volcano in the Canadian Beaufort Sea presented here expands our knowledge of the identities and thiotrophic metabolism of endosymbionts that are associated with hosts that dominate a wide range of methane seep habitats in the Arctic.

**Keywords** Siboglinid tubeworm · *Oligobrachia* · Endosymbiont · Canadian Beaufort Sea · Arctic · aprA gene

## Introduction

The family Siboglinidae contains tube-dwelling annelids that do not have a mouth or gut, and therefore rely on endosymbiotic bacteria for their nutrition and energy needs (Felbeck 1981; Southward et al. 1986). The worms supply oxygen, carbon dioxide, hydrogen sulfide, or methane to the endosymbionts inhabiting their trophosome (the symbiont-housing organ) via the circulatory system, and the endosymbionts produce organic matter for the host, usually through carbon fixation by the oxidation of reduced compounds (Felbeck 1981; Cavanaugh 1985). The tubes function as a bridge between the reducing deep sediments and the oxygenated water above the sediments, engineering the ecosystem with bioturbation and changing the sediment geochemistry (Dando et al. 2008). They also function as structural supports, allowing the worms to maintain a position spanning the oxic–anoxic interface (López-García et al. 2002; Stewart et al. 2005; Duperron et al. 2009). Among the three lineages

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00300-019-02599-w) contains supplementary material, which is available to authorized users.

✉ Yung Mi Lee
ymlee@kopri.re.kr

[1] Division of Polar Life Sciences, Korea Polar Research Institute, 26 Songdomirae-ro, Yeonsu-gu, Incheon 21990, Republic of Korea

[2] Inha University, 100 Inha-ro, Nam-gu, Incheon 22212, Republic of Korea

[3] Hanyang University, 55 Hanyangdaehak-ro, Sangrok-gu, Ansan 15588, Republic of Korea

[4] Monterey Bay Aquarium Research Institute, 7700 Sandholdt Rd, Moss Landing, CA 95039, USA

of the family Siboglinidae (Vestimentifera, Frenulata, and Osedax), Frenulata has highest diversity and is frequently recovered from cold seep environments (Halanych et al. 2001; Lösekann et al. 2008; Thornhill et al. 2008; Hilário et al. 2011; Rodrigues et al. 2011). Because the fluxes from cold seeps vary from diffusive to slow advective and/or pulsed rates, tubeworms inhabiting cold seeps may be flexible in the endosymbionts that they harbor to accommodate these environmental complexities (Naganuma et al. 2005; Thornhill et al. 2008). However, very little is known about the diversity of the bacterial endosymbionts of Frenulata because sampling is constrained by the small size of the individuals and their relatively inaccessible habitats (Kimura et al. 2003; Halanych 2005; Naganuma et al. 2005; Kubota et al. 2007; Lösekann et al. 2008; Thornhill et al. 2008; Hilário et al. 2011; Rodrigues et al. 2011; Sen et al. 2018).

Among the Frenulata, members of the genus *Oligobrachia* have been predominantly observed in Arctic and high-latitude Atlantic cold seeps, including the Haakon Mosby mud volcano (HMMV) in the Barents Sea and pingo and crater sites in the Norwegian Sea and Laptev Sea (Smirnov 2000; Paull et al. 2015; Sen et al. 2018). An exception is *O. mashikoi*, which was from the East Sea (Kimura et al. 2003). The endosymbionts of *O. haakonmosbiensis*, members of *Oligobrachia* sp. CPL-clade (which is named based on cytochrome c oxidase subunit I gene [*COI*] sequences from Arctic and high-latitude Atlantic cold seeps), and *O. mashikoi* all belong to the Gammaproteobacteria and contain functional genes such as those encoding ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisCO; *cbbL* and *cbbM*) and/or adenosine 1,5-phosphosulfate (APS) reductase (*aprA*), which are involved in chemoautotrophic sulfur oxidation (Kimura et al. 2003; Lösekann et al. 2008; Sen et al. 2018). High abundances of tubeworms that are closely related to *O. haakonmosbiensis* based on *COI* gene sequences (~ 97% identity) and highly negative $\delta^{13}C$ values in the bulk tissue of worms (− 55.0‰ Vienna Pee Dee Belemnite, VPDB) have been reported at an active mud volcano in the Canadian Beaufort Sea, supporting the notion that tubeworms in the genus *Oligobrachia* represent important chemosynthetic megafauna of Arctic seeps (Paull et al. 2015; Sen et al. 2018). However, no molecular analysis of the bacterial endosymbionts of the tubeworms or the metabolic potential of endosymbionts from the Canadian Beaufort Sea has been performed until now. These findings will extend our knowledge of the endosymbionts diversity and provide valuable information to better understand their symbiotic relationships with their hosts (*Oligobrachia*) across different geographic locations and settings in the Arctic.

First, we performed a phylogenetic analysis of the siboglinid tubeworm collected from an active mud volcano in the Canadian Beaufort Sea based on its 18S rRNA and *COI* genes. We then investigated the bacterial endosymbionts of the tubeworm, using Illumina MiSeq sequencing of 16S rRNA gene amplicons. We also examined the bacterial *aprA* gene involved in thiotrophy to confirm the sulfur-metabolizing capacity inferred from the phylogenetic analysis of 16S rRNA sequences.

## Materials and methods

### Site description and sampling

Tubeworms were collected with the push core (ARA08C-DIVE104-10) of a remotely operated vehicle operated by the Monterey Bay Aquarium Research Institute at a mud volcano on the continental slope of the Beaufort Sea (MV420, 70.791779°N, 135.55592°W, 420 m water depth) in September 2017 during the ARA08C expedition on the R/V ARAON (Fig. 1 and Online Resource 1). Tubeworms were about 12 to 20 cm in length. The color of tube was divided into black, red, and grayish white from the anterior to posterior part of the tube (Lee et al. 2019 and Online Resource 1). On board, the tubeworms were washed with distilled water and stored frozen at − 80 °C until analysis. The presence of methane, gas bubbles, and gas hydrates was observed in the proximity of the seafloor on top of MV420. Particularly, geochemical properties showed steep gradients in chloride and sulfate concentration with unusually low chlorinities (<210 mM) and sulfate (<0.1 mM) occurring within a few centimeters below the seafloor at MV420 (Paull et al. 2015).

### DNA extraction, PCR amplification, and sequencing

The worm was taken out from tube by holding down the posterior end of the tube with a pair of forceps and squeezing the tube behind the worm under a microscope. To obtain sufficient biomass for DNA extraction, worms obtained from four individuals were pooled. The worms were washed three times with autoclaved water. The genomic DNA was extracted using an Exgene™ Soil SV mini kit (Cambio, UK). The 18S rRNA gene and mitochondrial *COI* gene of the host were amplified with the primer pairs G01/G07 and LCO1490/HCO2198, respectively (Folmer et al. 1994; Wada and Satoh 1994), and sequenced with the same primers, on an ABI 3700 capillary sequencer (Applied Biosystem, CA) at Cosmo Genetech Co., Ltd (Korea). The V4–V5 region of the bacterial 16S rRNA gene was amplified with PCR using the primer pair 515F/926R (Walters et al. 2016) and the amplicons were sequenced at Integrated Microbiome Resource at Dalhousie University, Canada (https://cgeb-imr.ca) using the paired-end (2 × 300 bp) Illumina MiSeq system (Illumina, USA).

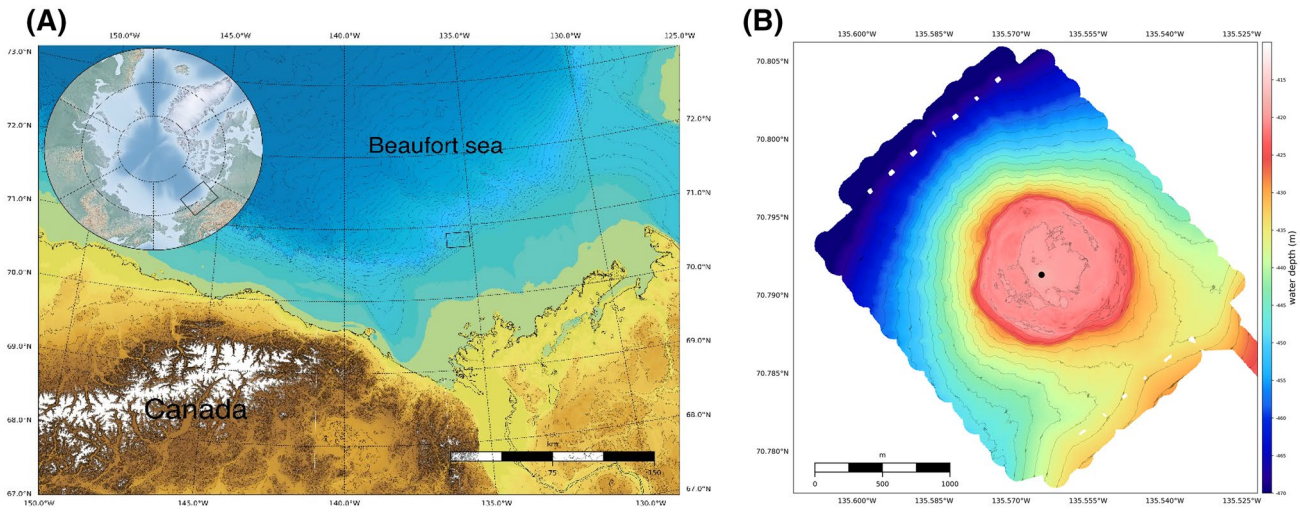


**Fig. 1** Bathymetric map of the continental slope of the Canadian Beaufort Sea (**a**) and the mud volcano at a water depth of 420 m (**b**). The black box in the globe inset represents the study area. The black dot indicates the siboglinid tubeworm sampling site

### aprA and pmoA gene cloning and sequencing

The *aprA* gene was amplified with the aps1F/aps4R primers (Blazejak et al. 2006) and *pmoA* gene encoding particulate methane monooxygenase was amplified with the A189gc and mb661 primers (Costello and Lidstrom 1999). The PCR product of *aprA* gene was cloned into the pGEM®-T Easy Vector (Promega, WI) according to the manufacturer's instructions, and five randomly selected clones were sequenced with the M13F/M13R primers at Cosmo Genetech Co., Ltd (Korea).

### Sequence processing

The paired-end sequences were trimmed based on the quality scores with Sickle (Schirmer et al. 2015), followed by error correction with BayesHammer (Nikolenko et al. 2013). The resulting quality-trimmed and error-corrected paired-end sequences were assembled with PANDAseq (Masella et al. 2012). Further sequence processing steps were performed according to MiSeq SOP (https://www.mothur.org/wiki/MiSeq_SOP) in the mothur program (Schloss et al. 2009). The assembled sequences were aligned against a SILVA alignment (https://www.arb-silva.de/), and then denoised with the 'pre.cluster' command. Chimeric sequences were removed with the 'chimera.uchime' command in de novo mode (Edgar et al. 2011). The sequences were further clustered into operational taxonomic units (OTUs) with 97% sequence similarity using the 'opticlust' clustering algorithm. The taxonomic assignments for each OTU were determined with sequence similarity searches against the EzBioCloud database (Yoon et al. 2017). 16S rRNA amplicon sequence data were deposited in the Sequence Read Archive at the National Center for Biotechnology Information (NCBI) under BioProject number PRJNA438412. The 18S rRNA and *COI* gene sequences of the host and the *apr*A gene sequences of the endosymbionts were submitted to NCBI GenBank under accession numbers MH393882 and MH393883, and MK598691–MK598695, respectively.

### Phylogenetic analyses

Phylogenetic trees of the host based on the 18S rRNA and *COI* gene sequences and of the bacterial symbiont based on 16S rRNA gene sequences were constructed using the maximum-likelihood (ML) algorithm (Felsenstein 1981) in MEGA X (Kumar et al. 2018). The robustness of the tree topologies was assessed by bootstrap analyses based on 1000 replications of the sequences. Phylogenetic tree of the protein-coding *aprA* gene was generated by ML from the deduced amino acid sequences.

## Results and discussion

### Host phylogeny

The 18S rRNA gene sequence of the tubeworm collected at MV420 in the Canadian Beaufort Sea shared 99.9% sequence similarity to that of *O. haakonmosbiensis* from the HMMV in the Barents Sea (Lösekann et al. 2008) and 93.9% identity with that of *O. mashikoi* from the shallow muddy sediments of Tsukumo Bay, Japan (20–25 m water depth) (Kimura et al. 2003) and that of *Siboglinum fiordicum* from Ypsemund, a marine inlet near Bergen, Norway (Winnepenninckx et al. 1995). It also clustered with other

species of Frenulata (Online Resource 2). The *COI* gene sequence showed 99.7–99.8% similarity to those of members of the *Oligobrachia* sp. CPL-clade from the Norwegian Sea and Laptev Sea (Sen et al. 2018), and 96.0–96.6% similarity to those of *O. haakonmosbiensis*. Based on *COI* gene sequences, the tubeworm from MV420 formed monophyletic clade with the *Oligobrachia* sp. CPL-clade while it formed a distinct clade with *O. haakonmosbiensis* (Online Resource 2). Because the 18S rRNA gene sequences of *Oligobrachia* sp. CPL-clade, the most closely related group to *O. haakonmosbiensis* based on *COI* gene sequences, are not available in the public databases, their similarity could not be compared. However, the *COI* gene results were consistent with previous results that indicated that the Arctic and high-latitude North Atlantic seeps are occupied by at least two species of *Oligobrachia* and support the notion that the *Oligobrachia* sp. CPL-clade is observed in more polar regions than *O. haakonmosbiensis* (Sen et al. 2018). Based on our phylogenetic analyses of the 18S rRNA and *COI* gene sequences, we designated this tubeworm '*Oligobrachia* sp. BS1.' As the first phylogenetic analysis of the genus *Oligobrachia* to include a tubeworm from the Canadian Beaufort Sea, this study provides important information that extends our understanding of the distribution of *Oligobrachia* at Arctic seep sites.

## Bacterial endosymbiont of Oligobrachia sp. BS1

A total of 6957 bacterial sequences were obtained, and the sequence reads, excluding the singletons, were clustered into 51 OTUs. The bacterial communities in the worms were dominated by the class Gammaproteobacteria (97.1%) (Online Resources 3 and 4). Among the 31 gammaproteobacterial OTUs, OTU001 constituted 91.5% of the bacterial sequences, suggesting that OTU001 is the endosymbiont of *Oligobrachia* sp. BS1. This OTU was closely related to the endosymbionts of *Oligobrachia* sp. CPL-clade, with sequence similarities of 98.4–99.4%, followed by the two phylotypes of *O. haakonmosbiensis* endosymbionts (16S rRNA gene sequence similarities of 98.4% and 97.8%), and it formed monophyletic clade with these sequences (Fig. 2 and Online Resource 5). It shared 16S rRNA gene sequence similarities of 94.4% and 90.3–91.3% with the endosymbionts of *S. fiordicum* and *O. mashikoi*, respectively. The endosymbionts of *S. fiordicum* and *O. mashikoi* formed a distinct clade with those of *Oligobrachia* sp. BS1, *Oligobrachia* sp. CPL-clade, and *O. haakonmosbiensis* (Fig. 2).

The *aprA* gene of the *Oligobrachia* sp. BS1 endosymbiont was successfully amplified and clustered with the *apr*A sequences from the endosymbionts of *Oligobrachia* sp. CPL-clade and *O. haakonmosbiensis* (Fig. 3). Molecular characterization of the *aprA* gene and electron micrographs of the rod-shaped bacteria, typical of siboglinid-associated sulfur-oxidizing bacteria in *Oligobrachia sp.* CPL-clade, suggested that these recently reported siboglinid tubeworms harbor thiotrophic endosymbionts (Sen et al. 2018). The molecular analysis of genes such as *cbbL* and *cbbM* for autotrophy and *aprA* for thiotrophy suggested that the endosymbionts of *O. haakonmosbiensis* are chemoautotrophic sulfur-oxidizing bacteria, although thiotrophy cannot explain the negative $^{13}C$ values for the bulk tissue and specific fatty acids of *O. haakonmosbiensis* (Lösekann et al. 2008). A possible explanation suggested earlier for these unusually negative $^{13}C$ values was the presence of dual symbioses with sulfur and methane oxidizers (Lösekann et al. 2008). However, despite the analysis of a large number of 16S rRNA gene sequences and genes involved in methanotrophy, no evidence for methane oxidizers among the endosymbionts of *O. haakonmosbiensis* was found (Lösekann et al. 2008). The phylogenetic proximity of the endosymbionts of *S. fiordicum* to known thiotrophs and the presence of sulfur-oxidizing activity in the trophosome region of *S. fiordicum* also support the thiotrophic potential of the endosymbionts of *S. fiordicum* (Dando et al. 1986; Southward et al. 1986; Thornhill et al. 2008). In contrast, it has been suggested that the endosymbiont of *O. mashikoi* is a possible methanotroph based on a phylogenetic analysis of the 16S rRNA gene that included the sequences of strains whose metabolic modes are known (Kimura et al. 2003). However, the endosymbiont of *O. mashikoi* fell between the thiotrophs and methanotrophs on a phylogenetic tree based on 16S rRNA gene sequences (Kimura et al. 2003). Moreover, no further evidence of methanotrophy, such as methane monooxygenase genes or the intracytoplasmic stacked membranes found in methanotrophs, was detected in the endosymbiont of *O. mashikoi* (Cavanaugh et al. 1992; Kimura et al. 2003). The close phylogenetic relationships between the endosymbionts of *O. mashikoi* and those of *S. fiordicum* based on their 16S rRNA genes detected in the present study supports the proposition that the endosymbionts of *O. mashikoi* are thiotrophic rather than methanotrophic.

The application of high-throughput 16S rRNA gene sequencing in this study to identify the endosymbionts of *Oligobrachia* sp. BS1 reduced the possibility of failing to detect methane oxidizers that have been suggested to explain the low $\delta^{13}C$ values of *O. haakonmosbiensis* and its endosymbionts (Lösekann et al. 2008). In the present study, no OTUs assigned to known methanotrophs were detected with the failure of *pmoA* gene amplification. Furthermore, bulk carbon isotopic compositions and the $^{13}C$ values of specific fatty acids such as $C_{16:1\omega7}$ and $C_{18:1\omega7}$ in the worm of *Oligobrachia* sp. BS1 indicated that sulfur-oxidizing symbionts inhabiting *Oligobrachia* sp. BS1 utilize anaerobic oxidation of methane (AOM)-derived dissolved inorganic carbon as a main carbon source (Lee et al. 2019). We cannot exclude the possibility that previously unknown methanotrophic

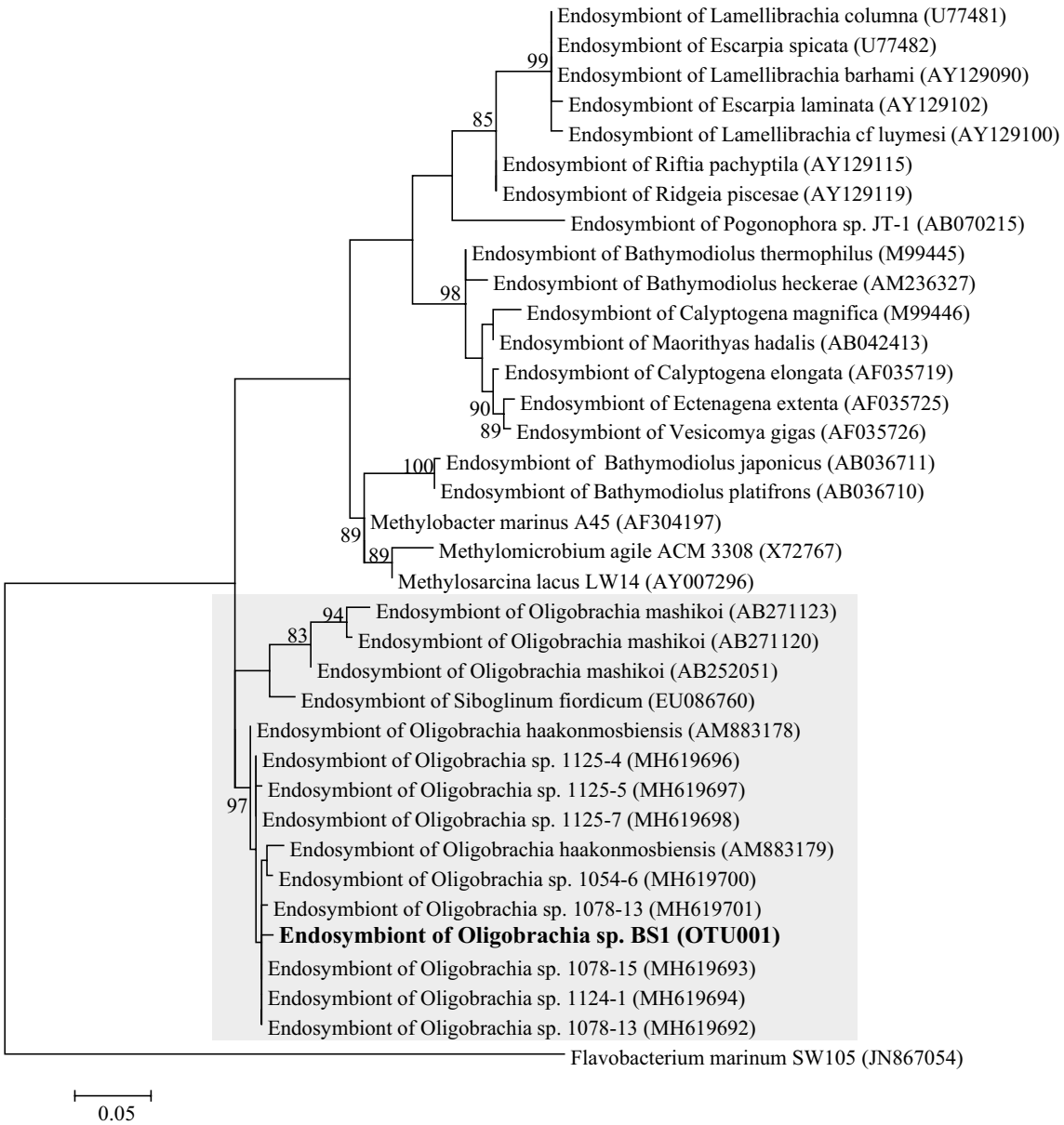


**Fig. 2** Phylogenetic tree of endosymbionts based on 16S rRNA gene sequences. The tree was constructed with a heuristic search using the maximum-likelihood criterion. Bold sequence was obtained with MiSeq sequencing in this study. Sequences of endosymbionts from other studies are indicated with GenBank accession numbers, together with the hosts from which the sequences were obtained. Percentage bootstrap support (> 60%) based on 1000 resamplings is given at each node. The gray box contains the sequences from endosymbionts of the genera *Oligobrachia* and *Siboglinum*. *Flavobacterium marinum* was used as the outgroup. Scale bar, 5 nucleotide substitutions per 100 nucleotides

endosymbionts occur in *Oligobrachia* sp. BS1 and other Frenulata species until the characterization of endosymbionts is complete. In addition, the application of fluorescence in situ hybridization experiment using the specific probes targeting OTU001 and its *aprA* gene with sulfur isotopic composition which was not performed in this study will give more direct evidence for the endosymbiont identity and its metabolic potential. However, molecular results of this study and isotopic compositions of bulk carbon and fatty acids performed with replication (Lee et al. 2019) seem to support the presence of sulfur-oxidizing *Oligobrachia* sp. BS1 endosymbionts that incorporate the $^{13}$C-depleted inorganic carbon produced by AOM.

In summary, this is the first report of a bacterial endosymbiont of siboglinid tubeworms collected at an active mud volcano in the Canadian Beaufort Sea, designated '*Oligobrachia* sp. BS1,' and its metabolic potential. High-throughput 16S rRNA gene sequence data combined with sequences

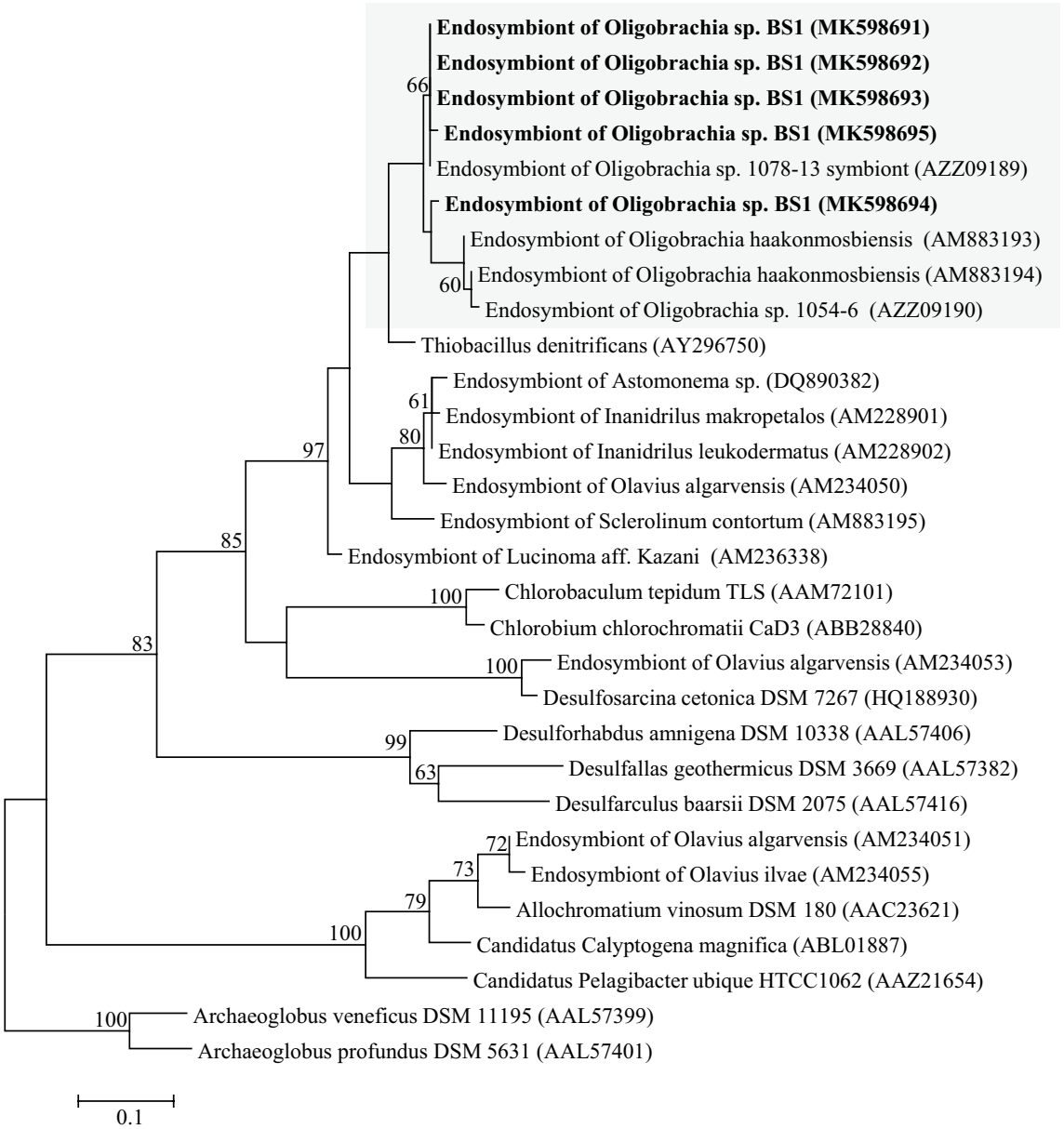


**Fig. 3** Maximum-likelihood tree based on derived amino acid sequences of *aprA* gene. Bold sequences were obtained by cloning in this study. Percentage bootstrap support (> 60%) based on 1000 resamplings is given at each node. *Archaeoglobus veneficus* and *A. profundus* were used as the outgroups. Bar, 1 nucleotide substitution per 10 nucleotides

of the *aprA* gene, which is involved in thiotrophy, show that *Oligobrachia* sp. BS1 harbors a thiotrophic endosymbiont closely related to other endosymbionts of the genus of *Oligobrachia*, extending our knowledge of the endosymbionts of siboglinid tubeworms predominant at Arctic seep sites.

**Acknowledgements** This research was supported by the Korea Polar Research Institute (Grant Nos. PM17050 and PM19050), the Korea Institute of Marine Science and Technology Promotion (Grant No. 20160247), and the David and Lucile Packard Foundation. We thank the captain and crew of RV ARAON for their support at sea. We are very grateful to Dale Graves, Frank Flores, and Roberto Gwiazda at Monterey Bay Aquarium Research Institute for the operation of the remotely operated vehicle and sample collection. We also thank Yeonjin Choi, Sang-Hee Kim, and Binu M. Tripathi of the Korea Polar Research Institute for providing maps, help in removing the worms from tubes, and stimulating discussions. We thank Janine Miller, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

## Compliance with ethical standards

**Conflict of interests** There are no conflicts of interest to declare.

## References


Blazejak A, Kuever J, Erséus C, Amann R, Dubilier N (2006) Phylogeny of 16S rRNA, ribulose 1,5-bisphosphate carboxylase/oxygenase, and adenosine 5′-phosphosulfate reductase genes from gamma- and alphaproteobacterial symbionts in gutless marine worms (Oligochaeta) from Bermuda and the Bahamas. Appl Environ Microbiol 72:5527–5536. https://doi.org/10.1128/aem.02441-05

Cavanaugh CM (1985) Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. Biol Soc Wash Bull 6:373–388

Cavanaugh CM, Wirsen CO, Jannasch HW (1992) Evidence for methylotrophic symbionts in a hydrothermal vent mussel (Bivalvia: Mytilidae) from the mid-Atlantic ridge. Appl Environ Microbiol 58:3799–3803

Costello AM, Lidstrom ME (1999) Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Appl Environ Microbiol 65:5066–5074

Dando PR, Southward AJ, Southward EC, Barrett RL (1986) Possible energy sources for chemoautotrophic prokaryotes symbiotic with invertebrates from a Norwegian fjord. Ophelia 26:135–150. https://doi.org/10.1080/00785326.1986.10421984

Dando PR, Southward AJ, Southward EC, Lamont P, Harvey R (2008) Interactions between sediment chemistry and frenulate pogonophores (Annelida) in the north-east Atlantic. Deep-Sea Res PT I 55:966–996

Duperron S, Beer DD, Zbinden M, Boetius A, Schipani V, Kahil N, Gaill F (2009) Molecular characterization of bacteria associated with the trophosome and the tube of *Lamellibrachia* sp., a siboglinid annelid from cold seeps in the eastern Mediterranean. FEMS Microbiol Ecol 69:395–409. https://doi.org/10.1111/j.1574-6941.2009.00724.x

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Felbeck H (1981) Chemoautotrophic potential of the hydrothermal vent tube worm, *Riftia pachyptila* Jones (Vestimentifera). Science 213:336–338

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376. https://doi.org/10.1007/bf01734359

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Halanych KM (2005) Molecular phylogeny of siboglinid annelids (a.k.a. pogonophorans): a review. Hydrobiologia 535:297–307. https://doi.org/10.1007/s10750-004-1437-6

Halanych KM, Feldman RA, Vrijenhoek RC (2001) Molecular evidence that *Sclerolinum brattstromi* is closely related to vestimentiferans, not to frenulate pogonophorans (Siboglinidae, Annelida). Biol Bull 201:65–75. https://doi.org/10.2307/1543527

Hilário A, Capa M, Dahlgren TG, Halanych KM, Little CTS, Thornhill DJ, Verna C, Glover AG (2011) New perspectives on the ecology and evolution of siboglinid tubeworms. PLoS ONE 6(2):e16309. https://doi.org/10.1371/journal.pone.0016309

Kimura H, Sato M, Sasayama Y, Naganuma T (2003) Molecular characterization and in situ localization of endosymbiotic 16S ribosomal RNA and RuBisCO genes in the Pogonophoran tissue. Mar Biotechnol 5:261–269. https://doi.org/10.1007/s10126-002-0073-2

Kubota N, Kanemori M, Sasayama Y, Aida M, Fukumori Y (2007) Identification of endosymbionts in *Oligobrachia mashikoi* (Siboglinidae, Annelida). Microbes Environ 22:136–144. https://doi.org/10.1264/jsme2.22.136

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Lee D-H, Kim J-H, Lee YM, Jin YK, Paull C, Kim D, Shin K-H (2019) Chemosynthetic bacterial signatures in Frenulata tubeworm *Oligobrachia* sp. in an active mud volcano of the Canadian Beaufort Sea. Mar Ecol Prog Ser 628:95–104. https://doi.org/10.3354/meps13084

López-García P, Gaill F, Moreira D (2002) Wide bacterial diversity associated with tubes of the vent worm *Riftia pachyptila*. Environ Microbiol 4:204–215. https://doi.org/10.1046/j.1462-2920.2002.00286.x

Lösekann T, Robador A, Niemann H, Knittel K, Boetius A, Dubilier N (2008) Endosymbioses between bacteria and deep-sea siboglinid tubeworms from an Arctic cold seep (Haakon Mosby Mud Volcano, Barents Sea). Environ Microbiol 10:3237–3254. https://doi.org/10.1111/j.1462-2920.2008.01712.x

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD (2012) PANDAseq: paired-end assembler for illumina sequences. BMC Bioinform 13:31. https://doi.org/10.1186/1471-2105-13-31

Naganuma T, Elsaied HE, Hoshii D, Kimura H (2005) Bacterial endosymbioses of gutless tube-dwelling worms in nonhydrothermal vent habitats. Mar Biotechnol 7:416–428. https://doi.org/10.1007/s10126-004-5089-3

Nikolenko SI, Korobeynikov AI, Alekseyev MA (2013) BayesHammer: Bayesian clustering for error correction in single-cell sequencing. BMC Genomics 14:S7. https://doi.org/10.1186/1471-2164-14-s1-s7

Paull CK, Dallimore SR, Caress DW, Gwiazda R, Melling H, Riedel M, Jin YK, Hong JK, Kim Y-G, Graves D, Sherman A, Lundsten E, Anderson K, Lundsten L, Villinger H, Kopf A, Johnson SB, Hughes-Clarke J, Blasco S, Conway K, Neelands P, Thomas H, Côté M (2015) Active mud volcanoes on the continental slope of the Canadian Beaufort Sea. Geochem Geophys Geosyst 16:3160–3181. https://doi.org/10.1002/2015gc005928

Rodrigues CF, Hilário A, Cunha MR, Weightman AJ, Webster G (2011) Microbial diversity in Frenulata (Siboglinidae, Polychaeta) species from mud volcanoes in the Gulf of Cadiz (NE Atlantic). Antonie Van Leeuwenhoek 100:83–98. https://doi.org/10.1007/s10482-011-9567-0

Schirmer M, Ijaz UZ, D'Amore R, Hall N, Sloan WT, Quince C (2015) Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform. Nucleic Acids Res 43:e37. https://doi.org/10.1093/nar/gku1341

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/aem.01541-09

Sen A, Duperron S, Hourdez S, Piquet B, Léger N, Gebruk A, Le Port A-S, Svenning MM, Andersen AC (2018) Cryptic frenulates are the dominant chemosymbiotrophic fauna at Arctic and high latitude Atlantic cold seeps. PLoS ONE 13:e0209273. https://doi.org/10.1371/journal.pone.0209273

Smirnov RV (2000) Two new species of Pogonophora from the Arctic mud volcano off northwestern Norway. Sarsia 85:141–150. https://doi.org/10.1080/00364827.2000.10414563

Southward AJ, Southward EC, Dando PR, Barrett RL, Ling R (1986) Chemoautotrophic function of bacterial symbionts in small

Pogonophora. J Mar Biol Assoc UK 66:415–437. https://doi.org/10.1017/S0025315400043046

Stewart FJ, Newton ILG, Cavanaugh CM (2005) Chemosynthetic endosymbioses: adaptations to oxic–anoxic interfaces. Trends Microbiol 13:439–448. https://doi.org/10.1016/j.tim.2005.07.007

Thornhill DJ, Wiley AA, Campbell AL, Bartol FF, Teske A, Halanych KM (2008) Endosymbionts of *Siboglinum fiordicum* and the phylogeny of bacterial endosymbionts in Siboglinidae (Annelida). Biol Bull 214:135–144. https://doi.org/10.2307/25066670

Wada H, Satoh N (1994) Phylogenetic relationships among extant classes of echinoderms, as inferred from sequences of 18S rDNA, coincide with relationships deduced from the fossil record. J Mol Evol 38:41–49. https://doi.org/10.1007/bf00175494

Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, Gilbert JA, Jansson JK, Caporaso JG, Fuhrman JA, Apprill A, Knight R (2016) Improved bacterial 16S rRNA gene (V4 and V4–5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. mSystems 1:e00009–e000015. https://doi.org/10.1128/mSystems.00009-15

Winnepenninckx B, Backeljau T, Wachter RD (1995) Phylogeny of protostome worms derived from 18S rRNA sequences. Mol Biol Evol 12:641–649. https://doi.org/10.1093/oxfordjournals.molbev.a040243

Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755