Teredinibacter haidensis sp. nov., Teredinibacter purpureus sp. nov. and Teredinibacter franksiae sp. nov., marine, cellulolytic endosymbiotic bacteria isolated from the gills of the wood-boring mollusc Bankia setacea (Bivalvia: Teredinidae) and emended description of the genus Teredinibacter

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Abstract
Here, we describe three endosymbiotic bacterial strains isolated from the gills of the shipworm, Bankia setacea (Teredinidae: Bivalvia). These strains, designated as Bs08T, Bs12T and Bsc2T, are Gram-stain-negative, microaerobic, gammaproteobacteria that grow on cellulose and a variety of substrates derived from lignocellulose. Phenotypic characterization, phylogeny based on 16S rRNA gene and whole genome sequence data, amino acid identity and percentage of conserved proteins analyses, show that these strains are novel and may be assigned to the genus Teredinibacter. The three strains may be differentiated and distinguished from other previously described Teredinibacter species based on a combination of four characteristics: colony colour (Bs12T, purple; others beige to brown), marine salt requirement (Bs12T, Bsc2T and Teredinibacter turnerae strains), the capacity for nitrogen fixation (Bs08T and T. turnerae strains) and the ability to respire nitrate (Bs08T). Based on these findings, we propose the names Teredinibacter haidensis sp. nov. (type strain Bs08T=ATCC TSD-121T=KCTC 62964T), Teredinibacter purpureus sp. nov. (type strain Bs12T=ATCC TSD-122T=KCTC 62965T) and Teredinibacter franksiae sp. nov. (type strain Bsc2T=ATCC TSD-123T=KCTC 62966T).

Cellulose is considered to be the most abundant biological material on Earth [1] and numerous organisms have exploited it for their nutritional needs [2]. It has been estimated that approximately 40% of bacteria for which genome sequence data is available encode a copy of a putative cellulase gene; however, only around 4% of these microbes are truly cellulolytic [3]. A number of cellulolytic bacteria form symbiotic partnerships with herbivorous animals. In terrestrial environments these animals include xylophagous insects such as termites and wood-eating roaches as well as a variety of herbivorous vertebrates, all of which harbour cellulolytic bacteria in their gut [4].

In marine environments, bivalves of the family Teredinidae (shipworms) are among the best-known hosts of cellulolytic bacteria [5]. Shipworms are worm-like wood-boring and wood-feeding bivalves that may be found in all oceans of the world, as well as in a variety of brackish to fresh water bays, rivers and estuaries [5]. Unlike the previously mentioned terrestrial animals, shipworms lack a conspicuous microbial community in the portion of the digestive tract in which wood...
digestion is thought to occur [6]. Instead, shipworms maintain dense populations of intracellular cellulolytic bacteria in specialized cells in their gills called bacterioocytes [7, 8]. Shipworm gill endosymbionts have been shown to secrete a variety of lignocellulolytic enzymes that are selectively transported to the cecum where they liberate simple sugars from the ingested wood that can readily be absorbed by the host. This form of nutritional symbiosis, wherein the principal enzyme producers are not in direct contact with the substrate, has only been observed in shipworms [9].

Shipworm gill endosymbionts are unique in that they are among the few intracellular endosymbionts that have been grown successfully in pure culture [10, 11]. To date, three of these endosymbiotic species have been formally described. Two of these, Teredinibacter turnerae and Teredinibacter waterburyi are microaerophilic cellulolytic bacteria placed within the family Cell vibrio naceae [11, 12]. The former is diazotrophic and is widely distributed within the members of the family Teredinidae [13], while the latter does not fix dinitrogen and has so far been found only in association with the feathery shipworm, Bankia setacea [12]. Consistent with their proposed function in supporting host xylotrophy, the genomes of these two bacterial species contain numerous genes encoding secreted carbohydrate-active enzymes (CAZymes) with predicted activities against plant cell-wall components [9, 14]. In addition to these cellulolytic endosymbionts, a sulfur-oxidizing chemosynthetic, nitrogen-fixing endosymbiont, Thi osocius teredinicola 2141T has been isolated from the gills of the giant mud-dwelling shipworm Kuphus polythalamius, indicating that non-cellulolytic bacteria can also form symbiotic partnerships with teredinids [15, 16].

In addition to T. waterburyi, three similar endosymbiont strains, designated Bs08T, Bs12T and Bsc2T were isolated from feathery shipworm Bankia setacea [9]. Together these four strains were shown to encode a significant portion of the bacterial genes detected in the gill metagenome of this host species, signifying that these bacteria are the dominant endosymbionts in the B. setacea gill community [9]. In this study, we characterize these bacteria and propose the names Teredinibacter haidensis sp. nov., Teredinibacter purpureus sp. nov. and Teredinibacter frankssiae sp. nov., with Bs08T, Bs12T and Bsc2T, as their respective type strains.

**ISOLATION**

Strains Bs08T, Bs12T and Bsc2T were isolated from the gill and cecum of the shipworm B. setacea collected from the Puget Sound, Washington State, USA as described by O'Connor et al. [9] and Altamia et al. [12]. Briefly, homogenates of gill or cecum tissues were streaked onto culture plates (motherplates) containing 1.0% Bacto agar shipworm basal medium (SBM) [10] at pH 7.0 or 8.0 supplemented with 0.2% w/v powdered cellulose (Sigmacell Type 101; Sigma-Aldrich) and 0.025% NH4 Cl. Plates were incubated at either 18 or 30 °C until individual colonies could be observed. To rapidly identify novel bacteria on the motherplates, 16S rRNA genes were amplified directly from the colonies using Phire Animal Tissue Direct PCR Kit (Finnzymes F-170S) using 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-TACG GYTACCTTGTTACGACTT-3’) primers [9] employing the manufacturer’s recommended protocol. Remnants of the colonies used for colony PCR were transferred to new plates and were further purified using at least two rounds of re-streaking to ensure clonal selection. On plates inoculated with either gill or cecum tissue homogenates colonies were initially slightly raised and translucent. However, upon prolonged incubation some colonies developed light brown or purple coloration.

Strain Bs08T was isolated from a motherplate inoculated with gill homogenate and incubated at 30°C. A purple colony growing on a motherplate incubated at 18°C was purified and designated as strain Bs12T. Strain Bsc2T was isolated from a motherplate inoculated with cecum homogenate and incubated at 18°C.

The 16S rRNA gene of Bsc2T is identical in sequence to that of symbiotic bacteria localized to the gills of B. setacea by metagenomic sequencing and fluorescent in situ hybridization [9] and to those previously amplified from gills by PCR [17]. Because Bsc2T was shown to be abundant in gills of B. setacea but has not been detected in cecum by in situ hybridization techniques [9] and because the cecum of B. setacea has previously been shown to be largely devoid of bacteria [6], we suggest that the presence of strain Bsc2T in cecum homogenates was likely due to contamination by neighbouring gill tissues during dissection.

For all the subsequent experiments, unless specified otherwise, strains were propagated in 6 ml of liquid SBM medium with either 0.2% cellulose (w/v) or 0.5% galactose (w/v) with 0.025% NH4 Cl in test tubes (18 mm × 150 mm) and shaken at 100 r.p.m. in an incubator set to 20°C. Preparation and revival of frozen stocks are detailed in Altamia et al. [12].

The ability of the strains to utilize carbon substrates was tested by transferring colonies to SBM liquid medium supplemented with 0.025% NH4 Cl or 5 mM NaNO3 with the following carbon sources and concentrations (% w/v): polysaccharides—cellulose (0.2%), carboxymethylcellulose (0.5%), D-galacto-D-mannan from locust bean (0.1%), β-D-glucan from barley (0.1%), lichenan (0.1%), starch (0.1%), pectin (0.1%), dextran (0.1%), curdlan (0.1%), pachymann (0.1%), laminarin (0.1%), pullulan (0.1%), chitin (0.1%) and chitosan (0.1%); sugars—cellobiose (0.5%), xylan (0.5%), sucrose (0.5%), xylose (0.5%) arabinose (0.5%), galactose (0.5%), glucose (0.5%); organic acids—formate (0.1%), acetate (0.1%), propionate (0.1%) and pyruvate (0.1%); and amines—N-acetylglucosamine (0.1%), gelatin (0.1%), yeast extract (0.1%) and casein hydrolysate (0.1%). All strains grew on cellulose, galactomannan, β-D-glucan, lichenan, pullulan, gelatin and casein hydrolysate and all sugars tested. Strains Bs08T, Bs12T and Bsc2T and previously described Teredinibacter species do not hydrolyse agar, distinguishing them from the closely related Saccharophagus degradans 2-40T [18] and Agarifytica rhodophytica strain 017T [19]. None of the tested strains grew on chitosan (see Table 1).
Table 1. Characteristics of strains Bs08<sup>T</sup>, Bs12<sup>T</sup>, Bsc2<sup>T</sup> and related type strains

| Strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---------|---|---|---|---|---|---|---|---|
| Cell shape | Rods | Rods | Rods | Rods | Rods | Pleomorphic | Rods | Rods |
| Cell length (µm) | 2–5 | 2–5 | 2–5 | 2–5 | 3–6 | 1–20 | 1.0–1.3 | 1.7–5.7 |
| Habitat | Endosymbiont of Bankia setacea | Endosymbiont of Bankia setacea | Endosymbiont of Bankia setacea | Endosymbiont of Bankia setacea | Widely occurring shipworm endosymbiont | Free-living, isolated from Spartina alterniflora | Free-living, isolated from surface seawater | Free-living, isolated from Glacilaria blodgettii |
| Marine salt (Mg, Ca) requirement | − | + | + | − | + | + | + | NR |
| Microaerobic | + | + | + | + | + | NR | NR | NR |
| Optimum temperature (range) for growth (°C) | 20 (15–30) | 20 (10–25) | 20 (15–20) | 20 (15–30) | 30–35 (~20–38)* | 30 (4–37) | 28–37 (15–37) | 28–33 (15–40) |
| Optimum pH (range) for growth | 8.0 (6.5–8.5) | 8.0 (6.5–8.5) | 8.0 (6.5–8.5) | 8.0 (6.5–8.5) | 8.5 (6.0–10.5) | 7.5 (4.5–10.0) | 7.0–8.0 (6.0–9.0) | 8.0 (6.5–8.5) |
| Optimum salinity (range) for growth (M NaCl) | 0.3 M (0.1–0.6 M) | 0.4 M (0.2–0.6 M) | 0.2 M (0.2–0.4 M) | 0.5 M (0.0–0.8 M) | 0.3 M (0.1–0.6 M) | 0.6 M (0.2–1.7 M) | 0.4 M (0.3–1.5 M) | 0.5 M (0.3–0.7 M) |
| Doubling time (h) | 5.2 | 8.8 | 11.5 | 7.2 | 9.7 | NR | NR | NR |
| Vitamin requirement | − | − | − | − | − | B cofactors stimulatory | − | NR |
| Colony colour | Beige | Purple | Beige | Beige | Beige | NR | NR | NR |
| Nitrogen fixation | + | − | − | − | + | − | − | − |
| Nitrate respiration | + | − | − | − | − | NR | NR | − |
| Cellulose hydrolysis | + | + | + | + | + | + | + | − |
| Agar hydrolysis | − | − | − | − | − | + | − | + |
| 0.1% Starch | + | − | − | − | − | + | NR | NR |
| 0.1% Pectin | + | + | + | + | + | NR | NR | NR |
| 0.1% Dextran | + | − | − | − | + | − | NR | NR |
| 0.1% Curdlan | + | − | − | − | + | NR | NR | NR |
| 0.1% Pachyman | + | − | − | − | + | NR | NR | NR |
| 0.1% Laminarin | + | − | − | − | + | NR | NR | NR |
| 0.1% Chitin | + | − | − | − | + | NR | NR | NR |

Continued
| Characteristic          | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|------------------------|----|----|----|----|----|----|----|----|
| 0.1% N-Acetyl-α-glucosamine | +  | −  | −  | +  | +  | NR | NR | NR |
| 0.5% Cellobiose         | +  | +  | +  | +  | +  | NR | −  | NR |
| 0.5% Xylan              | +  | +  | +  | +  | +  | +  | NR | NR |
| 0.5% Xylose             | +  | +  | +  | +  | +  | NR | −  | NR |
| 0.5% Arabinose          | +  | +  | +  | +  | NR | NR | NR | NR |
| 0.5% Galactose          | +  | +  | +  | +  | NR | NR | −  | +  |
| 0.5% Glucose            | +  | +  | +  | +  | +  | +  | NR | +  |
| 0.5% Sucrose            | +  | +  | +  | +  | +  | NR | −  | +  |
| 0.1% Formate            | −  | −  | −  | −  | −  | NR | NR | NR |
| 0.1% Acetate            | −  | −  | −  | −  | −  | +  | NR | +  |
| 0.1% Propionate         | −  | −  | −  | −  | −  | NR | NR | −  |
| 0.1% Pyruvate           | +  | +  | −  | +  | +  | NR | NR | NR |

*Data from [34].
The diazotrophic ability of these strains was tested by re-streaking colonies on SBM medium with 0.2% cellulose but without an added nitrogen source. Of these three strains, only Bs08T was found to be capable of nitrogen fixation. These results are supported by analyses of their respective genomes which indicate that only Bs08T encodes genes of the Nif operon which is diagnostic of nitrogen fixation [9]. It has been shown that endosymbionts of the shipworm *Lyrodus pedicellatus* fix nitrogen *in situ* within host cells, suggesting symbiont diazotrophy might help supplement their nitrogen deficient diet of wood [20]. Interestingly, of the six shipworm gill endosymbionts described to date, three are diazotrophic (*T. turnerae* T7902T, Bs08T and the thioautotrophic shipworm symbiont *Thiosocius teredinicola* 2141T) and three (Bs02T, Bs12T and Bsc2T) are incapable of fixing atmospheric dinitrogen. This indicates that although diazotrophy may be a common feature of shipworm gill endosymbionts, it is not a prerequisite for endosymbiotic existence. Notably, diazotrophy is not common among described species of the family *Cellibivirionaceae*. Of these, only *Cellivirbio diazotrophicus* [21] and *T. turnerae* [11] have been shown to fix nitrogen. Additionally, *nifH* homologs have been found in the genomes of *Agaribacterium halotis* feces2T and in the as-yet undescribed *Pseudomaricurvus* sp. HS19.

The ability of these three strains to respire nitrate was also tested. Briefly, cells grown in liquid SBM medium with 0.2% cellulose and 5 mM NaN03, were re-streaked on a solid version of this medium prepared with 1.0% Bacto agar. Control plates containing SBM medium with 0.2% cellulose and 0.0025% NH4Cl were also used. The inoculated plates were then placed inside an anaerobic pouch (BS GasPak EZ Anaerobic Pouch). Despite the presence of homologs of dissimilatory nitrate reductase (*nar*) in the genomes of all three strains, only strain Bs08T grew under anaerobic conditions in the presence of nitrate.

To determine the oxygen growth requirements of the three strains, stab cultures were prepared by inoculating soft agar tubes (18 mm×150 mm tubes containing 15 ml of 0.2% Bacto agar/w/v), 1x SBM medium, 0.2% cellulose and 0.025% NH4Cl with liquid cultures using sterile needles. For all strains, a faint lens of cells forms a few millimetres below the soft agar surface, indicating that, like *T. turnerae* and *T. waterburyi* Bs02T, strains Bs08T, Bs12T and Bsc2T, show increased growth under microaerobic conditions. Also, like *T. turnerae*, clearing of the medium was observed around the growth lens after prolonged incubation, due to the hydrolysis of cellulose.

Growth optima, ranges and doubling times were determined in an artificial seawater-based SBM liquid medium supplemented with 0.5% galactose (w/v) and 0.025% NH4Cl as previously described [12]. The data obtained for the three strains are shown in Table 1. Like *T. turnerae* T7902T, strains Bs12T and Bsc2T, require concentrations of Ca2+ and Mg2+ in the medium similar to those found in seawater (~10 mM Ca and 50 mM Mg), while *T. waterburyi* Bs02T and strain Bs08T do not.

Cellular fatty acid methyl ester (FAME) analysis was performed using the MIDI Sherlock Microbial Identification System. Briefly, week-old colonies were scraped from SBM medium agar plates supplemented with 0.5% galactose and 0.025% NH4Cl growing at 20 °C and submitted to MIDI (Newark, DE) for analyses. The three major fatty acids for all three strains are C15:0 iso, C15:0 anteiso and C17:0 anteiso. Strains Bs12T and Bsc2T have very similar FAME profiles wherein C15:0 anteiso and C17:0 anteiso account for more than 80% of fatty acids detected. In strain Bs08T, the total for these two fatty acids is less than 50%. FAME profiles of these three *B. setacea* endosymbionts are markedly different from recently described *B. setacea* symbiont *T. waterburyi* Bs02T which has dominant fatty acids composed of summed feature 5 (C16:0 ante and/or C18:ω6c). This is consistent with its more divergent phylogenetic relationship as indicated in Fig. 1. A detailed comparison of the FAME profiles among all the shipworm symbionts and related bacteria is shown in Table S1 (available in the online version of this article).

**MORPHOLOGY**

Colonies of strains Bs08T, Bs12T and Bsc2T, growing on SBM plates supplemented with cellulose initially appear as minute, slightly raised and translucent. Upon prolonged incubation, the colonies widen in the subsurface of the agar and produce a characteristic clearing zone due to the hydrolysis of cellulose. At this stage, colonies of strains Bs08T and Bsc2T appear off-white or beige, while those of strain Bs12T develop a deep-purple colour. Purple pigmentation is also observed in the culture supernatant when strain Bs12T was grown in liquid medium. No similar pigment production was observed in Bs08T and Bsc2T cultures or in previously described *Teredinibacter* species. Morphology of cells at mid-exponential phase in liquid medium was examined using phase contrast light microscopy (Nikon Eclipse Ni-U and Nikon NIS Elements). The cells of all the three strains appear as motile slightly curved rods that are 2–5 μm long and 0.4–0.6 μm wide, and are Gram-stain negative (Sigma-Aldrich).

**GENOMES**

Genomic DNA extracts used for whole genome sequencing and 16S rRNA gene sequence amplification were obtained from bacterial cell pellets grown on SBM liquid medium with 0.2% cellulose or 0.5% galactose using the method described in O’Connor et al, [9] and Altamia et al. [12]. The genomes of strains Bs08T and Bsc2T were sequenced at New England Biolabs using PacBio Model RSII platform, while the genome of strain Bs12T was sequenced by the Joint Genome Institute – Department of Energy (JGI-DOE) using PacBio Model RS II. All the genomes were assembled using HGAP version 2.0. Contigs from previously reported genome sequence data for these strains [9,12] were aligned with the new assemblies and those determined to overlap multiple contigs were used to identify sequence gaps and the contig termini flanking each gap. Contiguity across identified gaps was then confirmed by PCR amplification and the sequence spanning each gap was determined from the resulting PCR products by primer walking using the Sanger sequencing method.

The assembled genome of strain Bs08T is composed of a single circular chromosome of 4915572 bp with a G+C content of
47.2 mol%. The estimated sequencing coverage is 114×. The genome of strain Bsc2T was assembled to form nine scaffolds totaling to 5406307 bp with a G+C content of 47.31 mol% with estimated coverage of 62×. The assembled genome of Bs12T is composed of 2 circular elements, a chromosome of 4643613 bp with a DNA G+C content of 45.89 mol% and a large plasmid of 249467 bp with a DNA G+C content of 42.54 mol%. Estimated genome coverage is 210×. The cumulative GC-skew plots calculated for the two elements are typical of closed-circular DNA structures [22] (data not shown). The smaller of the two, which was identified as a putative conjugative element using the ICEfinder [23] web-based detection tool (https://db-mmnl.sjtu.edu.cn/ICEfinder/ICEfinder.html), differs from the chromosome both in mol% G+C and in tetranucleotide frequency. Additionally, it contains a copy of recA, a gene most commonly found as a single copy in bacterial chromosomes [24]. A BLASTp search of the GenBank nr database (7/1/2020) indicates that this recA copy is most similar to that found in the chromosome of Marinobacter confluentis, sharing 79.52% identity at 95% query coverage with this recA as compared to 75.9% identity at 95% query coverage with the recA copy found in the Bs12T chromosome. These data suggest that this plasmid was acquired recently via lateral gene transfer. Detailed analysis of the genomes of strains Bs08T, Bs12T and Bsc2T will be reported elsewhere. A table comparing the genomes of strains Bs08T, Bs12T and Bsc2T and related bacteria are shown in Table 2.

**PHYLOGENY**

Phylogenetic analysis was carried out by aligning the near-full length 16S rRNA gene sequences of the three strains with related reference taxa using mafft version 7 with Q-INS-I [25] setting, followed by manual adjustments by eye. The final trimmed dataset is composed of 1372 nucleotide positions. Phylogenetic analysis was carried out using Bayesian inference (MrBayes version 3.2.6) [26].
Markov chain Monte Carlo chains were set to 5 million with subsampling every 2000 generations utilizing the GTR+I+Γ nucleotide substitution model, with the first 20% of the results discarded as the analytical burn-in.

Here and in previous analyses [12, 27], phylogenetic relationships among examined species within the family Cellvibrionaceae are poorly resolved based on analyses of 16S rRNA gene sequences alone (Figs 2 and S2). In these analyses, many nodes, including those associating the three new strains presented here with previously named taxa, display posterior probabilities below those commonly accepted as thresholds for significance. However, approaches that utilize concatenated alignments of multiple protein-coding loci have proven to produce more robust phylogenetic topologies and to more accurately delimit natural phylogenetic groups [27]. For this study, we utilized the Genome Taxonomy Database Toolkit (GTDB-Tk 1.1.0) [28] to construct a concatenated alignment of 120 conserved single copy protein coding marker genes from the genomes of Bs08T, Bs12T and Bsc2T and 58 selected reference taxa (Table S2). Phylogenetic relationships among taxa were then inferred using RaxML 8.2.12 [29] employing the PROTGAMMALOGBASE62 substitution matrix and 250 bootstrap replicates were performed to generate a consensus tree. The resulting topology provides strong support for a clade containing strains Bs08T, Bs12T and Bsc2T and previously described species of the genus Teredinibacter. The same analysis places Cellvibrionaceae bacterium PMS-1162T.S.0a.05 basal to this clade, although with more modest bootstrap support. (Figs 1 and S1). This as yet uncharacterized strain was isolated from a specimen of the shipworm *Lyrodus pedicellatus* collected in the Philippines.

Increasingly, genomic comparisons are being used to guide delimitation of species and genera. For example, average amino acid identity (AAI) values less than 85 and 60% have been suggested as thresholds for delimitation of species and genera respectively [30]. Similarly, percent of conserved protein (POCP) values less than 50% have been proposed as a threshold for differentiating species [31]. Here, pairwise AAI and POCP values were calculated among shipworm symbionts and related taxa using the method described by Konstantinidis et al. [32] and Qin et al. [31] respectively. Pairwise AAI and POCP values observed among strains Bs08T, Bs12T, Bsc2T and the described *Teredinibacter* species *T. waterburyi* Bs02T and *T. turnerae* T7902T range from 62.50–70.18% (Table 3) and POCP values from 51.79–60.94% (Table 4). These values support the proposed designation of these strains as distinct species within the genus Teredinibacter. In consideration of physiology, genome composition and phylogenetic relationships to described taxa, we propose the new names *Teredinibacter haidensis* sp. nov., *Teredinibacter purpureus* sp. nov. and *Teredinibacter franksiae* sp. nov., with Bs08T, Bs12T and Bsc2T as their respective type strains.

**EMENDED DESCRIPTION OF THE GENUS TEREDINIBACTER DISTEL ET AL. 2020**

The description is as given by Distel et al. 2020 [12] with the following emendation. When grown on SBM agar plates supplemented with Sigmacell cellulose, mature colonies may be clear, white, yellow, beige, brown, or purple. The type species is *Teredinibacter turnerae*. 
### Table 3. Average amino acid identity matrix among strains Bs08<sup>T</sup>, Bs12<sup>T</sup> and Bsc2<sup>T</sup> and related bacteria

Values shown are for two-way AAI calculated using the method of Konstantinidis et al. [32]. Colour scale is from red (highest) to green (lowest)

|          | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
|----------|----|----|----|----|----|----|----|----|----|----|----|----|
| 1 Teredinibacter haidensis Bs08<sup>T</sup> | 70.18 | 69.11 | 64.62 | 64.06 | 59.57 | 58.14 | 57.34 | 55.88 | 55.52 | 52.94 | 52.08 |
| 2 Teredinibacter purpureus Bs12<sup>T</sup> | 70.12 | 67.67 | 64.16 | 62.83 | 59.09 | 57.78 | 56.62 | 55.41 | 55.35 | 52.68 | 51.69 |
| 3 Teredinibacter franksiae Bsc2<sup>T</sup> | 69.11 | 67.67 | 62.95 | 63.12 | 58.42 | 57.95 | 56.42 | 55.38 | 54.97 | 52.22 | 51.48 |
| 4 Teredinibacter waterburyi B<sup>o</sup>02<sup>T</sup> | 64.62 | 64.16 | 62.95 | 62.50 | 59.95 | 58.81 | 57.60 | 56.53 | 55.93 | 53.72 | 53.17 |
| 5 Teredinibacter turnerae T7902<sup>T</sup> | 64.06 | 62.83 | 63.12 | 62.90 | 57.91 | 57.85 | 56.65 | 54.89 | 55.23 | 52.53 | 51.55 |
| 6 Saccharophagus degradans 2–40<sup>T</sup> | 59.57 | 59.09 | 58.42 | 59.95 | 57.91 | 58.32 | 56.82 | 55.79 | 55.40 | 53.19 | 51.82 |
| 7 Cellvibrionaceae bacterium PMS-1162T.S.0a.05 | 58.14 | 57.78 | 57.95 | 58.81 | 57.85 | 58.32 | 56.57 | 54.99 | 54.94 | 52.36 | 51.93 |
| 8 Thalassocella blandensis ISS155<sup>T</sup> | 57.34 | 56.62 | 56.46 | 57.60 | 56.62 | 56.82 | 56.57 | 58.10 | 54.76 | 52.02 | 51.76 |
| 9 Agarilytica rhodophyticola 017<sup>T</sup> | 55.88 | 55.41 | 55.38 | 56.53 | 54.89 | 55.79 | 54.99 | 58.10 | 54.43 | 51.30 | 51.03 |
| 10 Agaribacterium halotis feces2<sup>T</sup> | 55.52 | 55.35 | 54.97 | 55.93 | 55.23 | 55.40 | 54.99 | 54.76 | 54.43 | 52.10 | 51.52 |
| 11 Simiduia agarivorans SA1<sup>T</sup> | 52.94 | 52.68 | 52.22 | 53.72 | 52.53 | 53.19 | 52.36 | 52.02 | 51.30 | 52.10 | 54.40 |
| 12 Pseudoteredinibacter isoporae SW-11<sup>T</sup> | 52.08 | 51.69 | 51.48 | 53.17 | 51.55 | 51.82 | 51.93 | 51.76 | 51.03 | 51.52 | 54.40 |
Table 4. Percentage of conserved protein matrix among strains Bs08\textsuperscript{T}, Bs12\textsuperscript{T} and Bsc2\textsuperscript{T} and related bacteria. Values shown were calculated using the method of Qin \textit{et al.} \cite{31}. Colour scale is from red (highest) to green (lowest).

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | Teredinibacter haidensis Bs08\textsuperscript{T} | 58.18 | 58.18 | 58.15 | 55.45 | 51.79 | 49.12 | 46.83 | 38.28 | 35.75 | 42.94 | 38.44 | 31.89 |
| 2  | Teredinibacter purpureus Bs12\textsuperscript{T} | 59.36 | 58.15 | 55.62 | 55.45 | 53.66 | 52.62 | 50.50 | 39.95 | 37.89 | 45.14 | 39.83 | 32.82 |
| 3  | Teredinibacter franksiae Bsc2\textsuperscript{T} | 59.10 | 51.79 | 53.66 | 56.70 | 54.99 | 54.99 | 50.50 | 39.95 | 37.89 | 45.14 | 39.83 | 32.82 |
| 4  | Teredinibacter waterburiy Bs02\textsuperscript{T} | 50.47 | 46.83 | 50.50 | 54.49 | 42.87 | 53.37 | 52.01 | 42.01 | 38.47 | 45.98 | 41.32 | 35.20 |
| 5  | Teredinibacter turnerae T7902\textsuperscript{T} | 52.59 | 49.12 | 52.62 | 54.72 | 46.64 | 49.67 | 42.33 | 38.71 | 45.27 | 40.15 | 33.77 | 35.88 |
| 6  | Saccharophagus degradans 2–40\textsuperscript{T} | 38.03 | 35.75 | 37.89 | 44.02 | 38.71 | 40.25 | 38.47 | 40.85 | 39.55 | 35.45 | 33.47 | 36.16 |
| 7  | Cellvibrionaceae bacterium PMS-1162T.S.0a.05 | 46.88 | 42.94 | 45.14 | 48.91 | 45.27 | 48.07 | 45.98 | 41.32 | 35.20 | 42.05 | 43.96 | 36.16 |
| 8  | Thalassocella blandensis ISS155\textsuperscript{T} | 41.48 | 38.28 | 39.95 | 42.47 | 42.33 | 43.57 | 42.01 | 40.85 | 39.44 | 38.47 | 31.99 | 36.16 |
| 9  | Agarilytica rhodophyticola 017\textsuperscript{T} | 41.49 | 38.44 | 39.83 | 43.17 | 40.15 | 44.30 | 41.32 | 38.47 | 35.45 | 42.05 | 43.96 | 36.16 |
| 10 | Agaribacterium halotis feces2\textsuperscript{T} | 34.38 | 31.89 | 32.82 | 36.06 | 33.77 | 35.88 | 35.20 | 31.99 | 33.47 | 36.16 | 43.96 | 43.96 |
DESCRIPTION OF TEREDINIBACTER HAIĐENSIS SP. NOV.

Teredinibacter haidensis (hai.den’sis. N.L. masc. adj. haiđen-sis named in recognition of the students and faculty of the Hydaburg City School District and members of the Haida Nation, Hydaburg, Alaska for their contributions to the study of shipworm biology).

In addition to the characteristics of the genus, capable of fixing atmospheric dinitrogen under microaerophilic growth conditions. Can use NH₄Cl or nitrate for growth under normoxic conditions. Has the ability to perform anaerobic nitrate respiration when grown in liquid or solid medium supplemented with cellulose as the sole carbon source. When grown on plates, small translucent colonies are initially observed on the agar surface, but over time the majority of growth forms an inverted dome of cells with off-white or beige coloration beneath the agar surface. The pH, temperature and salinity range for growth is approximately pH 6.5–8.5, 10–25°C and 0.2–0.6 M NaCl, respectively; with optimum growth recorded at pH 8.0, 20°C and 0.2 M NaCl. Under these conditions, the mean doubling time is approximately 11.5 h. The major fatty acids are C₁₅:0 anteiso, C₁₇:0 anteiso and C₁₅:0 iso. The genome is estimated to be 4.92 Mbp.

The type strain has been deposited as T. purpureus Bs₁₂T (=ATCC TSD-122T=KCTC 62965T).

DESCRIPTION OF TEREDINIBACTER PURPUREUS SP. NOV.

Teredinibacter purpureus (pur.pu’re.us. L. masc. adj. purpureus purple).

In addition to the characteristics of the genus, requires a source of combined nitrogen such as NH₄Cl or nitrate for growth. When grown on plates, small translucent colonies are initially observed on the agar surface, but over time the majority of growth forms an inverted dome of cells with pronounced purple coloration beneath the agar surface. The pH, temperature and salinity range for growth is approximately pH 6.5–8.5, 10–25°C and 0.2–0.6 M NaCl, respectively; with optimum growth recorded at pH 8.0, 20°C and 0.2 M NaCl. Under these conditions, the mean doubling time is approximately 8.8 h. The major fatty acids are C₁₅:0 anteiso, C₁₇:0 anteiso and C₁₅:0 iso. The total genome is estimated to be 5.41 Mbp.

The type strain has been deposited as T. purpureus Bs₀₈T (=ATCC TSD-121T=KCTC 62964T).

DESCRIPTION OF TEREDINIBACTER FRANKSIAE SP. NOV.

Teredinibacter frankiae (frank’si.æ. N.L. gen. n. frank-siae named in honour of microbiologist Diana Franks for her contributions to the characterization of the genus Teredinibacter).

In addition to the characteristics of the genus, requires a source of combined nitrogen such as NH₄Cl or nitrate for growth. When grown on plates, small translucent colonies are initially observed on the agar surface, but over time the majority of growth forms an inverted dome of cells with off-white or beige coloration beneath the agar surface. The pH, temperature and salinity range for growth is approximately pH 6.5–8.5, 15–20°C and 0.2–0.4 M NaCl, respectively; with the optimum growth recorded at pH 8.0, 20°C and 0.2 M NaCl. Under these conditions, the mean doubling time is approximately 5.41 Mbp with a G+C content of 47.3 mol%. The GenBank/EBML/DDBJ accession numbers for the bioprojects, chromosomal sequences, 16S rRNA genes for Teredinibacter haidensis sp. nov. Bsc₂T are PRJNA340180, CP060084 and MT416119, respectively. The type strain has been deposited as T. frankiae Bsc₂T (=ATCC TSD-123T=KCTC 62966T).

Funding information
Research reported in this publication was supported by the Fogarty International Centre of the National Institutes of Health under Award Number U19TW008163 (to M.G.H and D.L.D.), National Science Foundation awards IOS 1442759 (to D.L.D.) and DBI 1722553 and National Oceanic and Atmospheric Administration award NA190AR010303 (to D.L.D. and M.G.H.). DNA sequence data used in this investigation were produced by the US Department of Energy Joint Genome Institute through a grant from the Community Sequencing Program, JGI User Agreement No. FP00002075 and by New England Biosciences Inc. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Science Foundation.

Acknowledgements
The authors wish to thank Donald G. Comb, Richard J. Roberts, and Alexey Fomenkov (New England Biolabs) for support of and assistance with genome sequencing efforts and to the students and faculty of the Hydaburg City School District and members of the Haida Nation, Hydaburg, Alaska for their enthusiastic contributions to this research.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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